Aus dem Herzzentrum Wuppertal, Akademisches Lehrkrankenhaus der Universität Düsseldorf

(Direktor: Prof. Dr. H. Gülker)

C/T Polymorphism of the Intercellular Adhesion Molecule-1 Gene (Exon 6, Codon 469)- A Risk Factor for the Coronary Heart Disease and Myocardial Infarction

Dissertation

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Content

MY FAMILY

1. Introduction

1.1 Risk factors of atherosclerosis and coronary heart disease

Atherosclerosis is a disease of large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, resulting in coronary heart disease (CHD) and myocardial infarction (MI). The clinical consequences of atherosclerosis including CHD and acute myocardial infarction (AMI) are one of leading causes of morbidity and mortality in the most Western world despite the decline noted over the past several years, while most survivors are physically limited by their disease.

Large scale epidemiological studies have demonstrated that CHD, AMI and its complication are associated with a vary of risk factors. Coronary risk factors refer to conditions which have been demonstrated by statistical procedures to increase the susceptibility of an individual to the morbidity and mortality of coronary atherosclerosis. The Framingham Study in the United States was one of the first to describe the primary and secondary risk factors for CHD (Kannel et al., 1974). The primary alterable risk factors for CHD are hypercholesterolemia, hypertension, and tobacco smoking. Diabetes mellitus and low levels of high density lipoprotein (HDL) cholesterol are also well established as risk factors for CHD. Other alterable risk factors whose relative importance is still being established include serum triglyceride (TG) concentration, personality type, level of physical activity, and obesity. Potential benefit of identifying a risk factor is that risk factor may be altered through intervention, thus possibly preventing the formation of an atherosclerotic plaque, retarding its growth, or reducing its size. Direct evidence in humans for reversal of atherosclerosis by risk factor intervention is in its early stages. Several recent secondary intervention studies have shown that lowering levels

of cholesterol and low density lipoprotein (LDL) cholesterol will slow the progression of either femoral or coronary artery plaques.

However, atherosclerosis and CHD are multifactor disease caused by genetic and environmental factors, the clinician's ability to predict the development of a cardiovascular event in an individual patient is limited by the relatively low prognostic specificity of classical risk factors for atherosclerosis. A search for more specific atherosclerotic risk factors is therefore justified in order to facilitate identification of individuals with a high risk of cardiovascular disease.

1.2 Inflammation marker

The process of atherogenesis has been considered to consist largely of the accumulation of lipids within the artery wall, however, it does not result simply from that, Sato et al (1987) found increased infiltration of the coronary arteries by inflammatory cells in patients with unstable angina. It is also reported that inflammation is a fundamental component of atherogenesis, restenosis after percutaneous transluminal coronary angioplasty (PTCA), and reperfusion injury (Gerrity et al., 1981, Ross et al., 1993) and therefore plays an important role throughout all stages of the pathogenesis and progression of atherosclerosis in CHD (Entman et al., 1993, Alexander et al., 1994).

There are several potential sources of inflammatory parameters as well as the cytokines that promote their production. The most measured inflammatory markers are derived from the liver, including C-reactive protein (CRP), fibrinogen, and serum amyloid A. The acute-phase response of CRP and serum amyloid A protein are sensitive, nonspecific phenomenon of low-grade systemic inflammation. Which are mediated by cytokine. A second source of inflammatory markers are macrophages and related cells, for example monocyte including phospholipase A2, which stimulated synthesis of platelet activating factor (PAF, a potent pro-inflammatory phospholipid). A third

source of inflammatory markers is the arterial wall itself, particularly endothelial and vascular smooth-muscle cells, including cell adhesion molecular family (CAMs), the distal indicator of inflammation.

Liuzzo et al (1994) have been reported that the CRP and serum amyloid A protein were elevated in patients with unstable angina. Inflammation marker including fibrinogen, CRP, serum amyloid A, CAMs, cytokines are also elevated in acute and chronic inflammation states and at baseline among patients at risk for future coronary occlusion (Kugiyama et al., 1999, Packard et al., 2000, Lindahl et al., 2000). Therefore, inflammation may predict and increased the risk of events in patients with CHD (Libby et al., 1997, Ross et al., 1999, Ridker et al., 2000). Hence the effects of inflammatory markers and those of classic risk factors were clearly additive predictive of the risk of cardiovascular events .

1.3 Endothelial function

The impairment of normal endothelial activity by inflammatory responses may provide a link between inflammation and the risk of ischemic coronary syndromes (Vallance et al., 1997).

The vascular endothelium, located at the interface between the bloodstream and the vascular tissue, and serves as an important autocrine and paracrine organ, was considered to be a semipermeable membrane. In health, the endothelium usually provides potent antithrombotic, antiplatelet, anti-inflammatory, anti-adhesive and vasodilator properties on the vascular wall to maintain vascular homeostasis (Vane et al., 1990, Zeiher et al., 1991), in contrat chronic risk factors for CHD and the exposure of endothelial cells to proinflammatiory cytokines can adversely affect endothelium-dependent blood flow responses and impairs endothelium-dependent vascular relaxation in the human coronary and the forearm circulation (Chowienczyk et al., 1992, Johnstone et al., 1993,

Bhagat et al., 1996). These opposite properties of the vascular endothelium, lead to vasoconstriction, thrombosis, and proliferation of intima.

The injury of endothelium increased endothelial permeability to lipoproteins and other plasma components increase the adhesive capacity with up-regulated leukocyte adhesion molecules, including L-selectin, integrins, and platelet-endothelial-cell adhesion molecule 1 (PECAM-1); and with the up-regulated of endothelial adhesion molecules, including E-selectin, P-selectin, endothelial intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule (VCAM-1)(Alexander et al., 1995). Then continued migration of leukocytes into the artery wall, and proliferation of smooth muscle cells become intermixed with the area of inflammation to form an intermediate lesion. Continued thicken the artery wall, which compensates by gradual dilation, so that up to a point, the lumen remains unaltered, a phenomenon termed “remodeling”. All of these alterations in endothelial cell function termed “endothelial activation”, which is an earliest changes of the formation of lesions of atherosclerosis, and have been shown to result in coronary capillary plugging and impairment of coronary blood flow, implicated to promote acute events in atherosclerotic vascular disease.

1.4 Cell Adhesion molecular family (see Figure 1)

CAMs include the selectin family (E, P and L-selectin), immunoglobulin superfamily (ICAM, PECAM-1 and VCAM-1), and integrins (CD11/18). Leukocyte and endothelial adhesion molecules are members of one of above three major families, which facilitate the interactions between cells.

Selectins are uniquely suited for mediating the rapid cycle of attachment, detachment and reattachment underlying leukocyte rolling, the ligand for them are glycoprotein and glycolipids containing the sialyl-Lewis^x (sLe^x, CD 15s) structure (Foxall et al., 1992), sLe^x is found on neutrophils, monocytes, and

some lymphocytes. all three selectins have been clustered in tandem within a 220-kb region of chromosome 1q23 (Watson et al., 1990, Collins et al., 1991).

Endothelial leukocyte adhesion molecule-1 E-selectin (ELAM-1, SELE, CD62E) supports rolling of neutrophils, monocytes, eosinophils, and some lymphocytes. E-selectin is only expressed transiently on the endothelium after activation (Pober et al., 1986, 1987, Cumming et al., 1992). Its ligand is sLe^x .

P-selectin (also called granule membrane protein 140 KD, GMP-140, SELP, CD62P) is a platelet alpha-granule membrane protein of molecular weight 140,000 that redistributes to the plasma membrane during platelet activation and degranulation. P-selectin is found after stimulation with inflammatory mediators on the endothelial cells and platelets surface (Rubanyi et al., 1993), and is stored in Weibel-Palade bodies of the vascular endothelial cells or the alpha-granules of platelets, then release from that, supports rolling of neutrophils, monocytes, and some lymphocytes. Its ligand is also sLe^x .

Lymphocyte adhesion molecule-1 L-selectin (LAM1, SELL, CD62L) is a cell membrane surface receptor, expressed constitutively on the surface of most leucocytes (neutrophils, monocytes, and lymphocytes), but not endothelium, it may after stimulation transiently increase its ligand affinity without a concomitant increase of receptor density on the cell surface (Spertini et al., 1991), subsequently it is rapidly removed from the leukocyte surface (Griffin et al., 1990). It is important in the regulation of neutrophil movement and in the earliest contact between the leukocyte and the endothelial cell. Its Ligand is mucin-type molecules.

The ICAM immunoglobulin super family consists of five members, including ICAM-1, -2, -3, -4, -5 (Gahmberg et al., 1997), ICAM genes locates on chromosome 19 region p13.3-p13.2 (Trask et al., 1993, Bossy et al., 1994, Hermand et al., 1995, Mizuno et al., 1997), only ICAM-2 gene is mapped to

chromosome 17 region q23-25 (Sansom et al., 1991). They are a family of type I cell surface glycoproteins characterized by immunoglobulin-like extracellular C-type domains that vary in number between two and nine (ICAM–2, -4 have two, and ICAM-1, -3 have five, ICAM-5 have nine domains) followed by a transmembrane region and a cytoplasmic domain (Williams et al., 1984), all are the cell-surface ligands for the integrin lymphocyte function-associated antigen-1 LFA-1 (CD18/CD11a, $_{L2}$), and ICAM-1, -2, and -4 can also bind integrin Mac-1 (macrophage antigen-1) (CD18/CD11b, $_{M2}$), and ICAM-3 can also bind the novel integrin CD18/CD11d, $_{D2}$ (Van der Vieren et al., 1995) (Table 1), this integrins are all members of the CD 18 family of leukocyte integrin adhesion molecules.

Table 1 Ligands of ICAM family

ICAM-1	ICAM-2	ICAM-3	ICAM-4	ICAM-5	Ligand
+	+	+	+	+	LFA-1
+	+		+		Mac-1
		+			CD11d/CD18

ICAM-2 (CD102) is constitutively expressed by leukocytes, endothelial cells, platelets, but the expression remains at basal levels by inflammatory cytokines or other treatments under all conditions studies (de Fougerolles et al., 1991).

ICAM-3(CD 50) expressed also constitutively and highly on the surface of most resting leucocytes (neutrophils, monocytes, and lymphocytes), but not endothelium, ICAM-3 expression on endothelium was low in inflammatory

disease (Patey et al., 1996), it may be important in the generation of immune responses (Fawcett et al., 1992).

ICAM-4 is specifically expressed on red cells (Bailly et al., 1994).

ICAM-5 (telencephalin, TLN) expressed only by neurons within the telencephalon of mammalian brains (Yoshihara et al., 1994)

Platelet/endothelial cell adhesion molecule-1 PECAM-1 (CD31 antigen) mapped to chromosome 17 in the region 17q23 (Gumina et al., 1996), it is expressed on the surface of circulating platelets, monocytes, neutrophils, and particular T cell subsets, which is implicated in transendothelial migration of leukocytes, angiogenesis, and integrin activation.

Mononuclear cell-specific adhesion molecule VCAM-1 (CD106) mapped to 1p32-p31 (Cybulsky et al., 1991), which found only on the surface of mononuclear cells, not expressed on normal endothelium on baseline (Jang et al., 1994), after stimulation by cytokines can express on endothelium, epithelium, macrophages and dendritic cells, its counter receptor is the integrin very late antigen-4 (VLA-4, CD49d/CD29, $\alpha_4\beta_1$), which is expressed on monocytes, lymphocytes, and eosinophils, but not on neutrophils. interaction between VLA-4 and VCAM-1 plays major role in binding of this cells to activated endothelial cells.

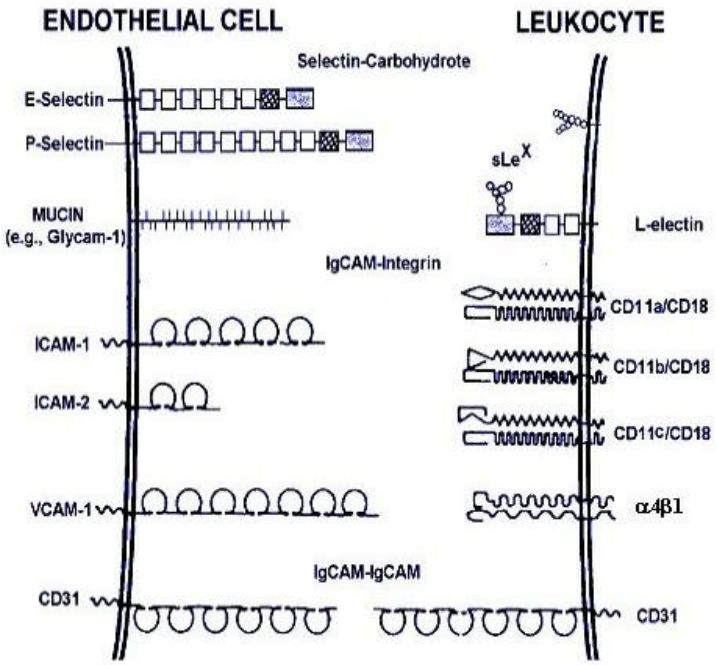

Figure 1 Cell adhesion molecules family. Three general classes of receptor-counter receptor binding interactions are illustrated. (a) Selectin-carbohydrate interactions include E- and P-selectin binding to sLe^x and related structures, possibly including some sugars on L-selectin. Leukocyte L-selectin can bind to mucin-type molecules, such as Glycam-1, expressed on the endothelial cell surface; (b) ICAM and VCAM are found on endothelial and integrins on leukocyte cell surfaces. ICAM-1 and ICAM-2 bind to activated CD11/CD18 integrins. VCAM-1 binds the $a_4ß_1$ integrin; (c) The CD31 antigen is capable of homophile interactions.

1.5 Role of the ICAM-1 gene in development of atherosclerosis

Atherosclerosis is regarded as a chronic inflammatory process (Ross et al., 1999), which may be present throughout a person's lifetime, the precursors of

advanced lesions are divided into three morphologically characteristic types. Type I lesions represent the microscopically and chemically detectable small lipid deposits in the arterial intima. They are common in infants and young children. Type I lesions is a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes (Stary et al., 1987), and type II includes those lesions generally referred to a so-called fatty streak, which on gross inspection may be visible, type III represents the stage that links type II to advanced lesions. The term “early lesions” is sometimes used for type I and II lesions, a smaller subgroup of type II will be to proceed to type III lesions and then to advanced lesions.

The pathogenesis of an advanced lesions is a complex and chronic process that involves the interaction among lipids, the endothelium, circulating and tissue inflammatory cells (leukocytes), platelets, and vascular smooth muscle cells and extracellular matrix in the intima of large arteries. A number of cell adhesion molecules in this complex interaction are one of the earliest and critical events.

One of the earliest detectable events in human and experimental atherosclerosis induced by hyperlipidemia is the slowing down of circulating leukocytes in the streaming blood and then the rolling and tethering of circulating leukocytes, including monocytes and lymphocytes along the arterial endothelial lining, which mediated by the selectin class of adhesion molecules (Ley et al., 1991, Abbassi et al., 1991, Lawrence et al., 1991), and subsequent firm attachment to endothelial cells, and transendothelial migration of these adherent leukocytes across the endothelial surface to the intima of the bloodvessels wall, and accumulation in the intima, which is mediated by ß2-integrins interacting with counterligands from the Ig superfamily including the ICAM-1 in activated endothelial cells (Smith et al., 1988), where monocytes transform into lipid-engorged “foam cells” by the uptake of lipids, which results in formation of

fatty streak lesions. (Faggiotto et al., 1984, Albelda et al., 1994), and secretion of cytokines and growth factors are also important events in the initiation and progression of atherosclerotic plaques (Ross et al., 1986, Munro et al., 1988, Hansson et al., 1989), hence participate in advanced lesions of atherosclerosis (Figure 2).

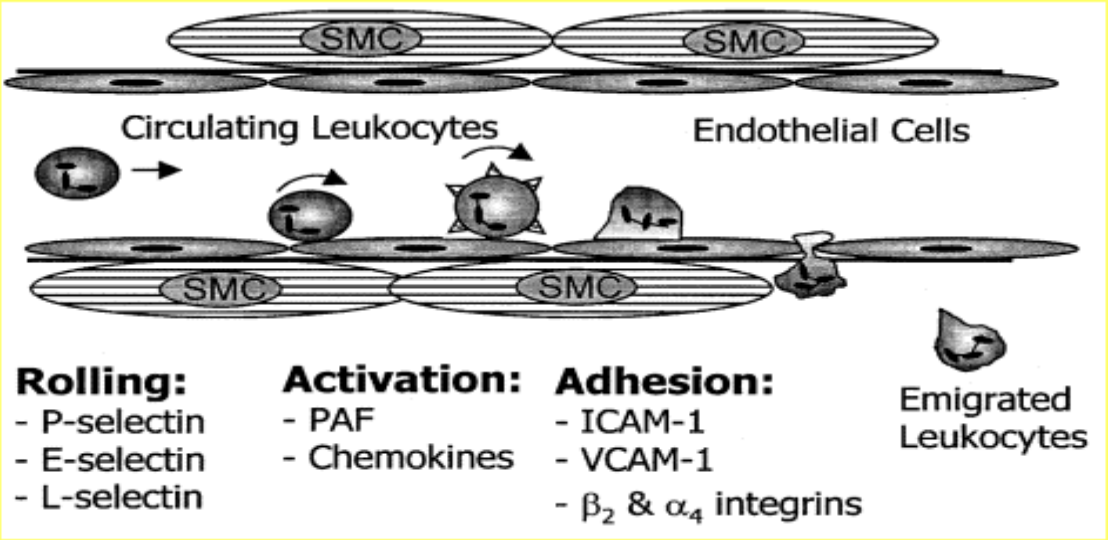

Figure 2 Interaction between leukocytes-endothelial cells

The ICAM-1 (CD54) gene is one of these adhesion molecules, which is a 95kD cell surface glycoprotein, Ridker et al (1998) found that sICAM-1 levels was inversely correlated with apoA-II level in boys and HDL cholesterol level in girls. Ma et al (1992, 1993) use of monoclonal antibodies directed against ICAM-1 and L-selectin reduce myocardial injury in experimental animals and may limit ischemic damage by blocking the interaction between ICAM-1 and its receptor LFA-1 (Haug et al., 1993, Hourmant et al., 1996). ICAM-1 can mediate adhesion of circulating leukocytes to the activated endothelium, it plays roles not only in the firm attachment but also in transendothelial migration of leukocytes (Hogg et al., 1993), therefore plays a role in

inflammation processes and is one of the earliest events in the pathogenesis of atherosclerosis.

ICAM-1 expressed widely on the surface of unhematopoitic and hematopoitic cells, including, fibroblasts, macrophages, activated monocytes lymphocytes, follicular dendrite cells, and epithelial cells (Dustin et al., 1988, 1989, Faull et al., 1989, Couffinhal et al., 1994), but at very low levels on normal endothelium, and could not detected on smooth muscle cells in the normal adult aorta. ICAM-1 expression can be rapidly upregulated several fold in atherosclerotic lesions by inflammatory mediators (Dustin et al., 1986, Munro et al., 1989, Davies et al., 1993).

Several studies have reported an increase in the expression of membrane bound ICAM-1 in all period of atherogenese, including early and developed lesions (Poston et al., 1992, Van der Wal et al., 1992, Printseva et al., 1992), membrane ICAM-1 expression was found in all blood vessels with fatty streak and fibrosis plaques in subjects of age from 25 to 61 years (Printseva et al., 1992), ICAM-1 can express on fibroblast, macrophages, circulating leukocytes, endothelial cells, smooth muscle cells in fatty streaks and fibrosis plaques, enhanced expression of ICAM-1 have also shown on macrophages, endothelial cells, and smooth muscle cells in human atherosclerotic plaque (Poston et al., 1992), on plaque of endothelium in CHD, myocardial infarction (MI) or in patients undergoing coronary angioplasty (Poston et al., 1992, Kamijikkoku et al., 1998, Porsch-Oezcueruemez et al., 1999, Inoue et al., 1999), its expression was not limited in the plaques, also on within the intima, including arterial luminal endothelial cells, neovascular endothelium, and non-endothelial cell type. (O´Brien et al., 1996), and the expression of ICAM-1 was also detected on medial, intima smooth muscle cells in the atherosclerotic vascular wall (Printseva et al., 1992, Poston et al., 1992), The expression of adhesion

molecules on smooth muscle cells may facilitate the accumulation of transmigrated leukocytes within the vascular wall (Libby et al., 1993).

Adhesion proteins may be released in soluble fragments into the circulating blood, the source of soluble forms of ICAM-1 (sICAM) is uncertain, assuming as the portions of membrane bound ICAM-1 molecule into the circulating blood, it may be derived from the shedding or proteolytic cleavage of vascular wall components, including endothelial and smooth muscle cells (Gearing et al., 1992, Pigott et al., 1992).

Two prospective cohort studies noted that baseline levels of sICAM-1 are elevated many years before a first MI occurs (Hwang et al., 1997, Ridker et al., 1998). The sICAM-1 were elevated in serum of patients with multiple risk factors for atherosclerosis (Fassbender et al., 1995), and with acute coronary syndrome within the first 24 hour (Shyu et al., 1996), it is reported that the sICAM-1 levels both in the coronary sinus and aortic root were higher in patients with unstable angina than in those with stable angina and than in healthy controls (Ghaisas et al., 1997, Miwa et al., 1997, Ogawa et al., 1999), patients with atherosclerosis, coronary heart disease, acute myocardial infarction, showed higher plasma concentration of sICAM than in health subjects (Kaikita et al.,1997, Hwang et al., 1997). During attacks of angina pectoris an increase in the plasma levels of the soluble ICAM-1 was noted, possibly reflecting activation of neutrophils and endothelial cells during myocardial ischemia (Siminiak et al., 1998), Patients with stable angina pectoris who developed cardiovascular death/myocardial infarction had elevated serum levels of sICAM-1, indicating increased inflammatory activity (Wallen et al., 1999).Thus suggest that ICAM-1 is involved both in acute phase of myocardial infarction and chronic process of atherosclerosis (Table 2).

Table 2 Indication of the importance for ICAM-1 at atherogenese

Membrane bound ICAM-1: -increased expression of ICAM-1 in all periode of atherogenese on endothelial cells, on circulating leukocytes and in atherosclerotic lesions (van der Wal et al., 1992, Davies et al., 1993)
Soluble forms of ICAM-1 in blood (Hwang et al., 1997, Ridker et al., 1998): -increased serum concentration in manifest atherosclerosis (carotisstenosis, coronary heart disease, acute coronary syndrome) -increased soluble ICAM-1 for many years before a first coronary syndrome

The factors that mediate expression of adhesion molecules in atherosclerotic plaques are not clear, Morisaki et al (1997) found that sICAM-1 are not affected by age and sex, expression of ICAM-1 is known to induces the infiltration of T cell and macrophages (Hamano et al., 1998), and enhance T helper cell activation (Van Seventer et al., 1990). O´Brien et al (1996) found also that ICAM-1 at the arterial luminal surface were correlated to the degree of macrophage infiltration, several studies (Wang et al., 1989, Springer et al., 1990, Hansson et al., 1991, Salomon et al., 1992) have demonstrated that atherosclerotic tissue contains a variety of macrophage and T lymphocyte derived cytokines, that may upregulate expression of one or all of the adhesion

molecules. On cultured endothelial cells (EC) and smooth muscle cells (SMC), endothelial cells surface upregulation of ICAM-1 expression is inducible at sites of inflammation by oxidized LDL and TNF-α, Interleukin-1, interleukin-4, interferon-gamma, lipopolysaccharide. (Kume et al., 1992, Jeng et al., 1993, Yokote et al., 1993, Ross et al., 1999).

It is also noted that diet (Li et al., 1993), hypercholesterolemia, minimally or extensively oxidized lipoproteins, and reactive oxygen species can induced ICAM expression. Kalra et al (1994) reported that cigarette smoke condense induced ICAM-1 expression in cultured human umbilical vein cells. PAF has been reported to mediate endothelial cell expression of ICAM-1 in response to some stimuli (Chihara et al., 1992). Pasceri et al. (2000) shows that CRP can induce ICAM-1 expression by human endothelial cells.

1.6 ICAM-1 gene and countereceptor of ICAM-1 gene

The ICAM-1 gene consists of 7 exons. Exon 1 encodes the signal sequence, exon 2 to 6 encode the Ig-like domains 1 to 5, respectively, and exon 7 encodes the transmembrane domain and the cytoplasmic tail (Ballantyne et al., 1991). (Figure 3)

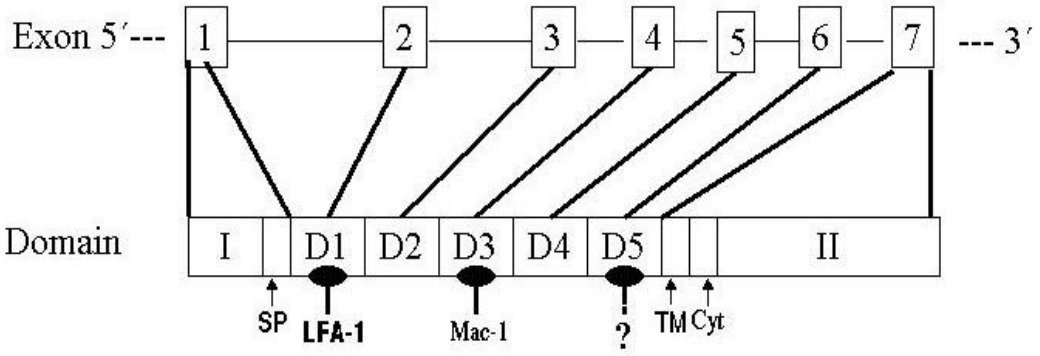

Figure 3 Exon-intron structure of ICAM-1 gene SP= Signal peptide, TM= transmembrane , Cyt= cytoplasmic

ICAM-1 is immunoglobulin-like transmembrane adhesion receptors, a heavily glycosylated polypeptide chain consists of extracellular region with five immunoglobulin-like (Ig) domains, a transmembrane domain, and a short cytoplasmic domain (Staunton et al., 1988). The first Ig domains is the recognition regions for LFA-1 (Staunton et al., 1990), the second domain can maintain the structure of the LFA-1 ligand-bind site in the first domain, but does not to have a direct role in ligand binding (Stanley et al., 2000), whereas the third Ig domain is involved in binding MAC-1 (Diamond et al., 1991). The correlation between other two domain and leukocyte integrin receptors were unknown.ICAM-1 is a ligand for LFA- I (Rothlein et al., 1986, Marlin et al., 1987), and Mac-1 (Smith et al., 1988, 1989). In addition to that, ICAM-1 is also a receptor for fibrinogen (Languino et al., 1993), ICAM-1 mediated adhesion is

mediated by binding to its counterreceptor. The process of adhesion is mainly determined by the action of the CAMs, Integrins and CAMs attach the cells to the vessel wall, allowing them to invade it (Hogg et al., 1993)

Integrin include three main groups, first ß1 integrins, very late appearing antigen-4 (VLA4) integrins (Maxfield et al., 1989), expressed in lymphocytes, second, ß2 integrins leukocyte integrins (leukocyte cell adhesion molecule), a family of four cell surface membrane glycoprotein, expressed only on leukocytes, which are transmembrane alpha-beta heterodimer proteins, they are all consist of a common ß-subunit (CD18) and homologous alpha- subunits (CD11a-d) (Arnaout et al., 1990), including 1) CD18/CD11a, referred to as LFA1, leuCAMa, and integrin beta-2/alpha-L, its ligand are ICAM-1, -2 , -3, -4, -5, is found on all leukocytes; 2) the integrin heterodimer CD18/CD11b, also referred to as Mac-1, leuCAMb, CR3, Mo-1, OKM-1, and integrin beta-2/alpha-M, expressed on monocytes or polymorphonuclear leukocytes, is a receptor for ICAM-1, -2, -4, on endothelium, contributing to vascular cell interactions in inflammation and atherosclerosis; 3) CD18/CD11c: also referred to as p150 (p150,95), LeuCAMc, and integrin beta-2/alpha-X (Sanchez-Madrid et al., 1983), ligand fibrinogen. present in myeloid cells and monocytes; and 4) CD18/CD11d, beta-2/alpha-d. Granulocytes and monocytes express all ß2 integrins , whereas most lymphocytes express only LFA-1. The integrin beta-2 gene is located on Chromosome 21 (Suomalainen et al., 1985, 1986). Third, cytoadhesins have a ß3-subset ß3 integrins - GpIIb/IIIa (CD41/CD61) expressed in platelet and endothelial cells. (Albelda et al., 1990). Association between several polymorphisms in the ICAM-1 gene and inflammation have been reported (Table 3), including a single base C to T transition polymorphism which results in an amino acid substitution glutamic acid (E) to lysine (K) in

the ICAM-1 protein in exon 6 codon 469, which has been found to be related to Behcet´s inflammation disease. However, their role in mediating the risk of CHD and MI is still unknown.

Table 3 Association of known Polymorphisms in Gene ICAM-1 for phenotype

Aminoacid position	Domain (region)	Asociation of polymorph.with the phenotype
G214R	Ig domain 3 (exon 4)	coronary and peripheral atherosclerosis(-) (Wenzel et al., 1996)
G241R	Ig domain 3 (exon 4)	rheumatoid arthritis (+) (Macchioni et al., 2000); polymyalgia rheumatica and giantcell arteritis (+) (Salvarani et al., 2000) inflammatory bowel disease (+) (Yang et al., 1995)
E469K	Ig domain 5 (exon 6)	Behcet´s inflammation disease (+) (Verity et al., 2000)

ICAM-1=intercellular adhesion molecule-1; polymorph. =polymorphisms;
(-) = no association; (+) = presence of association

Therefore, the purpose of our study was to determine whether C/T polymorphism of the ICAM-1 gene in exon 6 plays a role in CHD and MI in humans.

2. Objective

The main aim of the prospective study is to assess whether the common polymorphic allele (T) of the ICAM-1 gene in exon 6 domain 5 is associated with CHD or MI. ICAM-1 variants (one of genetic risk factors) is expected to enhance our understanding of the molecular basis for atherosclerosis, to enhance our ability to predict the high-risk patients for CHD and might be helpful in selecting patients who will benefit from specific therapies, provide new clues for clinicians, offers potential new interventional strategies for the treatment and prevention of atherosclerosis, and promises to contribute to a new era of preventive cardiovascular medicine, may in the future, allow therapies to be directed toward identified" high-risk" group.

3. Material and Methods

3.1 Study Populations

The study population consisted of three groups, a control group, a CHD group, a MI group (Table4).

The CHD group consisted of 327 patients (239 males, 88 females; median age 62,4± 11,0) with angiographically documented CHD. The inclusion criteria were stenosis of more than 50% of at least one major coronary vessel.

The MI consisted of 176 patients with an acute or chronic MI from CHD group. The diagnosis of MI was based on typical electro-cardiographic changes and increases in serum enzyme activities, including those of creatinine kinase (CK), CKMB, and lactate dehydrogenase (LDH).

Selected as control subjects were 107 patients (44 males, 63 females; median age 60,0 ±15,2) with normal left ventricle (LV) function and had no documented evidence of CHD. There were no significant differences in age between CHD, MI and controls. All patients and control subjects were Germans and joined written consent.

Table 4 Baseline Characteristics of the Study Population

Variable	Control group (n=107)	CHD group (n=327)	MI group (n=176)
Age (years)	60.0±15.2	62.4±11.0	61.4±11.8
Sex (female / male)	63/44	88/239*	38/138*
BMI (kg/m^2)	27.0±5.0	27.2±3.9	27.2±4.2
Habitual smoking (%)	27	54*	60*
Hypertension (%)	73	71	66
Diabetes mellitus (%)	17	24	20
Hypercholesterolemia (%)	57	82*	82*

The values are presented as mean±SD , BMI= body mass index, CHD= coronary heart disease, MI= myocardial infarction , *P<0.001 compared with control group

3.2 Determine of clinical parameter

Total cholesterol, TG were measured on fasting serum specimen by standard techniques at all visits.

3.3 DNA isolation from peripheral blood leukocytes

EDTA blood samples (5 ml) were obtained from all subjects after cardiac catheterization, all blood sample were stored at 4°C until DNA extraction were performed and DNA was prepared with an extraction kit (QIA amp DNA blood midi kit) from QIAGEN.

In detail, to isolate genomic DNA, 2 ml patients' whole blood (in 0.1 mM EDTA) was mixed at room temperature (R.T.) thoroughly with 200µl QIAGEN protease stock solution and 2.4 ml Buffer AL, then incubate at 70°C for 10 min, for optimal binding with 2 ml of ethanol (100%) to the sample and mix again by vortex, carefully transfer half of the solution (3.3 ml) onto the membrane of QIAamp Midi column placed in a 15 ml centrifugation tube, and centrifuge at 1850×g (3000rpm) for 3 min, DNA is adsorbed onto the QIA amp silica membrane, discard the filtrate, load the remainder of the solution onto the QIAamp Midi column and recentrifuge at 1850×g (3000rpm) for 3 min, then QIAamp Midi column was washed with 2 ml of Buffer AW1 and centrifuge at 4000rpm for 1 min, then washed with 2 ml of Buffer AW2 and centrifuge at 4000rpm for 15 min, followed incubate the QIAamp Midi column for 10 min at 70°C in an incubator to evaporate residual ethanol, thereafter DNA was dissolved with 300 µl of elution Buffer AE onto the membrane of the QIAamp Midi column, incubate at room temperature for 5 min and centrifuge at 4000rpm for 5 min, reload the 300 µl of elute containing the DNA onto the membrane of the QIAamp Midi column for maximum concentration, incubate at R.T. for 5 min, centrifuge at 4000rpm for 5 min, DNA stored at –20°C until analysis after DNA quantitation.

DNA samples were quantitated on a Lambda Bio Spectrophotometer (Perkin Elmer,überlingen, Germany). DNA concentration was determined by the

absorption value at 260nm. The ratio of absorption value at 260 nm and 280 nm was used as index of DNA purity.

3.4 Polymerase chain reaction (PCR) and Genotyping (Figure 4)

Material:

-10 x PCR-Reaktionsbuffer (Fa. Pharmacia):

500 mM KCI

15 mM $MgCl_2$

100 mM Tris/HCI, pH 9,0

-dNT'P-Mix: dATP 10 mM (Böhringer Mannheim)

dCTP 10 mM (Böhringer Mannheim)

dGTP 10 mM (Böhringer Mannheim)

dTTP 10 mM (Böhringer Mannheim)

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-Taq-Polymerase (5U/µl, Fa. Pharmacia) in storebuffer:

50 mM Tris/HCI, pH 7,5

-Mineralöl (Sigma)

-PCR-Cups

-PCR-Thermocykler:

Hybaid Omnigene Perkin Elmer DNA Thermal Cycler 480

Polymerase chain reaction (PCR) was performed in Omnigene Thermal Cycler (Hybaid) to amplify a 331 bp fragment of exon 6, analyses were performed using 25 ng template DNA in a final reaction volume of 25 µl, containing 1xPCR reaction buffer, 0.2 mM of each deoxynucleotide, and 2.0 U of TaqDNA polymerase (Pharmacia) with 15 pmol of each upstream and downstream primer (S1 and S2) as previously published (Mycko et al., 1998). Thermal cycling was carried out with an initial 95°C denaturation step for 5 min, then 2.0 Unit of TaqDNA polymerase was added, so-called"hot-start" method to minimized unspecific annealing and maximized Taq activity, then continues denaturation for 5 min, followed by 36 cycles of denaturation at 95°C 30 s, annealing at 60°C 30 s, extension at 72°C 30 s and a final extension of 7 min at 72° C. To determine the allelic type of the ICAM-1, we performed specially conditioned nested PCR with the use of allele--specific oligonucleotide primers (S-t, S-c). They have the specific sequence according to the allele-differentiating base located at the 3´position. The sequences of the primers are shown in Table 5. Thermal cycling was carried out with "hot-start" method and then continues an 95°C denaturation step for 5 min, followed by 15 cycles of denaturation at 95°C 30 s, annealing at 56°C 5 s, extension at 72°C 30s and a final extension of 7 min at 72° C.

Table 5 Oligonucleotide Primers for ICAM-1 K469E Genotyping

Gene	Primersequence	Productsize
ICAM-1	S1: 5´ CCC CGA CTG GAC GAG AGG 3´	331 bp
	S2: 5´ GGG GCT GTG GGG AGG ATA 3´	
	S-t: 5´ CAC ATT CAC GGT CAC CTT 3´	237 bp
	S-c: 5´ CAC ATT CAC GGT CAC CTC 3´	

ICAM-1=intercellular adhesion molecule –1, S1 and S2 upstream and downstream primer for PCR, S-t, and S-c = allele specific primer for nest PCR

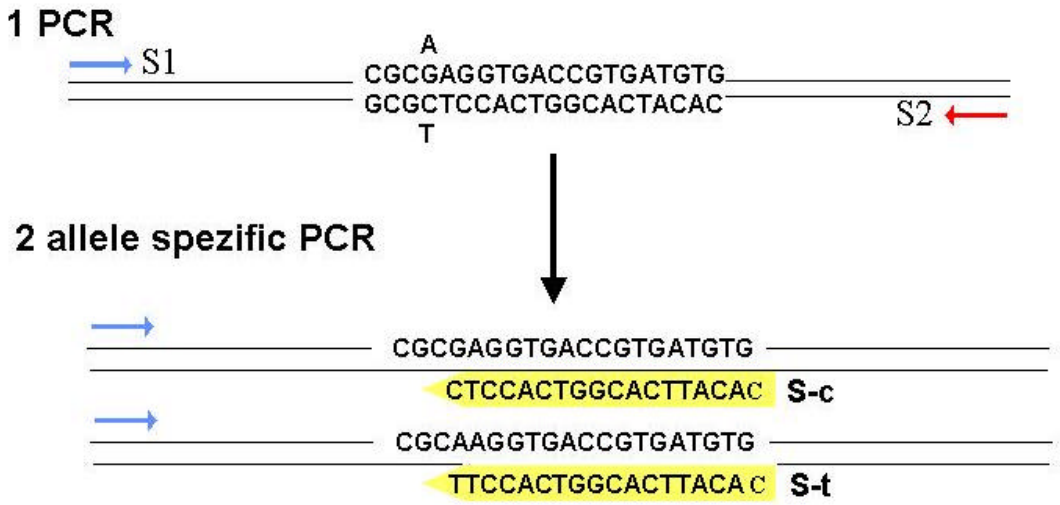

Figure 4 allele specific PCR for ICAM-1 polymorphism

3.5 Agarose gel

Used solutions and chemicals:

Agarose

1x TBE: 8,9mMTris

8.9 mM Boric acid

0.2 mM EDTA pH 8.0

Sample buffer: 7 M Urea

40% Glycerol

50 mM EDTA pH 8.0

10 MM Tris pH 8.0

0.1% Bromphenol blue

1 Kb DNA ladder (Gibcol BRL)

Ethidium bromide staining solution (10 µg/ml)

2 % agarose solution was made up in 1 x TBE. The solution was heated at max. for 3 min in a microwave oven to melt the agarose. The agarose solution was put in a prepared gel tray and left to set on a level surface for 40 min. After coagulation 20 µl samples (10 µl PCR products mixed with 10 µl sample buffer) were loaded on the gel. Electrophoresis was processed at 90 V for 100 min. Thereafter the gel was immersed in the ethidium bromide staining solution for 45-60 min followed by visualization under ultraviolet UV light visualization (lamda =312 nm).

The genotype was analyzed, according to the presence of the desired length PCR products (237bp) by 2% agarose gels, (Figure 5).

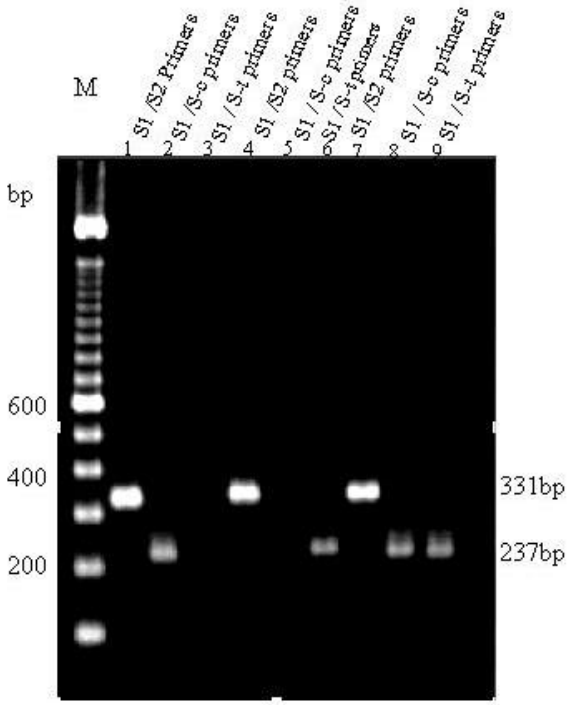

Figure 5 2% agarose gels. Electrophoresis image showing PCR amplification and digestion products. M indicates molecular weight marker; Numerals on left side correspond to molecular weight marker; Numerals on right side correspond to fragment sizes. Pat. 1 (line 2, 3, 4) is a CC homozygote; Pat. 2 (line 5, 6. 7) is a TT homozygote; Pat. 3 (line 8, 9, 10) is a CT heterozygote.

3.6 Statistical analysis

Statistical analyses were performed with the SPSS (version10.0) package. Data were expressed as mean ± SD. Continuous variables were compared by the unpaired Student´s t test. Frequencies were compared using the Chi squared test and Fisher's exact test. The ICAM genotype was calculated according to a dominant (TT+TC vs. CC) or additive (TC vs. CC, and TT vs. CC) genetic effect of the T allele. Allele frequencies were determined by counting the number of chromosomes bearing an allele. Associations between the genes and CHD or MI risk were assessed as odds ratio (OR) and 95% confidence intervals (CI), which are interpreted as the relative risk of disease for "exposed" compared with "unexposed" persons. Allele at each polymorphism were said to be in Hardy-Weinberg equilibrium if the observed homozygote frequencies did not differ significantly (p>0.05) from those expected when analyzed by x^2 test. We performed multivariable logistic regression analysis for the effect of the ICAM-1 polymorphism and other coronary risk factors for CHD, CHD was a dependent variable, whereas independent variables included ICAM-1 genotype (0 for CC, 1 for TC and TT combined [dominant effect of the T allele]), smoking status (0=nonsmoker, 1= smoker), hypercholesterolemia (0= for absence, 1= for presence), gender (0= female, 1= men). P <=0.05 was taken as the level of significance.

4. Results

4.1 Comparison of CHD, MI and Controls for Coronary Risk Factors

As expected, several classical risk factors for CHD and MI, including hypercholesterolemia, cigarette smoking were significantly increased in CHD and MI patients compared with controls.(Table 4).

4.2 Coronary risk factors characteristic by the ICAM-1 genotype

The CHD and MI patients were classified according to their ICAM-1 genotype. There were no statistically significant differences between the genotypes as regards coronary risk factors (Table 6, table 7).

Table 6 Characteristics of CHD Patients by ICAM-1 genotype

Variable	genotype		
	CC	CT+TT	p
Age (years)	62.9±10.1	62.4±11.2	0.74
Sex (female / male)	17/43	71/196	0.78
BMI (kg/m^2)	26.8±3.7	27.4±3.9	0.46
Habitual smoking (%)	50	55	0.56
Hypertension (%)	77	70	0.29
Diabetes mellitus (%)	21	24	0.66
Hypercholesterolemia (%)	88	81	0.24

The values are presented as mean±SD. BMI indicates body mass index, CHD= coronary heart disease, ICAM-1 =intercellular adhesion molecule –1

Table 7 Characteristics of MI Patients by ICAM-1 genotype

Variable	genotype		
	CC	CT+TT	p
Age (years)	61.6±12.2	0.84	
Sex (female / male)	8/30	30/108	0.93
BMI (kg/m^2)	26.3±3.4	27.5±4.2	0.22
Habitual smoking (%)	53	62	0.35
Hypertension (%)	71	65	0.55
Diabetes mellitus (%)	21	20	0.89
Hypercholesterolemia (%)	85	81	0.54

The Values are presented as mean±SD, BMI = body mass index, MI = myocardial infarction, ICAM-1 =intercellular adhesion molecule –1

4.3 Distribution of Allele and Genotype Frequencies

The distribution of C/T genotype, allele frequencies in exon 6 of the ICAM gene is shown in Table 8. We found that **t**he genotypes of the patients with CHD were: 18% (CC), 42% (TC) and 40% (TT), patients with MI were 21% (CC), 44% (TC) and 35% (TT), they differ from that for the control individuals, which were: 33% (CC), 40% (TC) and 27% (TT). Analysis assuming dominant and additive effects of T allele that between control individuals and the patients with CHD were TT+TC/CC: p=0,002, OR= 2,16, 95% CI 1,32-3,54; TC/CC: p=0,025, OR= 1,85, 95% CI 1,08-3,17; TT /CC: p= 0,001, OR=2,64, 95% CI 1,48-4,70), patients with MI were TT+TC/CC: p=0,03, OR= 1,82, 95% CI 1,06-3,14; TC/CC: p=0,07, n.s.; TT /CC: p= 0,03, OR=1,99, 95% CI 1,05-3,77.

The allele frequencies of T-allele were found to be significantly higher in the patients with CHD and in patients with MI than in controls. (0,61 vs. 0,57 vs. 0,47, control individuals vs. CHD, p<0,001; vs. MI P=0,03). Analysis of the genotype, allele frequencies (dominant effect) and additive effects revealed that those individuals with the T allele had a significantly increased risk of CHD and MI compared to those without this allele. The difference was due to the over-representation of the T homozygote and heterozygote for CHD, only T homozygote for MI, whereas heterozygote had a tendency (p=0.07).

Table 8 Allele and Genotype Frequencies of E/K 469 ICAM-1 Polymorphism in Patients and Controls

Variable		Controls (n=107)	CHD (n=327)	MI (n=176)
Genotype n (%)	TT	29(27)	131(40	61(35)
	TC	43(40)	136(42)	78(44)
	CC	35(33)	60 (18)	37(21)
dominant effects	TT+TC	0.67	0.82	0.79
	CC	0.33	0.18	0.21
	P		0.002*	0.03*
	OR (95% CI)		2.16(1.32-3.54)	1.83(1.06-3.14)
Additive effects	*P* (TC & CC)		0.03*	0,07
	OR (95%CI)		1.85(1.08-3.17)	
	P (TT&CC)		0001*	0.03*
	OR (95%CI)		2.64(1.48-4.7)	1.99(1.05-3.77)
Allele frequency (%)	T	0.47	0.61	0.57
	C	0.53	0.39	0.43
	p		<0.001*	0.03*

*Compared with controls. Presence of the T allele of ICAM-1 gene was significantly associated with CHD and MI. CHD= coronary heart disease, MI = myocardial infarction

4.4 Multiple variable logistic regression analysis (Table 9)

This analysis showed that the independent risk for CHD were cigarette smoking (OR=2.67, 95%CI=1.44-4.95), hypercholesterolemia (OR=4.44, 95%CI=2.41-8.19), gender (OR=4.0, 95%CI=2.21-7.22) and the T allele of ICAM-1 gene (OR=2.34, 95%CI=1.24-4.41).

Table 9 Results of multiple Logistic Regression Analysis: Final Significant Variables in Equation using Forward LR

Variable	Beta-Coeff.	SE	*p*	OR	95%CI
Cig. smoker	0.983	0.315	0.002	2.67	1.44-4.95
Hyperchole.	1.491	0.312	0.000	4.44	2.41-8.19
Gender	1.385	0.302	0.000	4.00	2.21-7.22
ICAM-1	0.850	0.324	0.009	2.34	1.24-4.41

Cig.=cigarette, Hyperchole.= Hypercholesterolemia, Coeff.= coefficient ICAM-1 =intercellular adhesion molecule –1, OR = odds ratio and CI =confidence intervals, SE= standard error

5. Discussion

5.1 Coronary heart disease and genetic

CHD and myocardial infarction are multifactorial disease caused by genetic and environmental factors. It is known, all hereditary information is transmitted from parent to offspring through the inheritance of deoxyribonucleic acid (DNA), DNA is a linear polymer composed of purine (A, G) and pyrimidine

(C, T) bases, the sequence of which ultimately determines the sequence of amino acids in every protein molecule. The four types of bases in DNA are arranged in groups of three, each group forming a codeword or codon that signifies a particular amino acid.

Gene polymorphisms in the DNA sequence have been identified that appear to play a role in blood pressure regulation, lipid metabolism, endothelial function, in the pathophysiology of coagulation or thrombosis, or in interventional cardiology by interfering with restenosis development, they not only be useful in early detection of individuals that are exposed to higher risk for myocardial infarction, but they also determine safety and effectiveness of commonly prescribed drugs, thus genetic polymorphisms seem to be clinically important.

A most commonly DNA polymorphism is change one of the purine or pyrimidine bases of a single gene, such single-base changes polymorphism consist of the substitution of one base for another, thus changing the meaning of the codon containing that base. These polymorphism cause the substitution of one amino acid for another in the protein specified by the polymorphism gene. Such substitution can have little effect on the function of the protein, or it can eliminate function.

Thus it is important to include genetic risk factors in the concept of the classical risk factor theory. Potentially in future a genetic risk profile including relevant polymorphisms may be an essential part of the clinicians' knowledge in primary and secondary prevention of coronary artery disease. In our study we found that one of the polymorphisms of the human ICAM-1 gene K/E469 may be involved in the pathogenesis of coronary atherosclerosis.

5.2 Known Polymorphisms of the ICAM-1 gene

ICAM-1 is known to contain at least 5 polymorphic sites, Fernandez-Reyes et al (1997) found , that ICAM-1 gene from children in Kilifi have A to T

transversion at nucleotide 179, causing a lys 29 to met substitution (K29M), called ICAM-1^{Kilifi}, this polymorphism in the N-terminal domain was associated with cerebral malaria, ICAM-1^{Kilifi} showed reduced affinity for LFA1 compared with $ICAM^{ref}$ (Craig et al., 2000).

Both the R241 and the K469 alleles have been reported to be a predetermined genetic risk factor for chronic allograf failure, in which upregulation of adhesion molecules in renal allograft biopsies has also been reported (McLaren et al., 1999), while in patients with multiple sclerosis a high frequency of K469 homozygote has been reported (Mycko et al., 1998). Hence, some polymorphisms have found associated with inflammation (Table 2), ICAM-1 polymorphisms R 241 (exon 4) is associated with rheumatoid arthritis in Italian patients (Macchioni et al., 2000, Salvarani et al., 2000), Significant differences have also been detected between patients with inflammatory bowel disease and controls at the G/R 241 locus (Yang et al., 1995). ICAM-1 K 469 has been found to be related to inflammatory processes of Behcet´s disease in Palestinian and Jordanian population (Verity et al., 2000). While G214R and T741T in domain 3 associated not with coronary and peripheral atherosclerosis (Wenzel et al., 1996)

Although a clear functional effect of this polymorphism has yet to be defined, this genetic variability may influence susceptibility to disease. For this reason, E469K polymorphism of ICAM-1 gene was investigated in the present study.

5.3 Detection technologies for polymorphisms

PCR is a method that typically utilizes two oligonucleotide primers to amplify a DNA segment >1 million-fold. We used a method termed polymerase chain reaction (PCR) amplification of the specific alleles (PASA)(Sommer et al., 1989, Sarkar et al., 1990). This rapid method is also known as allele-specific amplification (ASA), allele-specific PCR, and amplification refractory

mutation system (ARMS) (Newton et al., 1989, Nichols et al., 1989, Wu et al., 1989), which is a generally applicable method of genotyping single-base changes, the detection of known point mutations small deletions and insertions, polymorphisms; and other sequence variations. PASA is a modification of PCR that depends on the synthesis of a specifically designed PCR oligonucleotide primer that precisely matches with one of the alleles but mismatches with the other. Two PASA reactions must be performed, one reaction is required for each allele, in separate paired reactions, M or N allele-specific oligonucleotide primers were amplified with a common distal primer (Sommer et al., 1992), each PASA reaction provides information on the presence or absence of only one allele. When the mismatch occurs at the 3' end of the PCR primer, amplification is inefficient. Therefore, preferential amplification of the perfectly matched allele is obtained. The method should be generally applicable as our results indicate that with proper optimization all possible alleles can be reliably distinguished. The useful and technical simplicity of PASA make genetic analyses more accessible. PASA can be also adapted to accommodate specific requirements and can be extended by incorporating other techniques. Moreover, PASA shows promise for population screening because the technique is a simple and rapid, highly reproducible, inexpensive. Therefore, we have used this methode for ICAM-1 K469E polymorphism.

5.4 Analysis of our results and its capable mechanisms

The genotype distributions in our control group conformed with Hardy-Weinberg expectations, indicating our finding that German subjects with the T-allele of this polymorphism had a higher incidence of CHD and MI, was not due to selection bias. Although several classical risk factors for CHD and MI including habitual smoking, hypercholesterolemia were significantly increased compared with controls (Table 4), to rule out the possibility of relations between ICAM-1 and CHD are due simply to confounding by other risk-

factors, we determined genotype by risk factors, there was no difference between genotypes for any of the risk factors (Table 6, 7) in CHD and MI patients, therefore, our results could not be due to confounding by classic risk-factors. Hackman et al (1996) found that no correlation is between sICAM-1 and total cholesterol, and there was no evidence that lipid concentrations modified the effect of sICAM- 1 on risk of future myocardial infarction. Therefore, the risk associated with raised concentrations of sICAM-l was not limited to patients with hyperlipidaemia, and together with considering our multiple variable logistic regression analysis indicating that the T-allele of ICAM-1 K/E469 polymorphism is an independent risk factor of other known CHD risk factor for CHD and MI. In addition to classical risk factors, genetic risk factors may thus play an important role in the pathogenesis of CHD and MI.

Our study provides no information about mechanisms by which K469E polymorphism of the ICAM-1 gene predisposes patients to CHD and MI, however, a recent study showed that mice with ICAM-1 deficiency had normal endothelial function (vasorelaxation in response to acetylcholine (Ach)) after ischemia-reperfusion, whereas wild-type mice had impaired vasorelaxation in response to Ach (Banda et al., 1997), indicating that ICAM-1 gene may be related to impaired endothelium-dependent vasodilatation. This dysfunction of the endothelium plays a key role in all stages of atherosclerosis. ICAM-1 is produced as a consequence of inflammation. The mechanisms of the proinflammatory effects of ICAM-1 on endothelial cells are not completely clear, it may be that ICAM-1 binds and interaction with leukocyte integrin receptors such as LFA-1($\alpha_L\beta_2$, CD11a/CD18) and Mac-1 ($\alpha_M\beta_2$, CD11b/CD18) (Smith et al., 1989). ICAM-1 is found not only in the membrane form but also circulating in serum. This enables ICAM-1 to bind leukocyte integrin receptors such as LFA-1 and Mac-1 and therefore provide adaptive changes in the adhesion process between circulating cells and the endothelium and hence

provides an adaptive alternative in the adhesion process between circulating cells and the endothelium, leading to the attachment of leukocytes to endothelial cells and the transendothelial migration of leukocytes into the intima and thus to the accumulation of leukocytes in the vascular wall (Carlos et al., 1994, van de Stolpe et al., 1996). In addition to its above role in cell-to-cell adhesion by integrins/ICAM-1 pathway, ICAM-1 also serves as a receptor for soluble fibrinogen, and hyaluronic acid. Fibrinogen is one of inflammatory Marker, studies suggest that fibrinogen mediates leukocyte adhesion to the vascular endothelium through an ICAM- 1 dependent pathway. (Languino 1993 et al., Duperray et al., 1997), a significant correlation between sICAM-1 concentration and plasma fibrinogen concentrations is know, the interaction between fibrinogen and ICAM-1 observed in an vitro study provides evidence suggesting an association between ICAM-1 and thrombosis/ischemic events (Languino et al., 1995). Furthermore fibrinogen has been suggested to mediate cell proliferation via adhesion molecule (Plow et al., 1997, Gardiner et al., 1997), ICAM-1 has also at least two binding domains for hyaluronic acid (HA), HA is a primary component of the extracellular matrix (ECM), which was implicated in cell growth, adhesion, migration and differentiation (Entwistle et al., 1996), it play an important role for smooth muscle cell migration in resteosis (Savani et al., 1995). ICAM-1 may therefore play a major role in the pathogenesis of dysfunction of the endothelium and CHD.

It is reported, no expression of ICAM-1 was identified in exon 4 (domain 3) mutant mice , residual ICAM-1 expression could be identified in exon 5 (domain 4) mutant mice (van Den Engel et al., 2000), These studies indicated that the domain of ICAM-1 is important for maintaining protein-structure, The K/E469 polymorphism represents a change of amino acid, which occurs in Ig domain 5 of ICAM-1, an immunodominant epitope was found in domain 5 (Molgg et al., 1991). Joling et al (1994) reported that domain 5 was involved in adhesion of follicular dendrite cells and B-lymphocytes by others pathway

unlike LFA-1/ICAM-1. For all that, it is possible to hypothesize that this domain 5 may be important in maintaining normal protein structure, and might affect ICAM-1 ligand interactions and the strength and duration of the interaction with integrins , or mediates adhesion of circulating leukocytes to the activated endothelium by others pathway unlike LFA-1/ICAM-1 as follicular dendrite cells and B-lymphocytes, and therefor might have functional effects, but it remains to further study, more study are required to prove that hypothesis.

In conclusion, this study is the first to examine the K/E469 polymorphism of the ICAM-1 gene in patients with CHD and MI. as suggested a possible role for this polymorphism in the pathogenesis of CHD and MI. The exact mechanism(s) by which these effects are mediated is not clear, but Of particular importance is the fact that ICAM-1 K469E variants is expected to enhance our understanding of the molecular basis for atherosclerosis. They are useful in the identification of high-risk patients for CHD, furthermore enhances our ability to predict the risk of such events.

6. Summary

Background—The intercellular adhesion molecule-1 (ICAM-1) mediates the interaction of activated endothelial cells with leukocytes and plays a fundamental role in the pathogenesis of coronary atherosclerosis. ICAM-1 single-base C/T polymorphism, which determines an amino acid substitution in the ICAM-1 protein in exon 6 codon 469, has been described. Our purpose was to determine whether this C/T polymorphism influences the risk of coronary heart disease (CHD) and myocardial infarction (MI) in humans.

Methods and Results—We enrolled 327 patients with angiographically documented CHD, including a sub-group of 176 patients with acute or chronic MI. The control group consisted of 107 patients with normal left ventricular function and no documented evidence of CHD. All patients and controls were Germans genotyped by polymerase chain reaction and allele specific oligonucleotide techniques for the ICAM-1 polymorphism. The genotype for CHD were 18% CC, 42% TC, and 40% TT; for MI were 21% CC, 44% TC, and 35% TT, they differ from that for the control individuals, which were: 33% CC, 40% TC, and 27% TT. The frequencies of the T-allele according the dominant effect (TT+TC versus CC) in the control subjects and the patients with CHD and MI were 0.67 vs. 0.82 and 0.79 respectively (control subjects vs. CHD, p=0.002; vs. MI, P=0.03). Together with considering multivariable logistic regression analysis for CHD (TT+TC versus CC; *p*=0.009, odds ratio 2.34, 95% CI 1.24 to 4.41), we found a significant association between CHD, MI, and the T-allele of the ICAM-1 gene polymorphism**.**

Conclusions— **T**hese results suggest that the T allele of the ICAM-1 gene polymorphism in codon 469 might be a predictor of a new risk factor for CHD and MI in Germans.

Key Words: ICAM-1 ν genes ν coronary heart disease ν myocardial infarction

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Abbreviation

A	Adenine
Ach	Acetylcholine
AMI	Acute Myocardial Infarction
Aminoacide T	Threonine
ARMS	Amplification Refractory Mutation System
ASA	Allele-Specific Amplification
BMI	Body Mass Index
bp	Base Pair
C	Cytosine
CAMs	Cell Adhesion Molecular Family
CD102	ICAM-2
CD106	VCAM-1
CD15s	sialyl-lewis^{x}
CD18/CD11A	LFA-1
CD18/CD11B	Mac-1
CD31	PECAM-1
CD49d/CD29	VLA-4
CD50	ICAM-3

CD54	ICAM-1
CD62E	E-selectin
CD62L	L-selectin
CD62P	P-selectin
CHD	Coronary Heart Disease
CI	Confidence Intervals
CK	Creatine Kinase
CRP	C-Reactive Protein
dATP	2’-deoxyadenosine Triphosphate
dCTP	2’-deoxycytidin Triphosphate
dGTP	2’-deoxyguanosine Triphosphate
DNA	Desoxyribonucleic Acid
dNTP	Desoxyribonucleoside Triphosphate
dTTP	2’-deoxythamidine Triphosphate
E	Glutamic Acid
EC	Endothelial Cells
EDTA	Ethylenediaminetetraacetic Acid
G	Guanine
HA	Hyaluronic Acid
HDL	High Density Lipoprotein

ICAM-1	Intercellular Adhesion Molecule-1
IFN-gamma	Interferon-γ
Ig	Immunoglobulin
IL	Interleukin
K	Lysine
Kb	Kilobase
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LFA-1	Lymphocyte Function-Associated Antigen-1
LV	Left Ventricle
M	Methionine
Mac-1	Macrophage Antigen-1
MI	Myocardial Infarction
min	Minute
n.s.	No Significance
Nucleoid T	Thymidin
OR	Odds Ratio
PAF	Platelet Activating Factor
PASA	PCR Amplification of the Specific Alleles
PCR	Polymerase Chain Reaction

PECAM-1	Platelet-Endothelial-Cell-Adhesion Molecule-1
R	Arginine
R. T.	Room Temperature
rpm	Round per Minute
s ICAM-1	soluble Intercellular Adhesion Molecule-1
SD	Standard Deviation
SE	Standard Error
SLex	sialyl-lewis^{x}
SMC	Smooth Muscle Cells
sVCAM-1	soluble Vascular Cell Adhesion Molecule-1
Taq	Taq DNA Polymerase
TBE	Tris/Boric/EDTA
TG	Triglyceride
TNF	Tumor Necrosis Factor
Tris	Tris (hydroxymethyl) Aminomethane
U	Unit
VCAM-1	Vascular Cell Adhesion Molecule-1
VLA-4	Very Late Antigen-4
vs.	Versus

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Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation von mir selbstständig angefertigt wurde. Es wurden ausschließlich die von mir angegebenen Hilfsmittel verwendet.

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Publikationen:

auf Englisch oder Deutsch:

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C/T Polymorphismus des interzelluläre Adhäsionsmolekül-1 Gens (Exon 6, Codon 469) als Risikofaktor für eine koronare Herzerkrankung und Herzinfarkt

Zusammenfassung

Fragestellung: Entzündliche Mechanismen spielen in der Pathogenese der Atherosklerose eine bedeutende Rolle. Das interzelluläre Adhäsionsmolekül-1 (ICAM-1) stellt eine Gruppe von Membranproteinen dar, die die feste Adhäsion von Monozyten am Endothel mediieren. Für ICAM-1 ist ein single-base Polymorphismus (C- und T im Exon 6 Codon 469) beschrieben, dessen klinische Bedeutung für die koronare Herzerkrankung (KHK), und den Myokardinfarkt (MI) bisher nicht bekannt ist.

Methodik: Bestimmt wurde der Genotyp von 327 Patienten mit einer invasiv diagnostizierten KHK mittels Allel spezifischer PCR Analyse. 176 Patienten wiesen einen akuten oder früheren MI auf. Als Kontrollgruppe galten 107 Patienten mit normaler linksventrikulärer Funktion und unauffälligen Koronarien.

Ergebnisse: Die Genotypverteilung der Kontrollgruppe war: 33% (CC), 40% (TC) und 27% (TT), derjenigen Patienten mit KHK: 18% (CC), 42% (TC) und 40% (TT), und mit MI: 21% (CC), 44% (TC) und 35% (TT). Die Genotypverteilung von TT+TC mit CC (dominanter Effekt) der Kontrollgruppe (0,67) unterschied sich signifikant gegenüber Patienten mit KHK (0,82, p=0,002) und MI (0,79, p=0,03).Unsere multivariable logistische Regressionsanalyse für KHK zeigt: TT+TC verglichen mit CC, p=0.009, Odds Ration 2.34, 95% Konfident Intervall 1.24 bis 4.41).

Schlußfolgerung: Die Ergebnisse sprechen für eine Assoziation des T Allels des ICAM-1 Polymorphismus und der Entstehung einer koronaren Herzerkrankung und eines Myokardinfarktes in der deutschen Bevölkerung.

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