T.C.
YEDITEPE UNIVERSITY
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DEPARTMENT OF MOLECULAR MEDICINE

THE RELATIONSHIP BETWEEN CCR2 V64I POLYMORPHISM AND CCR5 DELTA DELETION AND SIGNIFICANCE OF CORONARY ARTERY DISEASE

PHD THESIS

MUSTAFA AYTEK ŞİMŞEK, M.D.

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for an award of any other degree except where due acknowledgment has been made in the text.

24/104/2022

Mustafa Aytek Şimşek

DEDICATION

I dedicate my thesis to my family and to the people that I love

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Mustafa Aytek Şimşek

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LIST OF SYMBOLS AND ABBREVIATIONS

ACS	Acute coronary syndrome
AGE	Advanced glycation end-products
AMI	Acute myocardial infarction
ApoB	Apolipoprotein B
AS	Atherosclerosis
ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
CAD	Coronary artery disease
Cx	Circumflex artery
DC	Dendritic cells
DM	Diabetes mellitus
EC	Endothelial cells
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EEM	External elastic membrane
ELR	Glutamic acid-leucine-arginine
EM	Internal elastic membrane
FH	Familial hypercholesterolemia
HDL	High density lipoprotein
HL	Hyperlipidemia
HT	Hypertension
IHD	Ischemic heart disease
IL	Interleukin
LAD	Left anterior descending artery
LDL	Low-density lipoprotein
MCP-1	Monocyte chemoattractant protein-1
MDSC	Myeloid derived suppressor cell
NK	Natural killer cell
NO	Nitric oxide
oxLDL	Oxidized LDL
PC	Plasma cell
PCI	Percutaneous coronary intervention

PCR	Polymerase chain reaction
PVAT	Perivascular adipose tissue
RANTES	Regulated upon activation, normal T cell expressed and secreted
RCA	Right coronary artery
SMC	Smooth muscle cell
SYNTAX	TAXUS Drug-Eluting Stent Versus Coronary Artery Bypass Surgery for the Treatment of Narrowed Arteries
TARF	Turkish adult risk factor
TG	Triglyceride
Th	T helper cell

ABSTRACT

Şimşek, M.A. (2022). The Relationship Between Ccr2 V64I Polymorphism, Ccr5 Delta Deletion and Significance of Coronary Artery Disease. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine, PhD thesis, İstanbul.

Inflammation plays an important role in the development and progress of atherosclerosis. Chemokines are small proteins secreted by certain cells to attract inflammatory cells such as macrophages, neutrophiles and lymphocytes to the inflammation site via their corresponding receptors. Ccr2 V64I polymorphism was shown to be involved in atherosclerosis in mice models. The human studies, however, found conflicting results. Ccr5 Delta 32 deletion results in a truncated protein that doesn't reach the surface and function as a receptor. This deletion was associated with coronary artery disease in animal and human studies. In this study we aimed to investigate the relationship between Ccr2 V64I polymorphism, Ccr5 Delta 32 deletion and significant coronary artery disease. Blood samples were collected before coronary angiography. Ccr2 V64I polymorphism and Ccr5 Delta 32 deletion was studied by PCR and then gel electrophoresis. Significance of coronary artery disease was evaluated by SYNTAX score. The patients were grouped into patient group (with significant coronary artery disease) and control group (without significant coronary artery disease). Median age was 61 and majority of subjects were male (54.5%). Patient group had significantly more hypertension, diabetes, and hyperlipidemia. Prevalence of Ccr2 V64I allele was 30.7% in overall population and did not differ between patient and control groups. Prevalence of Ccr5 Delta 32 mutation was low (5.9%) and no statistical comparison could be made between groups. Subjects with Ccr2 V64I polymorphism had significantly higher diabetes whereas subjects with Ccr5 Delta deletion had significantly higher body mass index. Linear regression analysis revealed male gender, hypertension, obesity and low HDL as independent predictors of significant coronary artery disease. Our study adds valuable information about the role of chemokine system in atherosclerosis. Inflammatory pathways should be thoroughly investigated to unravel potential therapeutic targets and preventive measures.

Keywords: coronary artery disease, atherosclerosis, chemokine, chemokine receptor, Ccr2 V64I polymorphism, Ccr5 Delta 32 deletion

ÖZET

Şimşek, M.A. (2022). Ccr2 V64I polimorfizmi ve Ccr5 Delta 32 delesyonu ile Koroner Arter Hastalığı Arasındaki İlişki. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Bölümü, Doktora tezi, İstanbul.

İnflamasyon ateroskleroz gelişimi ve ilerlemesinde önemli rol oynar. Kemokinler özel reseptörleri yoluyla makrofajlar, nötrofiller ve lenfositler gibi inflamatuar hücreleri inflamasyon bölgesine çeken ve belirli hücre gruplarından salgılanan küçük proteinlerdir. CC reseptör 2 (Ccr2) V64I gen polimorfizminin ateroskleroz ile ilişkili olduğu fere modellerinde gösterilmiştir. İnsan çalışmalarında ise çelişkili sonuçlar mevcuttur. Ccr5 Delta 32 delesyonu ise normalden kısa, yüzeye ulaşmayan ve reseptör olarak işlev göstermeyen bir protein sentezlenmesine neden olur. Bu delesyonun koroner arter hastalığı ile ilişkisi hayvan ve insan deneylerinde gösterilmiştir. Bu çalışmada Ccr2 V64I polimorfizmi ve Ccr5 Delta 32 delesyonu ile anlamlı coroner arter hastalığı arasında ilişkiyi araştırmayı amaçladık. Kan örnekleri coroner anjiyografi öncesi alındı. Ccr2 V64I polimorfizmi ve Ccr5 delta 32 delesyonu polimeraz zincir reaksiyonu ve jel elektroforez yöntemi ile çalışıldı. Koroner arter hastalığının anlamlılığı SYNTAX skoru ile değerlendirildi. Katılımcılar hasta grubu (anlamlı coroner arter hastalığı olanlar) ve control grubu (anlamlı coroner arter hastalığı olmayanlar) şeklinde gruplandırıldı. Ortanca yaş 61 ve katılımcıların çoğu erkekti (%54,5). Hasta grup anlamlı olarak daha fazla hipertansiyon, diyabet ve hiperlipidemiye sahipti. Ccr2 V64I alel prevalansı tüm popülasyonda %30,7 idi ve gruplar arasında anlamlı fark yoktu. Ccr5 Delta 32 delesyonu prevalansı ise düşüktü (%5,9) bu nedenle gruplar arasında istatistik karşılaştırma yapılamadı. Ccr2 V64I polimorfizmine sahip olanlarda anlamlı olarak daha fazla diyabet vardı, Ccr5 delta delesyonuna sahip olanlarda ise vücut kitle indeksi anlamlı olarak daha yüksekti. Doğrusal regresyon analizinde erkek cinsiyet, hipertansiyon, obezite ve düşük HDL anlamlı coroner arter hastalığı için bağımsız öngördürücüler olarak saptandı. Bu çalışmamız kemokin sisteminin aterosklerozdaki rolüne dair değerli bilgiler sağlamaktadır. Aterosklerozda inflamatuar yolaklar, tedavi hedeflerinin belirlenmesi ve koruyucu önlmelerin alınması için detaylı olarak araştırılmalıdır.

Anahtar Kelimeler: koroner arter hastalığı, ateroskleroz, kemokin, kemokin reseptörü, Ccr2 V64I polimorfizmi, Ccr5 Delta 32 delesyonu

1. INTRODUCTION AND PURPOSE

Ischemic heart disease (IHD) is the leading cause of death globally (1). Turkish adult risk factor (TARF) study revealed a high mortality due to coronary artery disease (CAD) in a sample population from Turkey (2).

Atherosclerosis is the underlying pathological mechanism in majority of the cases of CAD and it is a long term process that is thought to start at very early ages (3). The initiating factors of atherosclerosis are not well known however it has been showed that the progression of the atherosclerotic lesions is mainly associated with inflammation (4).

Chemokines are a group of proteins that mediate the inflammatory response by recruiting immune cells to the inflammation site. The interaction between chemokines and their corresponding receptors is the key element in chemokine action. The discovery of chemokines and chemokine receptors changed the perspective on inflammatory processes and made it possible to develop new therapeutic strategies (5).

As in other inflammatory diseases, several chemokines and their interaction with receptors were found to be associated with atherosclerosis (6). The polymorphisms and deletions that may affect this interaction may result in different pathological and clinical conditions.

In our study we aimed to investigate the association between the presence of CC chemokine receptor 2 Val64Ile polymorphism (Ccr2 V64I) and the presence of CC chemokine receptor 5 Delta 32 deletion (Ccr5 Delta 32) with presence of significant CAD in patients with suspected CAD.

2. LITERATURE REVIEW

2.1. Coronary Arteries and Atherosclerosis

2.1.1. Anatomy and Histology of Coronary Arteries

2.1.1.1. Anatomy of Coronary Arteries

Coronary arteries are the arteries that supply oxygen and required metabolites to the myocardium. The coronary artery system is divided into two segments: epicardial coronary arteries and coronary microcirculation.

Epicardial coronary arteries stem from right and left sinuses of Valsalva in the aortic root. Right coronary artery (RCA) after originating from right sinus of Valsalva, runs along right coronary sulcus and right margin of heart, goes inferiorly to the diaphragmatic surface of heart. Usually, it terminates with right posterior descending (posterior interventricular) branch and right posterolateral branch. Left main coronary artery, on the other hand, after originating from left sinus Valsalva, branches into left anterior descending artery (LAD) and circumflex artery (Cx). LAD gives diagonal and septal branches along its trajectory through anterior interventricular sulcus. Cx runs through left coronary sulcus and gives Optus margin and left posterolateral branches (7). (Figure 1)

After the epicardial segment, coronary microcirculation originates. The microcirculation is composed of arterioles and capillaries located intramyocardially. Since these structures are compressed by myocardium during ventricular systole, myocardial perfusion occurs only during diastole (8). (Figure 2)

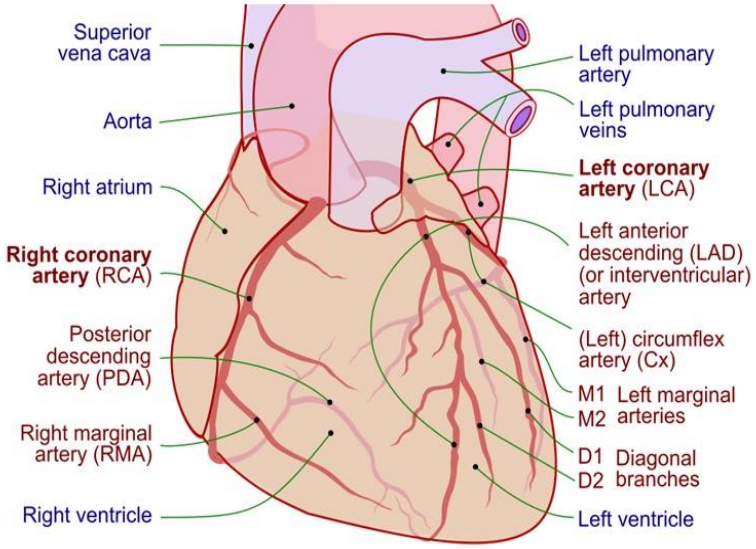

Figure 1. The Anatomy of cardiac circulation. Coronary arteries take their origin from left and righ sinuses of Valsalva which are located just superior to aortic valve. After giving their majör branches, epicardial segement of the circulation contiunes as the microcirculation which is located intramyocardially.

2.1.1.2. Histology of Coronary Arteries

In general, all arteries are composed of three layers: tunica intima, tunica media and tunica externa (adventitia) (Figure 3).

The innermost layer is tunica intima (intimal layer) and the luminal part of it is made up by endothelial cells (EC). Tunica intima is separated from tunica media by internal elastic membrane (IEM) which is composed of elastin fibers. Between endothelial cells and IEM, there's connective tissue formed by collagen, elastin fibers and other extracellular matrix (ECM) elements.

Tunica media is made by circular smooth muscle cell (SMC) layers. Up to 40 layers of SMCs may be observed in tunica media, which is higher in number than of other elastic arteries inside the body (9). The elastic fiber composition of coronary arteries is less than in other elastic arteries. Tunica media is separated from tunica adventitia by external elastic membrane (EEM) that is also made by elastin fibers.

Tunica externa consists of fibrous tissue, perivascular tissue and vasa vasorum (vessels that supply blood to arteries).

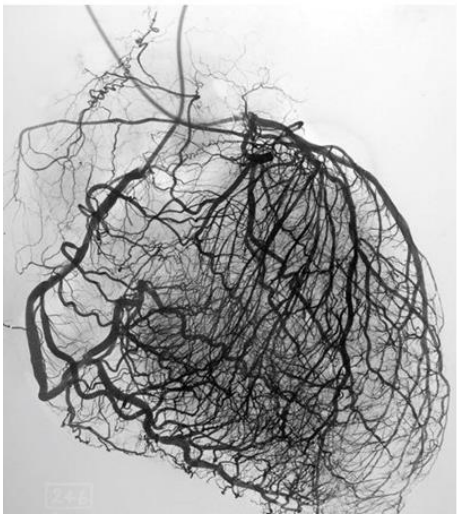

Figure 2. Coronary microcirculation after radiocontrast injection ex-vivo. Subsequent to the epicardial prortion, coronary microcirculation starts and it is usually not visible in imaging studies. However it is the main determinant of myocardial perfusion under normal conditions.

2.1.2. Atherosclerosis

2.1.2.1. Definition and Pathogenesis of Atherosclerosis

Atherosclerosis was previously defined as the accumulation of lipid, cellular products, smooth muscle cells, collagen, and occasional calcium inside the intimal layer of an artery. This definition, however, lacks the role of inflammation, oxidative stress and mechanical processes which have major roles in the pathogenesis of this chronical condition (10). Later new definitions included these factors and emphasized the role of endothelial injury. Contemporary definition of atherosclerosis is chronic inflammatory fibro-proliferative response of arteries against endothelial injury.

No matter what the definition is, the following elements should be present in all atherosclerotic lesions. (Figure 4)

a) Lipid core: The central part of every atherosclerotic lesion is made up by a lipid core which is the intimal deposition of low-density lipoprotein (LDL) particles

b) SMCs: These cells migrate from medial layer of the artery and secrete the extracellular matrix elements

c) Inflammatory cells: Inflammatory cells are recruited from blood stream in response to inflammatory mediators.

d) ECM elements: These are mainly consisted of collagen and non-collagenous proteins.

e) Fibrous cap: This structure covers the luminal surface of an atherosclerotic lesion. As the name suggests it consists of mainly collagen fibers.

Atherosclerosis is initiated by LDL. LDLs are structures that consist mainly cholesterol and are coated with phospholipids and apolipoprotein B (ApoB). The main function of LDL is to carry cholesterol to the tissues through blood stream. Exposure of arteries to LDL causes the accumulation of these particles in the intimal layer which is called fatty streaks.

The accumulated LDL particles are then transformed into oxidized LDLs (oxLDL) by metalloproteins in the ECM (The Fenton reaction). These oxLDLs are engulfed by macrophages and SMCs which transform them into foam cells. Foam cells secrete many inflammatory cytokines that recruit lymphocytes and promote the accumulation of new lipid particles. Matrix metalloproteinases that are secreted mainly by foam cells, degrade the collagen, elastin and proteoglycans which in turn make the atherosclerotic lesion more prone to acute changes.

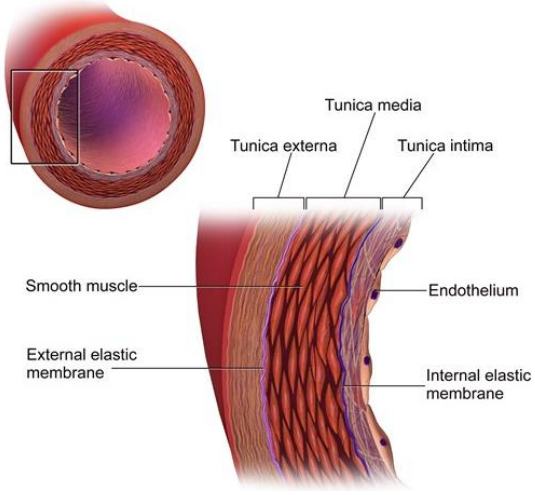

Figure 3. Illustrated histology of an elastic artery. The three layers are the sama efor all elastic arteries howewer coronary arteries differ by having thicker tunica media which makes them resistant to compression exerted by myocardium.

2.1.2.2. Risk Factors for Atherosclerosis

2.1.2.2.A. Metabolic Risk Factors

Diabetes Mellitus (DM), impaired fasting glucose and impaired glucose tolerance elevate the risk of atherosclerotic vascular disease (11). Hyperglycemia causes the increased production of advanced glycation end-products (AGE). AGEs promote lipid accumulation, inflammatory response and oxidative stress in arterial wall (12).

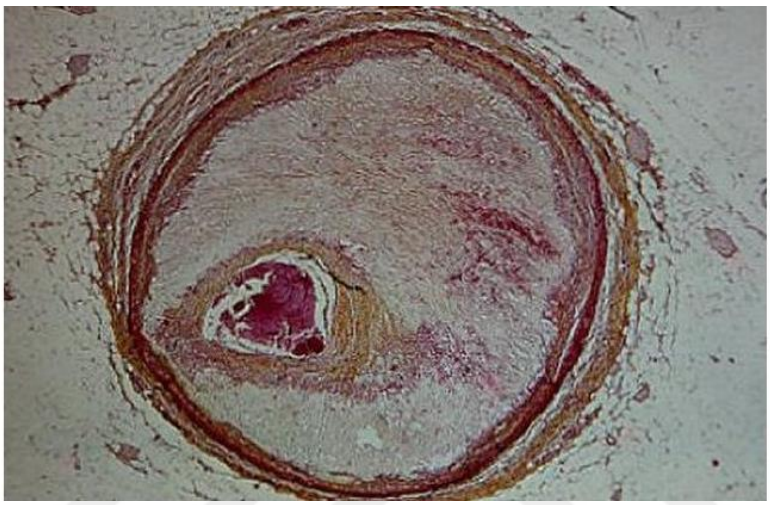

Figure 4. Histologic section of an atherosclerotic plaque. The most distinguished feature of atherosclerotic plaque is the accumulation of lipid and connective tissue elements in the intimal layer. Increased number of smooth muscle cells and inflammatory cells are also present in atherosclerotic plaques.

2.1.2.2.B. Lipid Risk Factors

Elevated LDL levels are directly related to atherosclerosis. In patients with familial hypercholesterolemia, high risk of mortality is present due to early onset atherosclerotic cardiovascular disease (ASCVD). Transcytosis of LDL into intimal layer of arteries is the initiating factor of atherosclerotic lesions. On the other hand, elevated levels of triglyceride (TG) rich lipoproteins were also found to be associated with increased cardiovascular risk (13).

2.1.2.2.C. Hypertension

Elevated blood pressure is known to affect vascular biology. Impaired shear stress, elevated oxidative stress, vascular damage, over-activation of renin angiotensin aldosterone system all contribute to endothelial dysfunction which in turn causes de novo atherosclerosis or accelerates the progression of the atherosclerotic lesions (14).

2.1.2.2.D. Lifestyle Risk Factors

Smoking is a well-established risk factor of atherosclerotic cardiovascular disease. Apart from more than 7000 chemicals that arise from the burning of tobacco, nicotine is solely responsible for the acceleration of atherosclerosis by inducing the dysregulation of endothelial cells, SMCs. It also causes the production of reactive oxygen species which disrupts lipid metabolism and handling of atherogenic lipids by vascular structures.

Sedentary lifestyle also increases the risk of incident ASCVD. Physically inactive (completely sedentary) individuals have 54% higher risk of developing ASCVD than physically active individuals (15).

2.1.2.3. Atherosclerosis and Inflammatory Cells

Inflammation is one of the major effectors in the pathogenesis of atherosclerosis and this relationship has been known since 19th century The “response to injury” theory by Ross and Glomset explained the initiation of atherosclerosis with the occurrence of endothelial dysfunction resulting from physical, chemical, inflammatory or infectious damages (16).

With the development of new molecular imaging techniques, the role of inflammation has gained more attention. It has been showed that response of innate immunity including recruitment and/or activation of antigen presenting cells such as monocytes and dendritic cells accelerated the progression of atherosclerotic lesions. The cytokines released from these cells trigger the adaptive immunity (17).

Monocytes as a part of innate immunity are recruited to atherosclerotic regions by adhesion and chemokine action. The presence of oxLDL activates the ECs to express adhesion molecules which capture monocytes. Once they enter the vascular wall, they transform into macrophages and dendritic cells (DC) (18). Not all of the macrophages that are present in the atherosclerotic lesion is from the monocyte pool in the blood stream (19). The self-renewal of macrophages also contributes to the macrophage population in the vessel wall (20).

The macrophages and also SMCs uptake oxLDL by scavenger receptors such as SR-A1, CD36 and lectin type oxidized LDL receptor 1 (21). After the uptake of oxLDL

macrophages and SMCs turn into foam cells. Expression of ABCA-1 on macrophages inhibit foam cell production and cause free cholesterol efflux from macrophage to high density lipoprotein (LDL) (22).

DCs can be grouped into three subsets: plasmacytoid DCs, type 1 conventional DCs, type 2 conventional DCs. Plasmacytoid DCs are in blood stream or lymphoid tissue, and they release interferon type 1 after encountering antigens. Conventional DC's are mainly responsible for cytotoxic actions or antigen presenting to T cells (23). It has been known that the atherosclerotic lesions that contain high amount of DCs are more susceptible to acute changes (24).

Neutrophils play a role in every stage of atherosclerotic process. The increase in number of neutrophils correlates with atherosclerosis in animal models (25). Neutrophils facilitate inflammation by releasing ROS and by attracting lymphocytes to the atherosclerotic regions. Also neutrophil extracellular traps cause cell lysis and atherosclerotic plaque destability (26).

Lymphocytes also act on the progression of atherosclerosis. T helper 1 cells are the predominant type in atherosclerotic lesions and they react to ApoB to release pro-inflammatory cytokines (27). T cells also stimulate B cells to produce certain antibodies which in turn maintains the inflammation. B cells, on the other hand, are not present in atherosclerotic lesion, instead they are located in lymph-node like structures located int tunica externa. B2 subset of B lymphocytes are known to be pro-atherogenic (28).

2.2. Chemokines and Chemokine Receptors

2.2.1. Structure and Functions of Chemokines

Chemokines (chemoattractant cytokines) are a group of proteins that are involved in leukocyte trafficking as well as normal leukocyte function (29). 47 different chemokines were discovered since 1970s. Chemokines exert their functions via binding to their G-protein coupled chemokine receptors located on leukocyte cell surface.

Chemokines share common structures such as at least 3 β sheets, C-terminal α helix and flexible N-terminal region (30) (Figure 5). 4 classes of chemokines were

defined according to the cysteine residues located 10 residues away from the N-terminal.

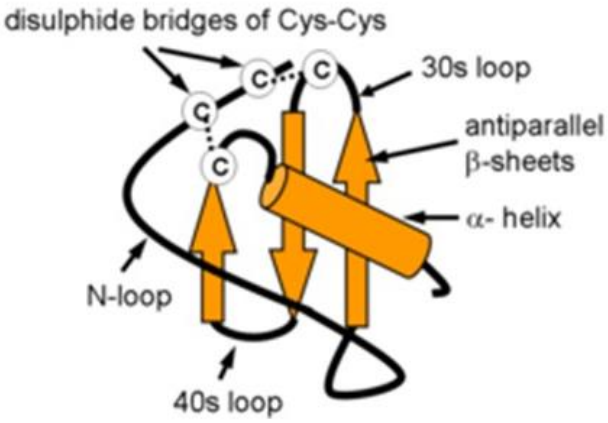

Figure 5. Three dimensional structure of chemokines. Common structures shared by all chemokines are β sheets, C-terminal α helix and N-termanal cysteine residue (s).

CXC chemokines (formerly α chemokines) have 2 cysteine residues separated by 1 extra amino acid. Two different groups of CXC chemokines exist. The first group has ELR (glutamic acid-leucine-arginine) motif (ELR+) just before the first cysteine residue. The second group lacks this motif (ELR-). Most of the ELR+ CXC chemokines are angiogenic and attractant to neutrophils. On the other hand, ELR- CXC chemokines are monocyte and lymphocyte attractant.

CC chemokines (formerly β chemokines) have 2 adjacent cysteine residues in their N-terminal. They are usually chemoattractant to monocytes, basophils, eosinophils, and lymphocytes but not neutrophils. CX_3C chemokines (formerly δ chemokines) have 3 extra amino acids between 2 cysteine residues. Fractalkine is the only member of this group that has been discovered until today. It has a unique structure with a mucin-like stalk at the N-terminal. It is a transmembrane glycoprotein and may exist both soluble and bound form. XC chemokines (formerly γ chemokines) have only one cysteine residue in their N-terminal and they are mainly T lymphocyte attractant (31).

Table 1 lists CC chemokines, their corresponding receptors and key roles.

CXC coding genes are located on chromosome 4 in human genome with an exception of CXCL12 which is located on chromosome 12 (32). CC chemokine genes

are generally located on chromosome 17, however gene coding CCL19 is located on chromosome 9 and gene coding CCL 20 is located on chromosome 2 (33).

The chemokines bind strongly to glycosaminoglycans that are located on certain cell surfaces and in EC matrix. This way they are presented to their corresponding receptors on migrating leukocytes. (Figure 6)

Chemokines are released after contact with antigens or in response to certain cytokines such as interleukin 1 (IL-1) and IL17.

The most important function of chemokines is to attract inflammatory cells to inflammation area. Upon release chemokines form a gradient which attracts circulating leukocytes. Chemokines induce the leukocytes to express integrins which bind to adhesion molecules on endothelium. Also, chemokine signaling is necessary for leukocytes to migrate from intravascular space to inflammation area.

Chemokines also play a role in normal development and physiology of lymphoid organs. Their expression and secretion are necessary to maintain and regulate the trafficking of immune cells through lymphoid tissues.

The antigen presentation function of DCs is dependent on chemokines. Certain chemokines induce DCs to leave the inflammatory site and reach to lymphoid tissues, there they present the antigens to T cells.

As mentioned previously ELR+ CXC chemokines also play a role in angiogenesis.

Table 1. Chemokines of CC family, their corresponding receptors and key roles

Chemokine	Typical Chemokine receptor	Expression and Key Roles
CCL1	CCR8	-Produced by activated monocytes, macrophages and T cells -Activates NK cells
CCL2	CCR2	-Promotes the migration and trafficking of monocytes, DCs and NK cells -Involved in the pathogenesis of atherosclerosis
CCL3	CCR1 CCR5	-Involved in wound healing, inhibition of stem cells -Induces NK cells
CCL4	CCR5	-Mainly secreted by macrophages -Chemoattractant for macrophages, immature DCs
CCL5	CCR5	-Expressed by T cells, macrophages -Acts in the pathogenesis of human immunodeficiency virus infection -Involved in lipid metabolism
CCL6	CCR1	-Produced by macrophages, fibroblasts, vascular SMCs -Chemotaxis of macrophages, monocytes and T cells
CCL7	CCR1, CCR2, CCR3, CCR5, CCR10	-Expressed by stromal cells, airway SMCs, tumor cells -Chemotaxis of monocytes, basophils, eosinophils, T cells
CCL8	CCR1, CCR2, CCR3, CCR5	-Leukocytes chemotaxis, HIV entry -Highly expressed by tumor-associated macrophages, and its presence promotes tumor growth
CCL9	CCR1	-Produced by macrophages and osteoclasts -Functions as a cell survival factor
CCL11	CCR2, CCR3, CCR5	-Produced by endothelial cells, eosinophils, fibroblasts -Highly expressed in brain injury and associated with neurodegeneration
CCL12	CCR2	-Highly expressed by macrophages, involved monocyte derived macrophage and fibroblast recruitment -Inhibits wound healing
CCL13	CCR1, CCR2, CCR3	-Chemoattractant for eosinophils, basophils, macrophages, T cells -Acts on epithelial, muscular and endothelial cells by inducing immunomodulatory responses

DC: dendritic cell; HIV: human immunodeficiency virus; NK: natural killer; SMC: smooth muscle cell

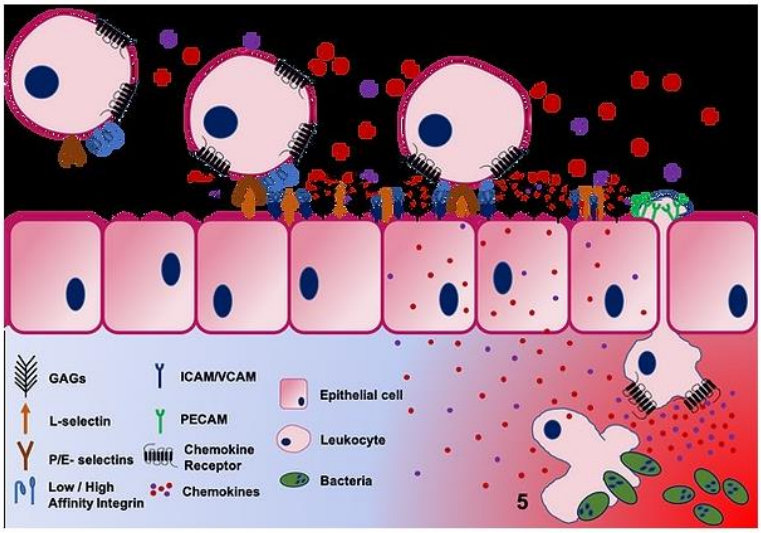

Figure 6. Schematic represantation of the chemotaxis of a leukocyte in response to bacterial infection. The relase of certain chemokines attracts the corresponding leukocytes to the inflammation area. Glycoproteins and glucosaminoglycans are also involved in the attachment and diapedesis of leukocytes.

2.2.2. Structure and Functions of Chemokine Receptors

Chemokine receptors are seven-transmembrane domain G-protein coupled proteins (Figure 7). The genes encoding these receptors are located on chromosome 2 and 3 in human genome.

After binding of chemokines to extracellular N-terminal portion of the receptor, phosphorylation of serine and threonine residues of intracellular C-terminal occurs. The resulting effect is the change in cytoskeleton organization, activation of integrins, biochemical processes that result in adhesion and migration. Chemokine receptors are not unique to only one certain chemokine type, different chemokines may bind to the same receptor (34).

CXCR1 and CXCR2 receptors are expressed on neutrophils, monocytes, mast cells, NK cells and T lymphocytes. CXCR2 can also be found on certain nervous system cells. CXCR3 is expressed mainly on lymphocytes, specifically T lymphocytes.

CCR1 is the receptor for CCL3, CCL5 and CCL7. It is located on lymphocytes and monocytes but not neutrophils. CCR1 also is expressed on certain nervous system cells. CCR2 binds to CCL2, CCL7 and CCL13 and is expressed on monocytes, myeloid precursor cells, activated T lymphocytes and basophils. CCR3 is located mainly on

eosinophils. CCR5 binds to CCL3, CCL4 and CCL5. IT is located on monocytes, effector/memory T cells., macrophages and microglia. It is also a co-receptor for HIV-1.

The receptor for XCL1 is XCR1. It is located on CD8 T lymphocytes and B lymphocytes.

The receptor of fractalkine is CX3CR1 and it is located on NK cells, monocytes and CD8 T lymphocytes.

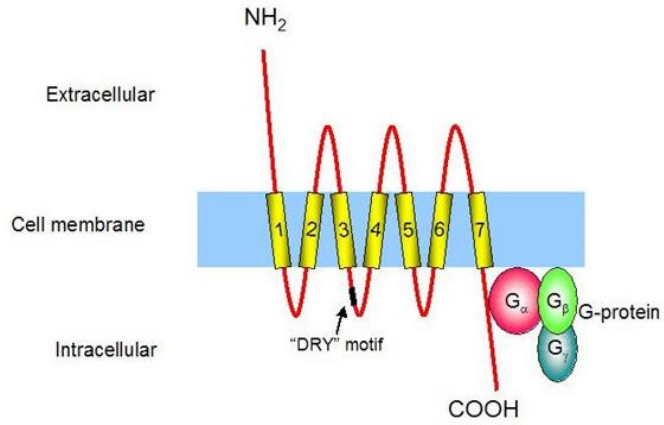

Figure 7. Common structure of chemokine receptors. Chemokine receptors are G-protein coupled proteeins and contain highly connservative glutamic acid/aspartic acid-arginie-tyrosine (E-DRY) motif that is thought to play a major role in receptor's conformational charge upon activation/deactivation.

2.3. Chemokines, Chemokine Receptors, and Atherosclerosis

2.3.1. Chemokines in the Pathogenesis of Atherosclerosis

2.3.1.1. CC Chemokines

CCL2 (monocyte chemotactic protein 1, MCP-1) accelerates atherosclerotic process by recruiting inflammatory cells to the arterial wall. The release of CCL2 increases during nighttime (35).

CCL3 (macrophage inflammatory protein 1α) is involved in chemoattraction of monocytes and T lymphocytes. Certain treatments such as statins were shown to decrease CCL3 levels and atherosclerotic lesions in rodent models (36).

CCL5 (regulated upon activation, normal T cell expressed and secreted; RANTES) forms heterodimers with other peptides and chemokines such as human

neutrophil peptide 1 (HNP1) and CXCL4 to recruit neutrophils into atherosclerotic lesions (37).

CCL17 is mostly located on DCs and involved in recruitment of T cells. It also forms heterodimers with other chemokines such as CCL5. Inhibition of these heterodimers cause atherosclerosis regression in rodent models (38).

CCL19 and CCL21 are both involved in chemotaxis of T cells and macrophages. Their dysregulation may cause both atherosclerosis progression and regression.

2.3.1.2. CXC Chemokines

CXCL1 levels increase after oxLDL accumulation in atherosclerotic lesions. CXCL1 recruits leukocytes mainly macrophages into the inflammatory site Inhibition of CXCL1 reduced plaque burden in mice models (39).

CXCL4 and CCL5 form heterodimers and recruit monocyte and neutrophils into the atherosclerotic site. Also, NET formation is accelerated by this heterodimer.

CXCL12 (stromal cell derived factor 1 alpha) acts through its receptor CXCR4 and has athero-protective role. The release of CXCL12 is associated with plaque stabilization and fibrous cap thickening.

CXCL16 has a mucin stalk like fractalkine. It can be found both bound and soluble form. Soluble form was found to be associated with increased atherosclerosis in mice.

Table 2 summarizes some chemokines as biomarkers in atherosclerosis.

2.3.2. Chemokine Receptors in the Pathogenesis of Atherosclerosis

2.3.2.1. CC Chemokine Receptors

CCR1 functions as pro-atherogenic. It has a role in monocyte chemotaxis into atherosclerotic lesion. It has been showed that atherosclerotic mice lacking *CCR1* gene showed higher plaque burden compared to the mice having the gene (40).

CCR2 and its ligand CCL2 together act on monocyte recruitment in atherosclerosis. Blocking of CCR2 may disrupt atherosclerosis progression and may be a suitable target in the treatment of atherosclerosis.

CCR5 is involved in proatherogenic processes. CCR5 blockade results in decreased recruitment of monocytes into atherosclerotic lesions.

CCR7 has been shown to exert both atherogenic and anti-atherogenic properties (41). It has a role in DC maturation and T cell homeostasis.

Table 2. Chemokines as biomarkers in atherosclerosis

Chemokine	Clinical condition	Clinical significance
CCL2	Early stage of AS	Simvastatin significantly decreases CCL2 levels in hypercholesterolemia
		Postprandial decrease in CCL2 after an oral unsaturated fat load tested in FH
	CAD	Increased risk for clinical adverse events predicting CAD
		Higher in AMI and unstable angina pectoris
		Predictor of restenosis after percutaneous transluminal coronary angioplasty
CCL3	Early stage of AS	Postprandial decrease in CCL3 after an oral unsaturated fat load tested in FH
CCL4	Early stage of AS	Postprandial decrease in CCL4 after an oral unsaturated fat load tested in FH
CCL5	Stable angina pectoris	Patients with elevated CCL5 prone to AS increase of CCL5 might represent a sign of restenosis
	Refractory unstable angina pectoris	Predictor of clinical adverse events
		Higher in AMI and unstable angina pectoris
CCL17	CAD	positively associated with the type and severity of CAD
CXCL12	AMI	Predictor of clinical adverse events
CXCL16	ACS	Predictor of clinical adverse events
CCL18	Refractory unstable angina pectoris	Predictor of clinical adverse events
CX3CL1	CAD	Higher in AMI and unstable angina pectoris elevated early after PCI

AS: atherosclerosis; FH: familial hypercholesterolemia; CAD: coronary artery disease; AMI: acute myocardial infarction; ACS: acute coronary syndrome; PCI: percutaneous coronary intervention.

2.3.2.2. CXC and CX_3C chemokine receptors

CXCR2 is involved in neutrophil activity in atherosclerosis. Its interaction with certain peptides results in pro-atherogenic effects. However, CXCR2-CXC5 interaction decreases foam cell formation and shows athero-protective effect (42).

CXCR4 has dual action pattern on atherosclerosis. It is located on B lymphocytes and CXCR4 activation ends up in increased IgM production. Vascular CXCR4 has endothelial protective effect (43).On the other hand CXCR4-macrophage migration inhibitory factor (MIF) interaction has atherosclerotic effects.

CXCR6 has pro-atherosclerotic effects via T cells and macrophages. ApoE -/- mice lacking CXCR6 was shown to have decreased level of atherosclerosis (44).

CX3CR1 activation is very important in macrophage trafficking and activation inside atherosclerotic lesions. Its interaction with CX3CL1 (fractalkine) was shown to increase atherosclerotic burden in vivo mice models (45).

2.3.3. CCR2 and CCR5

CCR2 is the common receptor for CCL2 (MIP-1), CCL8 (MIP-2), CCL7 (MIP-3), CCL13 (MIP-4). It is mainly expressed on the surface of blood monocytes but also found on dendritic cells and T helper cells. CCR2/CCL2 axis is the main route for chemotaxis of macrophages. *CCR2* knock out or CCL2 inhibited mice exhibited impaired mobilization of monocytes from bone marrow to blood stream which resulted in impaired accumulation of monocytes in the inflammatory sites (46, 47). The discovery of the role of CCR2 in the monocyte recruitment ignited several studies which showed the activity of CCR2/CCL2 axis in romatoid arthritis (RA), transplant rejection and CAD

CCR5, on the other hand, is located mainly on macrophages differentiated from blood monocytes and T helper 1 lymphocytes (48). It is the common receptor of CCL5 (RANTES), CCL4, CCL3 and CCL8. CCR5 is responsible for macrophage survival during inflammation. CCR5 also functions in Th1 recruitment to the inflammation site. As CCR2, CCR5 was also studied in RA and CAD (49, 50).

2.3.3.1. Ccr2 V64I polymorphism

Ccr2 is an approximately 8 kb gene which contains 3 exons and is located on chromosome 3. It forms a cluster with *CCR1, CCR3*, *CCR4* and *CCR5*. The most studied polymorphism of *Ccr2* gene is V64I. This polymorphism was first described by Smith et.al (51). They screened the entire *Ccr2* gene and found G-to-A nucleotide substitution in position 190 which corresponded to substitution of valine in position 64 to isoleucine. The allelic frequency of this polymorphism was found to be 0.098 in Caucasians, 0.151 in African Americans, 0.172 in Hispanics and 0.250 in Asians out of a cohort composed of 3003 individuals.

Ccr2 V64I polymorphism was studied first in subjects with HIV infection due to the role of this receptor in virus entry into the T lymphocytes (52). Since its association with inflammation, many investigators studied this polymorphism in coronary artery disease. Gonzalez et al. studied 670 subject (310 patients, 360 control) and found that *Ccr2* V64I polymorphism did not affect the age of onset in patients with myocardial infarction.

Pai et al. compared incident CAD cases with matched individuals from a Nurses' Health Study cohort (53). The allelic frequency did not differ between groups and the V64I polymorphism was found not to be associated with incident CAD.

2.3.3.2. Ccr5 Delta 32 deletion

Ccr5 gene is also located on chromosome 3 (3p21.3-p24) in human genome. It spans almost 8kb and includes 3 exons and 2 introns. *Ccr5* Delta 32 deletion was first discovered in 1996 as a mutation that protects cells from being infected by HIV. Genetic analysis of the gene revealed that the mutation consists of a 32 base pair deletion from nucleotide 794 to 825 (54). The mutation causes a truncated receptor protein that is unable to reach cell surface (Figure 8). Heterozygous individuals carry 20-30% less CCR5 molecules on the cell surface (55). It is known that *Ccr5* Delta 32 deletion causes a reduced ability of HIV-1 into the cells. The frequency of this mutation reaches up to 10% in Caucasians however it is almost not seen in Asian populations.

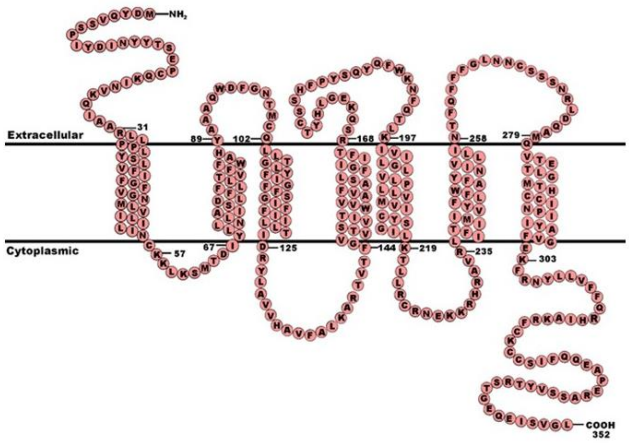

Figure 8. Schematic represantation of CCR5. Delta 32 deletion results in a truncated protein which prevents the receptor from reaching the cell surface.

2.3.4. CCL2-CCR2 Axis and Atherosclerosis

The evident role of inflammation in atherosclerosis drove researchers to find the mechanisms underlying the development and progression of atherosclerosis as well as the treatment of atherosclerosis. The anti-inflammatory treatments such as interleukin (IL) 1β antagonist canakinumab and colchicine showed promising results in patients with CAD (56, 57).

However, the side effects and the lack of mortality benefit of these treatments forced scientists to look for other mechanisms that may play a role in the inflammation underlying atherosclerosis.

The preclinical studies that were mainly based on mice models showed that deletion of *Ccl2* and *Ccr2* genes resulted in reduction of atherosclerotic burden (58, 59). On the other hand overexpression of *Ccl2* in *ApoE* deficient mice increased atherosclerosis (60).

Recent observational human studies showed that CCL2 levels in atherosclerotic plaques were associated with some features that were related to plaque vulnerability such as increased number of macrophages, larger lipid core etc. (61). Large genetic studies showed that genetically determined high levels of CCL2 was strongly associated with ischemic stroke and large atherosclerotic plaques (62).

Very few interventional studies that targeted CCL2-CCR2 axis have been conducted until today. Bindarit, a drug which inhibits CCL2 production (63), showed

efficacy in preventing in stent late loss in a phase II trial (64). Another molecule named MLN1202 is an monoclonal antibody which has anti-CCR2 effect. In study with 108 patients who had 2 or more CVD risk factors and elevated C-reactive protein (CRP), MLN1202 administration showed significantly decreased CRP levels (65). However, this study was not designed for clinical end points.

Another striking feature of CCL2-CCR2 axis is its circadian rhythmicity. The release of CCL2 has a diurnal feature and blocking CCL2 in its active release phase reduced atherosclerosis in mice models (65).

2.3.5. CCL5-CCR5 Axis and Atherosclerosis

CCL5 acts through multiple receptors including CCR1, CCR3 and CCR5. CCR5 distinguishes itself from other receptors of CCL by being expressed on macrophages, T cells, ECs and SMCs (66). The expression on SMC is somewhat important because of the critical role of SMCs in the development and progression of atherosclerotic plaque. Early studies showed that dyslipidemia increased the CCL5 levels and patients with early atherosclerotic lesions had elevated CCL 5 levels (67, 68). Studies on animal models found that CCL5 and CCR5 deficiency significantly reduced atherosclerotic burden (69). On the other hand, CCR5 was found to be involved in atherosclerotic plaque stabilization and pharmacological blockade of CCR5 reduced plaque formation (70, 71). These findings suggest that CCL5-CCR5 axis is important in both atherosclerosis development and progression. It is a potential target for the treatment of the atherosclerosis.

3. MATERIALS AND METHODS

3.1. Study Population and Data Acquisition

101 subjects who had suspected or known CAD and positive myocardial ischemia evidence were enrolled in our study. All subjects were chosen non randomly and consecutively from the patient population who admitted for coronary angiography in Yeditepe University Hospital Cardiology Department, Turkey. Signed and oral informed consent were obtained from all participants. Demographical, clinical, and angiographic data were obtained from the patient files, hospital records and imaging software retrospectively. The peripheral blood samples were obtained before the coronary angiogram.

3.2. Inclusion and Exclusion Criteria

Inclusion criteria were:

a) Subjects who are 18 years of age or older
b) Subjects with suspected or known CAD and objective evidence of myocardial ischemia

Exclusion criteria were:

a) Subjects with chronic renal disease
b) Subjects who had a history of coronary artery bypass surgery
c) Subjects with moderate to severe chronic obstructive pulmonary disease

3.3. Evaluation of Coronary Angiograms

All coronary angiograms were evaluated by two cardiologists using TOMTEC® diagnostic imaging software (TOMTEC Imaging Systems GMBH, Unterschleissheim, Germany). TAXUS Drug-Eluting Stent Versus Coronary Artery Bypass Surgery for the Treatment of Narrowed Arteries (SYNTAX) score was calculated for each participant except the control group by using commercially available SYNTAX score calculator (72). SYNTAX score was developed from the data of SYNTAX trial (73) and used to establish the significance and extent of coronary artery disease using several parameters such as narrowing in main coronary arteries and their major branches, certain

anatomical features of the coronary circulation and presence of coronary calcification. SYNTAX score of zero means that there's no significant coronary artery disease.

3.4. Study groups and cardiovascular risk factors

Subjects who had a SYNTAX score of zero formed the control group whereas subjects with a SYNTAX score greater than zero formed the patient group. Also, subgroups according to cardiovascular risk factors were formed.

Hypertension (HT) was defined as using an anti-hypertensive drug; diabetes mellitus (DM) was defined as being treated for diabetes mellitus or hemoglobin A1c level over 6.0; hyperlipidemia (HL) was defined as being treated for hyperlipidemia or total cholesterol level over 200 mg/dl; cigarette smoking was defined as subjects who were actively smoking during study enrollment period or who quitted less than 3 months ago; family history of CAD was defined as having a first degree relative who was diagnosed with CAD before 50 years of age for males and 55 years of age for females; history of CAD was defined as having an established CAD either by invasive or computerized tomography coronary angiography; obesity was defined as body mass index (BMI) over 30 kg/m^2

BMI was calculated using Deveraux formula and body surface area was calculated by Du Bois formula.

3.5. Blood sample acquisition and DNA Isolation

All peripheral blood samples were taken into 5 ml sample tubes containing ethylenediaminetetraacetic acid (EDTA) and stored in 4°C until the analysis. Invitrogen DNA isolation Kit was used for isolation of DNA blood (iPrep ™ PureLink ™ performed Blood Kit). This system has the capacity of isolating DNA from 100 samples of 350µl for one instance. The steps recommended by the manual of the kit were applied. iPrepTM Insulation Device ChargeSwitch ® Technology (CST ®) was also utilized to purify the DNA extracts. This system can be adjusted via the buffer pH, surface load and relies on the magnetic bead-based technology. The negatively charged nucleic acid backbones bind to positively charged beads so the remaining molecules such as proteins can't bind and washed away by the buffer solution. When the pH of the

mixture was raised to 8.5, bound nucleic acids were freed and were ready to be isolated. Until the genomic analysis DNA extracts were stored in 4°C.

3.5.1. Measurement of DNA purity

The optical density of 50ng/l (g/ml) double-stranded DNA at 260 nm wavelength was found to be 1. The Nanodrop2000 device was used to determine the purity of DNA samples. The OD260/OD280 ratio was used to evaluate the purity of DNA samples. Clean samples were those with an OD260/OD280 ratio of 1.7 to 1.9, which were recognized as acceptable for genotyping.

3.6. Genomic analysis

DNA samples from the subjects were amplified by polymerase chain reaction (PCR) method which used Ccr2 and Ccr5 Delta 32 primes under the protocols described below. The products of Ccr2 PCR were restricted by BsaBI enzyme. The restriction products of Ccr2-BsaBI and PCR products of Ccr5 Delta 32 were moved in 2% agarose gel by electrophoresis and visualized under UV light. Fort he PCR, i-star Taq® PCR kit (iNtRON Bio, Korea) was used.

3.6.1. PCR mix protocol

Table 3. PCR mix protocol for DNA samples

dH_2O	17.4 µL
10x Buffer	2.5 µL
dNTP	2 µL
Forward Primer	0.8 µL
Reverse Primer	0.8 µL
Taq	0.25 µL
DNA	1.25L

3.6.2. Primers for *Ccr2*

Forward primer: 5’-TTGGTTTTGTGGGCAACATGATGG-3’

Reverse primer: 5’-CATTGCATTCCCAAAGACCCACTC-3’

Length of PCR Product: 173 bp

3.6.3. PCR conditions for *Ccr2* gene

Table 4. PCR conditions for Ccr2 gene

Step	Temperature	Time	Number of cycles
First Denaturation	94°C	5 minutes	1 cycle
Denaturation	94°C	30 seconds	33 cycles
Binding	56°C	30 seconds	
Lengthening	72°C	30 seconds	
Final lengthening	72°C	5 minutes	1 cycle

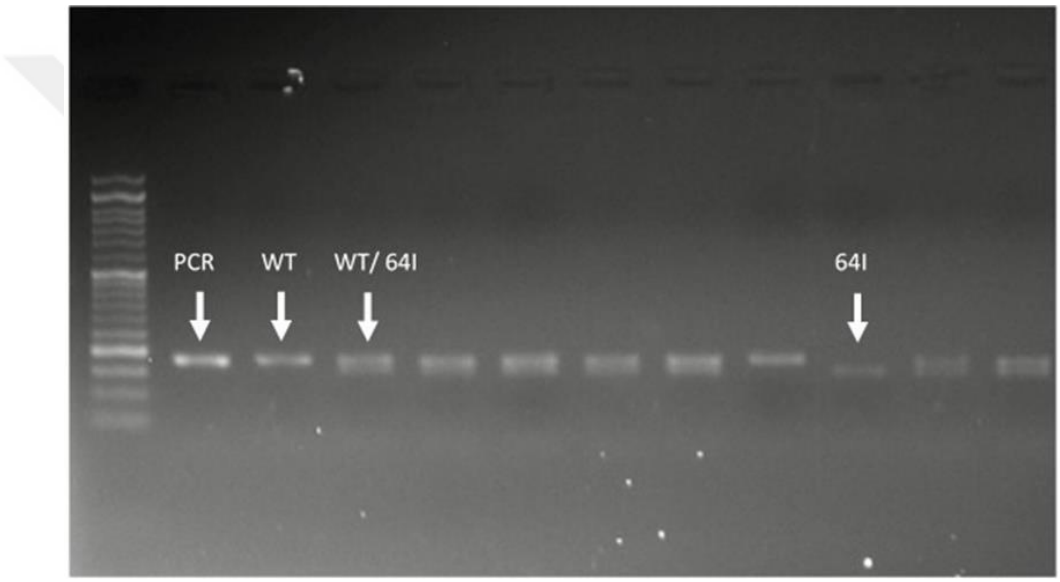

Figure 9. PCR results of *Ccr2* V641. WT refers to wild type and 64I refers to valine to Ileucine polymorphism

3.6.4. BsaBI enzyme restriction protocol (Fermentas Catalog number: ER1711)

Table 5. BsBI enzyme protocol for Ccr2-V64I polymorphism

dH_2O	8.5 µL
10x-Buffer 0	1 µL
BsaBI restriction enzyme	0.5 µL
PCR product	10 µL

3.6.5. BsaBI enzyme restriction conditions

Table 6. BsaBI enzyme restriction conditions

Temperature	Time
65°C	3 hours

Restriction products:

WT/WT: 173 bp

WT/64I: 173 bp, 149 bp, 24 bp

64I/64I: 149 bp, 24 bp

Results:

50 bp marker, PCR product (173 bp), homozygote WT (173 bp), heterozygote (173 bp, 149 bp), homozygote mutant 64I (149 bp)

3.6.6. Primers for *Ccr5*-Delta 32

Forward primer: 5'-TGTTTGCGTCTCTCCCAG-3'

Reverse primer: 5'-CACAGCCCTGTGCCTCTT-3'

3.6.7. PCR conditions for *Ccr5* gene

Table 7. PCR conditions for Ccr5 gene

Step	**Temperature**	**Time**	**Number of cycles**
First Denaturation	94°C	4 minutes	1 cycle
Denaturation	94°C	30 seconds	35 cycles
Binding	52°C	30 seconds	
Lengthening	72°C	30 seconds	
Final lengthening	72°C	7 minutes	1 cycle

PCR products

II: 233 bp

DD: 201 bp

ID: 233 bp, 201 bp

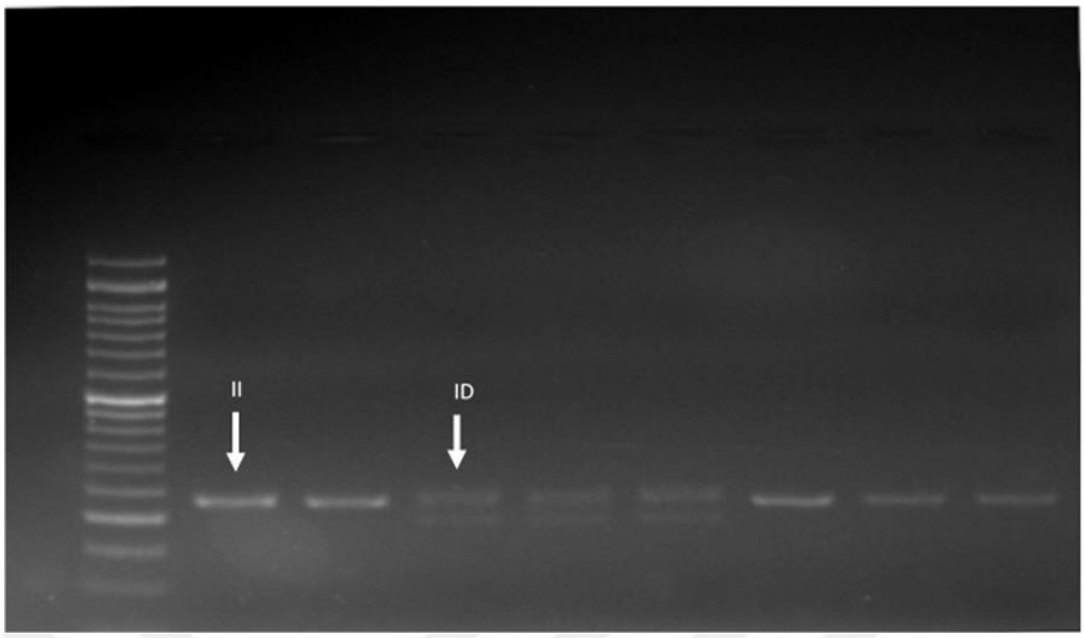

Figure 10. PCR results of *Ccr5* Delta Deletion. II refers to wild type and ID refers to 32 aminoacid deletion in only one copy of the gene

3.7. End points

The primary end point of this study is to establish the frequencies of *Ccr2* V64I polymorphism and *Ccr5* Delta 32 deletion in patients with and without significant coronary artery disease. Secondary end point is to establish independent risk factors for significant coronary artery disease.

3.8. Statistical analysis

Continuous variables were expressed as median (interquartile range-IQR) and categorical variables were expressed as percentages. Categorical variables were compared by Chi Square test or Kruskal-Wallis test and continuous variables were compared by Student t test or Mann Whitney U test between groups. Logistic regression analysis was used to determine independent variables that were related to significant CAD (SYNTAX score over zero). P values below 0.05 at 95% confidence interval were accepted statistically significant in every statistical analysis. All analyses were done in SPSS (Statistical Package for the Social Sciences) software version 25.0 (IBM Corp., Armonk, NY, USA)

4. RESULTS

4.1. General characteristcs of the study population

101 subjects were enrolled in the study. 51 patients had zero SYNTAX score and therefore constituted the control group. 75% of the study population were younger than 67 years of age and 54.5% were male. Demographical and clinical features of the study population were outlined in Table 8 .

When compared to subjects with zero SYNTAX score, subjects with SYNTAX score over 0 were significantly more hypertensive, diabetic and male gender and history of CAD were more prevalent in this group (Table 8).

Table 8. Baseline characteristics of the study population

Demographical and clinical characteristics (median-IQR or n-%)				
	Overall n:101 (%)	**Patient group n:51 (%)**	**Control group n:50 (%)**	***p value***
Age	61 (15)	62 (10)	56.5 (16)	0.09
Gender (male)	55 (54.5)	34 (66.7)	21 (42)	**0.02**
HT	57 (56.4)	39 (76.5)	18 (36)	**<0.01**
DM	34 (33.7)	23 (45.1)	11 (22)	**0.03**
HL	49 (48.5)	31 (60.8)	18 (36)	**0.02**
Smoking	35 (34.7)	17 (33.3)	18 (36)	0.57
Family history of CAD	35 (34.7)	16 (31.4)	19 (38)	0.57
CAD history	24 (23.8)	24 (47.1)	0 (0)	**<0.01**
Obesity	42 (41.6)	23 (45.1)	19 (38)	0.62
BMI	29.40 (6.1)	29.8 (5.0)	29.0 (6.3)	0.53
Laboratory analysis (median-IQR)				
LDL	114 (49)	110 (47)	116 (52)	0.15
HDL	45 (14)	46 (16)	44 (13)	0.74
Total cholesterol	191 (50)	193 (63)	186.5 (47)	0.20
TG	159 (91)	157 (85)	159(101)	0.67

BMI: body mass index; CAD: coronary artery disease; DM: diabetes mellitus; HDL: high density lipoprotein; HL: hyperlipidemia; HT: hypertension; IQR: interquartile range; LDL: low density lipoprotein; TG:triglyceride

4.2. Prevalance of *Ccr2* V64I polymorphism and *Ccr5* Delta 32 deletion

4.2.1. *Ccr2* V64I polymorphism

70 (69.3%) subjects had wild type gene whereas 22 (21.8%) subjects were heterozygote, and 9 subjects (8.9%) were homozygote for *Ccr2* V64I polymorphism. The prevalence of wild type, heterozygote and homozygote subjects were not significantly different between the patient and control groups. (Table 9). Genotype distribution was consistent with that predicted by Hardy–Weinberg equilibrium in patients and controls (p> 0.05)

Table 9. Prevalence of Ccr2 V64I polymorphism

Gene type	**Patient group n:51 (%)**	**Control group (%) n:50 (%)**	***p value***
WT	36 (70.6)	34 (68)	0.107
WT-V64I	8 (15.7)	14 (28)	
V64I	7 (13.7)	2 (4)	
WT	80(78.43)	82(81.2)	
V64I	22(21.5)	18(19.80)	0,52

n is the number of subjects per group. Comparison between groups made by chi-square analysis. WT: wild type; WT-V64I: heterozygote for V64I polymorphism; V64I: homozygote for V64I polymorphism

4.2.2. *Ccr5* Delta 32 deletion

95 (94.1%) subjects did not have *Ccr5* Delta 32 deletion whereas 6 subjects (5.9%) were heterozygote. No homozygote subject was found. Both patient and control groups had 3 subjects with heterozygote mutation. Genotype distribution was consistent with that predicted by Hardy–Weinberg equilibrium in patients and controls (p> 0.05) Since the number was below 5, no statistical analysis could be performed.

Table 10. Prevalence of Ccr5 Delta 32 deletion

Gene type	Patient group n:51 (%)	Control group (%) n:50 (%)	p value
II	48 (94.1)	47 (94)	
ID	3 (5.9)	3 (6)	
DD	0 (0)	0 (0)	
I	99(97.05)	97(97.02)	
D	3(2.94)	3(2.97)	0.98

n is the number of subjects per group. Comparison between groups made by chi-square analysis. II: no Delta 32 deletion, ID: heterozygote Delta 32 deletion, DD: homozygote Delta 32 deletion

4.3. *Ccr2* V64I polymorphism and cardiovascular risk factors

Heterozygous and homozygous subjects of V64I polymorphism were grouped together as *Ccr2* V64I polymorphism carriers. The prevalence of cardiovascular risk factors was compared between subjects who carried V64I polymorphism and who did not carry the polymorphism. Presence of HT, HL, obesity, family history of early CAD and history of CAD did not differ between groups however DM was significantly more prevalent in patients without V64I allele (Table 11).

Table 11. Prevalence of cardiovascular risk factors in subjects with and without Ccr2 V64I allele

Cardiovascular risk factor	V64I (+) n: 31 (%)	V64I (-) n:70 (%)	*p value*
HT	16 (51.6)	41 (58.6)	0.515
DM	6 (19.4)	28 (40)	**0.043**
HL	14 (45.2)	35 (50)	0.654
Family history of CAD	12 (38.7)	23 (32.9)	0.569
Obesity	13 (41.9)	29 (41.4)	0.962
CAD history	8 (25.8)	16 (22.9)	0.748

CAD: coronary artery disease; DM: diabetes mellitus; HL: hyperlipidemia; HT: hypertension

4.4. *Ccr2* V64I polymorphism and lipid profile and BMI

Mean LDL level was significantly higher in V64I allele carriers but mean HDL, TG, total cholesterol were not different between groups. Mean BMI was also not different between groups (Table 12)

Table 12. Lipid profile and BMI values in subjects with and without Ccr2 V64I allele

Lipid profile and BMI	V64I (+) (median-IQR)	V64I (-) (median-IQR)	*p value*
LDL (mg/dl)	109 (58)	118.5 (46)	0.068
HDL (mg/dl)	45 (13)	44.5 (14)	0.714
TG (mg/dl)	161 (57)	156.5 (97)	0.477
Total cholesterol (mg/dl)	193 (51)	190 (51)	0.248
BMI (kg/m^2)	28.5 (7.7)	29.4 (4.1)	0.109

BMI: body mass index; HDL: high density lipoprotein; IQR: interquartile range; LDL: low density lipoprotein; TG: triglyceride

4.5. *Ccr5* Delta 32 deletion and cardiovascular risk factors

No difference in the prevalence of cardiovascular risk factors was found between subjects with or without *Ccr5* Delta 32 deletion (Table 13).

Table 13. Prevalence of cardiovascular risk factors in subjects with and without Ccr5 Delta 32 deletion

Cardiovascular risk factor	Delta (+) n: 6 (%)	Delta (-) n:95 (%)	*p value*
HT	3 (50)	54 (56.8)	0.743
DM	2 (33.3)	32 (33.7)	0.986
HL	2 (33.3)	47 (49.5)	0.443
Family history of CAD	3 (50)	32 (33.7)	0.415
Obesity	13 (41.9)	21 (41.4)	0.666
CAD history	3 (50)	21 (22.1)	0.119

CAD: coronary artery disease; DM: diabetes mellitus; HL: hyperlipidemia; HT: hypertension

4.6. *Ccr5* Delta deletion and lipid profile and BMI

Mean BMI level was significantly higher in *Ccr5* Delta 32 deletion carriers, but mean lipid profile was not different between groups. (Table 14)

Table 14. Lipid profile and BMI values in subjects with and without Ccr5 Delta Deletion

Lipid profile and BMI	**Delta (+) (median-IQR)**	**Delta (-) (median-IQR)**	***p value***
LDL (mg/dl)	102.5 (44)	115 (52)	0.328
HDL (mg/dl)	48 (14)	44 (14)	0.921
TG (mg/dl)	140.5 (68)	159 (94)	0.223
Total cholesterol (mg/dl)	172 (70)	191 (46)	0.661
BMI (kg/m^2)	30.1 (14.4)	29.4 (5.3)	**0.002**

BMI: body mass index; HDL: high density lipoprotein; IQR: interquartile range; LDL: low density lipoprotein; TG: triglyceride

4.7. Linear Regression Analysis of Factors Related to the Presence of Significant CAD

A regression model was formed to establish the factors related to the presence of significant CAD. Model included classical cardiovascular risk factors such as HT, DM, HL plus the presence of any allele of *Ccr2* V64I polymorphism or *Ccr5* Delta 32 deletion (either heterozygote or homozygote). Table 15 depicts the effects of variables on the presence of significant CAD. Age, male gender, obesity, and HDL were significantly associated with significant CAD. On the other hand, neither the V64I allele nor the Delta allele was found to be associated with significant CAD.

Table 15. Linear Regression Analysis of Factors Affecting The Presence of Significant CAD

Variable	Standard coefficient Beta	95,0% Confidence Interval for B		P value
		Lower bound	Upper bound	
Age	0.254	0.003	0.020	0.008
Male gender	0.383	0.192	0.577	<0.001
HT	0.312	0.121	0.507	0.002
DM	0.120	-0.125	0.244	0.186
HL	0.156	-0.035	0.348	0.109
Family history of CAD	-0.011	-0.197	0.173	0.897
Smoking	0.010	-0.173	0.194	0.909
Obesity	0.416	0.147	0.698	0.003
LDL	-0.075	-0.005	0.003	0.633
HDL	-0.208	-0.021	-0.001	0.030
Total Cholesterol	0.016	-0.003	0.003	0.922
V64I	0.055	-0.125	0.244	0.521
Delta	0.071	-0.217	0.517	0.418

CAD: coronary artery disease; DM: diabetes mellitus; HL: hyperlipidemia; HT: hypertension, V64I: presence of any Ccr2 V64I polymorphism allele, Delta: presence of any Ccr5 Delta 32 deletion

5. DISCUSSION

In this study, we couldn't find any difference of *Ccr2* V64I polymorphism and *Ccr5* Delta 32 deletion prevalence between subjects with and without significant coronary artery disease. Linear regression analysis revealed that age, male gender, obesity, and HDL were significantly associated with significant CAD, however the presence of any *Ccr2* V64I polymorphism (heterozygote or homozygote) or any *Ccr5* Delta 32 deletion (heterozygote or homozygote) was not associated with significant CAD.

CCL2 is an inducible chemokine and is secreted upon the release of several toher inflammatory factors such as ILs 1, 4 and 6; tumor necrosis factor alpha; transforming growth factor beta; interferon gamma and several growth factors.

The main target for CCL2 is monocytes, however it also exerts its effects on T cells, DCs, NK cells (74). After binding to CCR2, JAK2/STAT3 (75) and MAP kinase signaling pathways are activated. This results in cytoskeleton reorganization which results in chemotaxis of the leukocyte to inflammation area.

CCL2 was shown to play role in the pathogenesis of inflammatory diseases such as rheumatoid arthritis (76), multiple sclerosis, and atherosclerosis. It also has been studied in infectious diseases and malignancies.

The role of CCL2-CCR2 axis in obesity and atherosclerosis is very important. Obesity is an increased inflammatory state characterized by inflammatory cell infiltration (mainly macrophages) in adipose tissue and elevated serum inflammatory markers. Elevated CCL2 levels in adipose tissue were demonstrated in animal models (77). Some studies showed decreased adiposity and insulin resistance in *Ccr2* knock out animal models, other studies revealed conflicting results (78). These findings suggested additional chemokine pathways such as CCL5-CCR5 axis might play a role in the pathogenesis of obesity.

CCL2 and its corresponding receptor CCR2 were studied extensively in CAD. CCL2 is a major player in the regulation of monocyte trafficking as it is a highly effective signal for monocytes to be recruited into the inflammation site (79). Secreted CCL2 mainly acts on through CCR2 to mobilize monocytes from bone marrow (80). de

Lemos et al. studied CCL2 serum levels in 2270 patients with acute coronary syndrome and 279 healthy volunteers. A CCL2 level of 75th percentile was found to be associated with increased cardiovascular events during follow up (81). Kervinen et al. studied 183 patients with high-risk coronary artery disease and found that increased levels of CCL2 was associated with adverse cardiovascular outcomes (82). Combadiere et al. showed that combined inhibition of CCL2, CXCR1 and CCR5 in hypercholesterolemic mice resulted in 90% decrease in atherosclerosis (83).

The association between *Ccr2* gene polymorphisms and CAD, however, was not as clear as CCL2 and CAD. Karaali et al. studied 146 patients with acute myocardial infarction from Turkey (84). The allelic frequency of *Ccr2* V64I was 25.3% and *Ccr5* deletion was 13.4%. Similar to our study, they could not find any association between *Ccr2* V64I polymorphism and acute myocardial infarction, however the presence of *Ccr5* Delta deletion increased the risk 89%.

Gonzalez et al. investigated the role *Ccr2* V64I polymorphism in development of myocardial infarction before 55 and 60 years of age. Similar to our study, the prevalence of the V64I heterozygote subjects were 22% and the allele frequency of V64I did not differ between patient and control groups (85).

Kallel et al. studied 290 Tunisian male patients with MI. Similar to our study no relationship between V64I allele and myocardial infarction risk was found (86). In a meta-analysis of 24 studies and *Ccr2* V64I polymorphism was found to be not associated with CAD (87). To our knowledge, our study is the first study that investigated the relationship between *Ccr2*V64I polymorphism and the significance of CAD.

CCL5 mainly stimulates T cells and macrophages. It binds to CCR1, CCR3, CCR4 and CCR5, but the highest affinity of CCL5 is to CCR5. Upon activation of CCR5 by CCL5, downstream intracellular pathways including JAK/STAT, NF-κB and PI3K/AKT are recruited. These downstream pathways increase the expression of certain genes, affect apoptosis and angiogenesis (88).

CCL/CCR5 axis recruits T cells to atherosclerotic lesions. Also by acting on SMCs this axis extends the cell mediated immune process (89). Overexpression CCL5 is associated with plaque instability and neointimal thickening. Hypertension and

obesity both are associetd with CCL5-related atherosclerosis which signifies a role of CC5 in these disease processes.

CCL5 is present in very high amounts in the granules of platelets (90). Upon activation, platelets secrete CCL5 and other molecules and attract monocytes and lymphocytes to the area. ABCB6-an ATP cassette transporter- knock out animal models showed increased levels of CCL5 and P-selectin, increased platelet count and leukocyte-platelet complexes (91).

The CCL5-CXCL4 heteromer was also found to play a role in atherogenesis. Inhibition of this heteromer resulted in prevention of atherosclerotic lesions (92). In animal models, P-selectin mediated CCL5-CXCL4 heteromerization was found to be involved in atherosclerosis.

As mentioned before, the main chemotaxis exerted by CCL2-CCR2 axis is on monocytes, however atherosclerotic lesions contain adaptive immune response elements such as T cells, B cells and NK cells. The recruitment of T cells are partly done by CCL5-CCR5 axis. The atherosclerotic plaques in *Ccr5* and *ApoE* knock out mice show reduced T cell infiltration and and Th-1type immune response (93).

TAK-779, an inhibitor of CCR5 and CXCR3, was shown to reduce atherosclerotic burden in *Ldlr* knock out mice. The most interesting result of this study is that the T cell component of the plaques reduced up to 95%. This shows the importance of CCR5 in the recruitment of T cells into the atherosclerotic plaques (94).

CCL5 was shown to be involved in myocardial reperfusion injury and progression of atherosclerosis in previous studies (71). The receptors of CCL5 are CCR1, CCR3 and CCR5. CCL5 like CCL2, is involved in the chemotaxis of monocytes/macrophages however it also recruits T cells and activates platelets and SMCs(95). The versatility of this chemokine led to various results in the studies. CCL5 also forms heterodimers with other cytokines such as CXCL4 (38). These heterodimers were found to be involved in atherosclerotic plaque progression (92). CCR5 is one of the receptors of CCL5 and was shown to play a role in CAD development (85). Delta 32 deletion of *Ccr5* gene results in a truncated protein and loss of CCR5 from leukocyte cell surfaces. Homozygous subjects of *Ccr5* Delta 32 deletion were shown to be

protected against atherosclerosis however some studies showed that early stages of atherosclerosis were not dependent on CCR5 activity (96).

The largest study so far regarding *Ccr5* Delta 32 deletion and atherosclerosis was conducted by Hyde et al. 5748 subjects were genotyped, the frequency of *Ccr5* Delta allele was similar to our study. *Ccr5* Delta 32 deletion was found to be associated with increased HDL and decreased TG both of which are protective against atherosclerosis (97). In a study by Dinh et al. blood samples from 15.206 patients from The Danish Blood Donor Study was studied for CRP levels and *Ccr5* Delta 32 deletion (98). *Ccr5* Delta 32 deletion was not associated with low grade inflammation which was defined as CRP levels between 3-10 mg/L. A non-significant trend was observed towards increased cardiovascular hospitalization in subjects with *Ccr5* Delta 32 deletion (heterozygote or homozygote). In our study *Ccr5* Delta 32 deletion carriers were 6 in total, all of which were heterozygous. Heterozygous *Ccr5* Delta 32 deletion was not found to be associated with the presence of significant CAD.

In our study DM was more prevalent in *Ccr2* V64I allele carriers. Insulin resistance and inflammation are two main factors that are related to type 2 DM (99, 100). Many studies investigated the role of chemokine in diabetes and its complications. It was shown that macrophages were recruited in the renal injury area early in the development of diabetic nephropathy mainly via CCL2 (101). CCX140-B, a CCR2 inhibitor, was administered to patients with diabetic nephropathy. After 52 weeks of treatment, the urinary/albumin creatinine ratio reduced with the treatment (102). On the other hand, the relationship between CCR5 and diabetes is not clear. Kochetova et al. studied 940 Tathar subjects (440 diabetic, 500 non diabetic). They showed that *Ccr 5* rs2107538 −471G/A single nucleotide polymorphism was associated with 80% increase in type 2 DM risk (103).

In our study *Ccr5* Delta 32 deletion allele non-carriers had higher LDL levels compared to mutant allele carriers but the difference was statistically non-significant. The non-significance may have occurred because of low subject numbers in *Ccr5* Delta 32 deletion carrier group. CC5-CCR5 axis was known to be involved in lipid synthesis and increased adiposity and CCR5 inhibition resulted in improved TG content in a murine model of non-alcoholic fatty liver disease (104). *Ccr5* Delta 32 deletion was also associated with improved lipid profile in epidemiological studies (97).

Another important finding of our study is that age, hypertension, and HDL were found to be associated with significant CAD.

Age is a well-known atherosclerotic risk factor. Also, high inflammatory state plays a major role in vascular aging and atherosclerosis (105). Perivascular adipose tissue (PVAT) was shown to be involved in vascular aging. Several factors affect the function of this tissue. Inflammation is one of these factors that affect the content and secreted product of PVAT. Chemokines and adipokines are the key factors and potential therapeutic targets in maintaining healthy PVAT and reduced vascular aging (106).

Hypertension induces several anatomical and physiological changes in arterial wall. Elevated blood pressure stimulates the secretion of extracellular matrix elements, which in turn accelerates vascular aging. Also, hypertension causes endothelial dysfunction, by doing so, promotes atherosclerosis. It has been showed that, mechano-transduction was involved in atherosclerosis. Hypertension disrupts laminar flow and high pressure induces inflammatory response (107).

HDL is associated with nitric oxide (NO) dependent vasodilation in animal models. It has been shown that HDL binds to scavenger receptors in endothelium which in turn promotes NO release (108). The anti-atherogenic properties of HDL is linked to its protein components such as apolipoprotein A1, apolipoprotein E and glutathione peroxidase.

6. CONCLUSION

In our study we found no association between *Ccr2* V64I polymorphism and significant coronary artery disease. Also, no association was found between *Ccr5* Delta 32 deletion and significant coronary artery disease. There was a trend for higher LDL levels and HL prevalence in subjects with *Ccr5* Delta 32 deletion. CAD is a multifactorial disease any many pathological mechanism interplays in the progression of atherosclerosis. Our results were in accordance with previously published studies and may be considered hypothesis generating. Future studies are needed to outline the true nature of the relationship between inflammatory factors and CAD.

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7. APPENDICES

7.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 2353 **12/05/2022**
Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Mustafa Aytek Şimşek)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İsbir'in proje koordinatörü olduğu, Tıp Fakültesi Kardiyoloji AD. Dr. Öğr. Üyesi Mustafa Aytek Şimşek'in sorumlu araştırmacı olduğu, **"Koroner Anjiyografi Uygulanan Hastalarda Kemokin 2 ve 5 Gen Polmorfizmi Sıklığı İle Koroner Arter Hastalığı Arasındaki İlişkinin Araştırılması"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(2418)** kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **11.05.2022** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir. **(KAEK Karar No: 1616)**

Prof. Dr. Turgay ÇELİK

Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurul Başkanı

7.2. CIRRUCULUM VITAE

Personal Informations

Name	MUSTAFA AYTEK	**Surname**	ŞİMŞEK

Educational Informations

	Name of Institution	**Year**
Doctorate	Yeditepe University Health Institute Molecular Medicine	**2017- 2022**
Residency Training	Dokuz Eylul University School of Medicine, Department of Cardiology, İzmir	**2007-2012**
Medical School	Hacettepe University, School of Medicine (Curriculum in English)	**2001-2007**

Work Experience

Cardiologist **(Ass. Proffesor)**	Yeditepe University School of Medicine	2015- 2022
Cardiologist	Nevşehir State Hospital	2012-2015

Language	Reading*	Speaking*	Writing*	KPDS/UDS Score	(109) Score
English	Very good	Very good	Very good	88.75	

*****Excellent**,** Very Good, Good, Basic

Computer skills

Program	Ability to use
Microsoft Office	Good

Publications / Notices Certificates / Awards

A –Articles published in international journals

A1. TÜRER CABBAR AYÇA, DEĞERTEKİN MUZAFFER MURAT, ŞİMŞEK MUSTAFA AYTEK, ÖZVEREN OLCAY, GÜLEÇ YILMAZ GÜLSÜM SEDA, YANARTAŞ MEHMED, GEZER TAŞ SERPİL, OLGUN YILDIZELİ ŞEHNAZ, MUTLU BÜLENT, İSBİR TURGAY, YILDIZELİ BEDRETTİN (2022). Evaluation of Asymmetric Dimethylarginine Levels in Patients With Chronic Thromboembolic Pulmonary Hypertension Undergoing Pulmonary Endarterectomy. Heart, Lung and Circulation, 31(1), 110-118., Doi: 10.1016/j.hlc.2021.05.090

A2. BARUT ZERRİN, TÜRER CABBAR AYÇA, GÜLEÇ YILMAZ GÜLSÜM SEDA, AKDENİZ FATMA TUBA, ŞİMŞEK MUSTAFA AYTEK, ÇAPAR BETÜL, DEĞERTEKİN MUZAFFER, DALAN ALTAY BURAK, YEREBAKAN HALİT, İSBİR TURGAY (2021). Investigation of Circulating miRNA-133, miRNA-26, and miRNA-378 as Candidate Biomarkers for Left Ventricular Hypertrophy. Invivo, 35(3), 1605-1610., Doi: 10.21873/invivo.12417

A3. ŞİMŞEK MUSTAFA AYTEK, KORKMAZ BETÜL, EZİCİ ADNAN, TÜRER CABBAR AYÇA, ASLANGER EMRE, ÖZKALAYCI FLORA, KARABAY CAN YÜCEL, DEĞERTEKİN MUZAFFER MURAT (2021). The Association between Serum Heme Oxygenase-1 Levels and Coronary SYNTAX Score. Cardiology, 146(3), 288-294., Doi: 10.1159/000513144

A4. YILDIRIMTÜRK ÖZLEM, ASLANGER EMRE, BOZBEYOĞLU EMRAH, ŞİMŞEK BARIŞ, ŞİMŞEK MUSTAFA AYTEK, AYDIN SİNAN, KARABAY CAN YÜCEL, DEĞERTEKİN MUZAFFER MURAT (2020). Does electrocardiogram help in identifying the culprit artery when angiogram shows both right and circumflex artery disease in inferior myocardial infarction?. ANATOLIAN JOURNAL OF CARDIOLOGY, 23(6), 318-323., Doi: 10.14744/AnatolJCardiol.2020.24583

A5. ASLANGER EMRE, YILDIRIMTÜRK ÖZLEM, ŞİMŞEK BARIŞ, BOZBEYOĞLU EMRAH, ŞİMŞEK MUSTAFA AYTEK, KARABAY CAN YÜCEL, SMITH STEPHEN W, DEĞERTEKİN MUZAFFER MURAT (2020). DIagnostic accuracy oF electrocardiogram for acute coronary OCClUsion resuLTing in myocardial infarction (DIFOCCULT Study). IJC Heart & Vasculature, 30, Doi: 10.1016/j.ijcha.2020.100603

A6. ŞİMŞEK MUSTAFA AYTEK, DEĞERTEKİN MUZAFFER MURAT, TÜRER CABBAR AYÇA, HÜNÜK BURAK, AKTÜRK SERKAN, ERDOĞMUŞ SİYAR, MUTLU BÜLENT, KOZAN ÖMER (2020). NT-proBNP Levels in Stage 3-4 Chronic Kidney Disease and Mortality In Long Term Follow Up: HAPPY study sub group analysis. Turk Kardiyoloji Dernegi Arsivi-Archives of the Turkish Society of Cardiology, 48(5), 454-460.

A7. BOZBEYOĞLU EMRAH, ASLANGER EMRE, YILDIRIMTÜRK ÖZLEM, ŞİMŞEK BARIŞ, KARABAY CAN YÜCEL, ŞİMŞEK MUSTAFA AYTEK, TEKKEŞİN AHMET İLKER, DEĞERTEKİN MUZAFFER MURAT, KOZAN ÖMER (2018). A tale of two formulas: Differentiation of subtle anterior MI from benign ST segment elevation. Annals of Noninvasive Electrocardiology, 23(6), 12568, Doi: 10.1111/anec.12568

A8. ŞİMŞEK MUSTAFA AYTEK, DEĞERTEKİN MUZAFFER MURAT, TÜRER CABBAR AYÇA, ASLANGER EMRE, ÖZVEREN OLCAY, AYDIN SİNAN, MUTLU BÜLENT, EROL ÇETİN (2018). NT-proBNP levels and mortality in a general population-based cohort from Turkey: a long-term follow-up study. Biomarkers in Medicine, 12(10), 1073-1081., Doi: 10.2217/bmm-2018-0120

A9. ŞİMŞEK MUSTAFA AYTEK, TÜRER CABBAR AYÇA, ÖZVEREN OLCAY, AYDIN SİNAN, TAŞCILAR ÖĞE, DEĞERTEKİN MUZAFFER MURAT (2018). Short term effects of sleeve gastrectomy on weight loss and diastolic function in obese patients. Turk Kardiyoloji Dernegi Arsivi-Archives of the Turkish Society of Cardiology, 47(3), 162-167., Doi: 10.5543/tkda.2018.95994

A10. DUMAN ÖMER ÖZKAN, GÖLDELİ ÖZHAN, GÜRSUL ERDAL, BARIŞ NEZİHİ, ÖZPELİT EBRU, ŞİMŞEK MUSTAFA AYTEK (2017). The value of aortic pulse wave velocity in predicting coronary artery disease diagnosis and severity. Acta Cardiologica, 70(3), 315-322., Doi: 10.1080/AC.70.3.3080636

B-Article that were published in national journals

B1. ŞİMŞEK MUSTAFA AYTEK, TÜRER CABBAR AYÇA, ÖZVEREN OLCAY, KOCA ÇİĞDEM, DURMUŞ ERDAL, DEĞERTEKİN MUZAFFER MURAT (2021). Factors Affecting The Change in Agatson Score in Follow Up Multislice Coronary Ct Angiograms. Harran Üniversitesi Tıp Fakültesi Dergisi(3), 485-488., Doi: 10.35440/hutfd.997349

B2. TÜRER CABBAR AYÇA, ŞİMŞEK MUSTAFA AYTEK, HÜNÜK BURAK, ÖZVEREN OLCAY, GÜZELBURÇ ÖZGE, DEĞERTEKİN MUZAFFER MURAT (2021). Akut koroner sendrom olmayan tip 2 diyabetik hastalarda koroner arter hastaligi yayginligi ile MPV iliskisinin arastirilmasi. SDÜ Tıp Fakültesi Dergisi, 28(3), 411-417., Doi: 10.17343/sdutfd.820120

B3. ŞİMŞEK MUSTAFA AYTEK, BARIŞ NEZİHİ, ASLAN ÖZGÜR, GÜNERİ SEMA (2019). İlaç Salınımlı Stent İmplante Edilen Hastalarda Stent Tipi İle Takipte Gelişen Kardiyak Advers Olayların Arasındaki İlişkinin İncelenmesi. İzmir Eğitim ve Araştırma Hastanesi Tıp Dergisi, 23(4), 269-273.

B4. ŞİMŞEK MUSTAFA AYTEK, ÖZVEREN OLCAY, ŞENGÜL CİHAN, TÜRER CABBAR AYÇA, ASLANGER EMRE, HÜNÜK BURAK, AYDIN SİNAN, DEĞERTEKİN MUZAFFER MURAT (2019). Comparison of Left Main Coronary Disease Prevalance in Patients with or without Chronic Kidney Disease: A Propensity Score Match Analysis. Akdeniz Medical Journal, 5(2), 370-375., Doi: 10.17954/amj.2019.1721

C – Oral presentations in international congresses

C1. EREN MURAT, ÖZPELİT EBRU, ŞİMŞEK MUSTAFA AYTEK, GÜNGÖR HASAN, GÜNERİ SEMA (2012). Neutrophil to Lymphocyte Ratio on Admission: As a Predictor of Cardiovascular Outcome and Mortality in Patients with Acute Coronary Syndrome. 3rd World Heart Failure Congress (Özet Bildiri/Sözlü Sunum)

C2. ŞİMŞEK MUSTAFA AYTEK (2020). TIP 2 DİYABET HASTALARINDA NT-proBNP DÜZEYİ İLE MORTALİTE ARASINDAKİ İLİŞKİ. II. Uluslararası 29 Ekim Bilimsel Araştırmalar Sempozyumu (Özet Bildiri/Sözlü Sunum)

C3. YAZICI ELMAS BURCU, TÜRER CABBAR AYÇA, ŞİMŞEK MUSTAFA AYTEK, KANTARCI GÜLÇİN (2020). RADYOKONTRAST MADDENİN SANTRAL KAN BASINCI, VASKULER YATAK, KALP RİTMİ VE RENAL FONKSİYONLAR ÜZERİNE ETKİSİ. 4. Uluslararası Tıp ve Sağlık Bilimleri Araştırmaları Kongresi, 325-339. (Özet Bildiri/Sözlü Sunum)

C4. HÜNÜK BURAK,ÇAĞAÇ ÖZGÜR,ŞİMŞEK MUSTAFA AYTEK,ASLANGER KARTAL EMRE,ÖZVEREN OLCAY,TÜRER CABBAR AYÇA,MUTLU BÜLENT,EROL ÇETİN,DEĞERTEKİN MUZAFFER MURAT (2019). The impact of QT interval duration on mortality in a large cohort of community based adultpopulation with a long term followup. The European Heart Rhythm Association 2019 Annual Congress

C5. HÜNÜK BURAK, ÇAĞAÇ ÖZGÜR, ŞİMŞEK MUSTAFA AYTEK, MUTLU BÜLENT, DEĞERTEKİN MUZAFFER MURAT, EROL ÇETİN (2018). Gender specific effects of metabolic syndrome on mortality in a large cohort of Turkish middle aged adult population with long term follow up. 34th Turkish Cardiology Congress with International Participation (Özet Bildiri/Sözlü Sunum)

C6. ŞİMŞEK MUSTAFA AYTEK, ÖZVEREN OLCAY, KAHVECİ GÖKHAN, AKPEK MAHMUT, TÜRER CABBAR AYÇA, BAKAL RUKEN BENGİ (2016). Prognostic role of procalcitonin and other infection parameters in patients with left sided infective endocarditis. ESC Congress 2016 (Özet Bildiri/Poster)

C7. TANRIVERDİ ZÜLKİF, ŞİMŞEK MUSTAFA AYTEK, ÜNAL BARIŞ, DURSUN HÜSEYİN, KOZAN ÖMER, KAYA DAYİMİ (2013). Can Fragmented QRS On 12 Derivation ECG Predict Thrombolytic Therapy Success In Acute ST Elevated Myocardial Infarction?. 29th Turkish Cardiology Congress with International Participation, 62(18), 21-22., Doi: 10.1016/j.jacc.2013.08.073 (Özet Bildiri/Sözlü Sunum)

C8. ÖZKURT YALÇIN, ASLAN ÖZGÜR, ÖZPELİT EBRU, ARSLAN ABDURRAHMAN, KOYUNCU İLHAN, ŞİMŞEK MUSTAFA AYTEK, EKİNCİ MEHMET AKİF, ÇİL ALPER, EYÜBOĞLU MEHMET, AYTEMİZ FATİH, ÜNAL BARIŞ, YILDIZ ZEYNEP JALE (2012). THE RELATIONSHIP BETWEEN BNP, DIASTOLIC PARAMETERS AND HEART RATE IN PATIENTS WITH PERSISTANT ATRIAL FIBRILLATION. 8th International Congress of Update in Cardiology and Cardiovascular Surgery (Özet Bildiri/Sözlü Sunum)

D – National Oral Presentations

D1. TÜRER CABBAR AYÇA, DEĞERTEKİN MUZAFFER MURAT, ŞİMŞEK MUSTAFA AYTEK, ÖZVEREN OLCAY, YANARTAŞ MEHMED, TAŞ SERPİL, OLGUN YILDIZELİ ŞEHNAZ, YILDIZELİ BEDRETTİN (2018). Kronik tromboembolik pulmoner hipertansiyonda pulmoner tromboendarterektomi sonrası takipte asimetrik dimetilargigin bir belirteç olabilir mi?. 2. Ulusal ADHAD Kongresi (Özet Bildiri/Sözlü Sunum)

D2. ŞİMŞEK MUSTAFA AYTEK, TÜRER CABBAR AYÇA, HÜNÜK BURAK, AYDIN YUSUF SİNAN, KOZAN ÖMER, EROL ÇETİN, MUTLU BÜLENT, DEĞERTEKİN MUZAFFER MURAT (2016). Prognostic value of electrocardiographical parameters in a large population based cohort: A long term (7 year) follow up study. 32nd Turkish Cardiology Congress with International Participation (Özet Bildiri/Sözlü Sunum)

E – Chapters in books published by national publishers

E1. KARDİYOLOJİDE SAĞKALIM REHBERİ, Bölüm adı: (KORONER KALP HASTALIĞINA YAKLAŞIM) (2021)., ŞİMŞEK MUSTAFA AYTEK, Nobel Tıp Kitabevleri, Editör: ASLANGER EMRE; YILDIRIMTÜRK ÖZLEM; AKASLAN DURSUN, Basım sayısı:1, Sayfa Sayısı 879, ISBN:978-605-335-654-7, Türkçe(Bilimsel Kitap)

E2. KALP, Bölüm adı:(VENOGRAFİ) (2019)., ŞİMŞEK MUSTAFA AYTEK, Nobel Tıp Kitabevleri, Editör:Ömer Kozan; Gönenç Kocabay; Özgen Şafak, Basım sayısı:1, Sayfa Sayısı 1804, ISBN:978-605-4335-450-5, Türkçe(Bilimsel Kitap)

E3. Kritik Durumlarda Klinik Uygulama Rehberi, Bölüm adı:(Akut Koroner Sendromlara Yaklaşım) (2015)., ÖZVEREN OLCAY, ŞİMŞEK MUSTAFA AYTEK, Yeditepe Üniversitesi Yayınevi, Editör:Yaşar Küçükardalı; Başar Atalay, Basım sayısı:1, Sayfa Sayısı 402, ISBN:978-975-307-067-6, Türkçe(Bilimsel Kitap)

F –Awards

Archives of Turkish Society of Cardiology Best Original Article Award, 2021