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GENETICS (AJ MARIAN, SECTION EDITOR)

Pro-Inflammatory Genetic Markers of Atherosclerosis

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Abstract Atherosclerosis (AS) is a chronic, progressive, multifactorial disease mostly affecting large and medium-sized elastic and muscular arteries. It has formerly been considered a bland lipid storage disease. Currently, multiple independent pathways of evidence suggest this pathological condition is a peculiar form of inflammation, triggered by cholesterol-rich lipoproteins and influenced both by environmental and genetic factors. The Human Genome Project opened up the opportunity to dissect complex human traits and to understand basic pathways of multifactorial diseases such as AS. Population-based association studies have emerged as powerful tools for examining genes with a role in common multifactorial diseases that have a strong environmental component. These association studies often estimate the risk of developing a certain disease in carriers and non-carriers of a particular genetic polymorphism. Dissecting out the influence of pro-inflammatory genes within the complex pathophysiology of AS and its complications will help to provide a more complete risk assessment and complement known classical cardiovascular risk factors. The detection of a risk profile will potentially allow both the early identification of individuals susceptible to disease and the possible discovery of potential targets for drug or lifestyle modification; i.e. it will open the door to personalized medicine.

Keywords Atherosclerosis · Cardiovascular disease · Coronary heart disease · Genetics · GWA · Inflammation · Meta-analysis · Myocardial infarction · SNP

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Introduction

Inflammation is a well-conserved process, evolved to enhance the organism's resistance to infections, and to lead to tissue repair after various forms of injury. It is considered as a complex and potentially life-threatening condition, orchestrated by a variety of chemical mediators, signaling pathways, and cell types. Its initiation and perpetuation are the result of several characteristic functional and structural changes of the microcirculation, ranging from the vasomotor dysfunction (impaired vessel dilation and constriction), adhesion and transendothelial migration of leukocytes, to endothelial barrier dysfunction (increased vascular permeability), and blood vessel proliferation (angiogenesis). Changes in physiological hemostasis also accompany inflammation as consequence of the action of pro-inflammatory cytokines, chemokines, adhesion molecules, tissue factor expression, platelet and endothelial

activation. Its evocation may be caused by chronic inflammation associated-endothelial damage, leading to the loss of physiologic anticoagulant, antiaggregant and vasodilatory properties of endothelium. On the other hand, coagulation also augments inflammation, causing a vicious cycle. This is mainly achieved by means of thrombin-induced secretion of pro-inflammatory cytokines and growth factors [1].

When chronic inflammation moves beyond local tissues and into the lining of blood vessels and organs, systemic inflammation occurs. Chronic systemic inflammation is the result of release of pro-inflammatory cytokines from immune-related cells and the chronic activation of the innate immune system. In atherosclerosis (AS), inflammation in the vascular wall and the release of inflammatory cytokines from macrophages is considered the main mechanism. However, infection (i.e. Chlamydia or Helicobacter pylori infection and periodontal disease) is considered as an underlying cause of systemic inflammation [2]. It can contribute to the development or progression of certain conditions. A characteristic biomarker of systemic inflammation is C-reactive protein (CRP), an acute phase protein, whose levels increase only during an inflammatory process in the body [3••].

Inflammation in Atherosclerosis as the Prevalent Process

AS is a chronic progressive disorder, principally affecting large and medium-sized elastic and muscular arteries. It has formerly been considered a bland lipid storage disease. Currently, multiple independent pathways of evidence suggest this pathological condition as a peculiar form of inflammation, triggered by cholesterol-rich lipoproteins and other noxious factors, such as cigarette smoke, diabetes mellitus and hypertension. Inflammation seems, indeed, to be the prevalent process of AS, evocated by multiple risk factors and responsible for the altered arterial biology associated with AS complications [3••].

The initial stimulus inducing the inflammatory process has not been fully identified. However, endothelial dysfunction plays a crucial role in inflammation evocation. Several causes are associated with endothelial dysfunction, including the major number of traditional AS risk factors, i.e. elevated low density lipoprotein (LDL) values, free radicals caused by cigarettes smoking, hypertension, diabetes, elevated levels of homocysteine, but also infections caused by Chlamydia pneumoniae, Herpes simplex virus, Cytomegalovirus and Helicobacter pylori [4]. Thus, AS may be considered as a characteristic response both to endothelium injury and to the consequent endothelial dysfunction. Endothelial dysfunction leads to compensatory responses modifying the normal homeostatic properties of the endothelium [5] and favouring the expression of adhesion molecules, which bind to various classes of leukocytes [6, 7].

Both innate and clonotypic immune cells are involved in this clinical inflammatory disorder. Monocytes are the prominent cellular component of innate immune response during atherogenesis. They, recruited through activated endothelium, differentiate into macrophages. This step is critical for the onset of AS, and is associated with up-expression and regulation of pattern-recognition receptors, including scavenger receptors (able to internalize a broad range of molecules) and Toll-like receptors (TLRs) (whose activation can initiate a signal cascade leading to cell activation) [8, 9]. The activated macrophages produce inflammatory cytokines, proteases, cytotoxic oxygen and nitrogen radical molecules.

Accumulating evidence also supports the involvement of clonotypic immune cells in AS and its complications. In particular, a T cell infiltration is always present in AS lesions. Several CD4+ T cell subsets have been observed. A type-1 helper T (Th1) response seems to be involved in the activation of macrophages and the consequent evocation of an inflammatory response similar to delayed hypersensitivity and with the typical functions of response against intracellular pathogens. The AS lesions contain cytokines promoting a Th1 response. Activated Th1 cells differentiate into Th1 effector cells and produce the macrophage-activating cytokine interferon(IFN)-γ, which augments the synthesis of the inflammatory cytokines, such as Tumor Necrosis Factor(TNF)-α and Interleukin (IL)-1. These cytokines stimulate the production of numerous inflammatory and cytotoxic molecules in macrophages and vascular cells. Thus, Th1 response appears to exacerbate AS [8]. Another T cell subtype, regulatory T cell (T_{reg}), seems to play a modulatory role in AS, controlling inflammatory responses through the secretion of anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β [10]. Thus, the development of AS could be the effect of the imbalance among different T cells populations.

Several data from human and animals studies confirm the key role of inflammation in the atherogenesis: significant elevation of plasma levels of inflammatory biomarkers predicts outcomes in patients with acute coronary syndromes, independently on myocardial damage. In particular, levels of CRP or IL-6 have been suggested to be significant predictive risk factors for future cardiovascular events [8, 11]. In addition, some treatments able to reduce the risk of cardiovascular events seem to limit inflammation: this is the case of statins, whose anti-inflammatory effects are not only related with the lipid-lowering effect [12, 13].

Furthermore, several clinical conditions characterized by a chronic systemic inflammatory state have recently been associated with an increased cardiovascular risk, not explained by traditional risk factors. Many epidemiologic observations have linked some diseases with inflammatory systemic pathophysiology with cardiovascular events, such

as autoimmune diseases as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). In fact, an increasing body of evidence supports the hypothesis that atherosclerosis and autoimmune diseases share many similarities, such as the presence of an inflammatory infiltrate in the unstable coronary lesion that is indistinguishable respect to inflammatory synovitis seen in RA [14]. Moreover, an increased mortality has been demonstrated, largely due to cardiovascular diseases (CVD), in subjects affected by RA or SLE [15–17]. Interestingly, the treatment with methotrexate, able to reduce immune-inflammatory burden in these diseases, seems to limit cardiovascular risk in patients with chronic inflammatory diseases [18, 19].

Several other clinical conditions characterized by a chronic inflammatory state have been found to be associated with increased risk of AS and CVD. For example, coronary heart disease (CHD) occurs much more frequently in patients with end-stage renal disease (ESRD) than in the general population, accounting for 45–50 % of deaths in patients receiving dialysis [20]. It has been hypothesized that chronic inflammation characterizing ESRD may accelerate the development and progression of AS.

Other clinical conditions, associated with a low grade chronic inflammation often due to chronic infection, have been associated with an increased risk of CVD, even if in lesser measure. An example is periodontal disease, which is actually considered a possible cause of cardiovascular disease [21, 22•]. Thus, the concept of inflammation as a cardiovascular risk factor has spread in the scientific community [23].

AS arises as a multistep clinical condition, characterised by a series of events whose individual probability and timing vary with risk factors and intrinsic ability of self-repair for the artery. Its progression is generally assumed as irreversible and one-directional as a function of time. However, for each step, a small reverse probability exists [24]. It has been, indeed, evidenced that some individuals with a genetically substantial ability for self-repair of their arteries, even in the presence of potent risk factors, remain sheltered from the consequences of AS and related thromboembolic events [24].

Genetic Polymorphisms which Modulate Inflammation, AS Onset and Progression, and CVD Complications

A study performed 25 years ago for identifying genetic and environmental influences of premature death in adult adoptees concluded that premature death in adults has a strong genetic background, especially death due to infections and vascular causes [25]. Therefore, the inflammatory process, involved both in response to infection and in AS, has a strong heritable component. Thus, the analysis of the genes that are key nodes of the inflammatory response might in part clarify by the pathophysiology of AS and its complications.

The Human Genome Project opened up the opportunity to dissect complex human traits and to understand basic pathways of health and disease. Population-based association studies have emerged as powerful tools for examining genes with a role in common multifactorial diseases that have a strong environmental component. These association studies often estimate the risk of developing a certain disease in carriers and non-carriers of a particular genetic polymorphism. The majority of the genetic variants studied are common single nucleotide polymorphisms (SNPs) that occur with a frequency of>1 % in the normal population. It is estimated that approximately 3.5 million SNPs are present in the human individual genome [26, 27]. Most of these SNPs do not occur in the coding sequences. However, although they are not associated with alteration in the amino acid sequence, they may have functional consequence, if, for example, they are localized in the promoter region. There has been an exponential increase in the number of published genetic association studies. Quite often, a report of a single genetic marker is published with great promise; however, it is followed by several negative studies that fail to reproduce the original observation. There is no doubt that the strategy of genetic association studies could be a powerful tool for dissecting human diseases, provided that certain principles are observed to minimize the chances of false positive and negative reports [28].

In the following sections, we discuss the role of SNPs that modulate inflammation and AS and CVD risk. Three genetic approaches: candidate gene approach, meta-analysis and genome-wide association studies have been used to assess SNPs and AS and CVD, including CHD and Myocardial Infarction (MI) risk.

Candidate Gene Approach and Meta-Analysis

The candidate gene approach is a hypothesis-driven method widely employed by case–control studies. In these studies, the genotype and allele frequencies of two populations are compared: one affected and one unaffected by a complex disease. If the identified allelic variants are more prevalent in the affected population as compared to unaffected, these genotypes are associated with the disease. The number of reported studies on the association between one or multiple SNPs in inflammation-related pathways and CVD risk is greatly increasing, event though a large number of these studies show inconsistent results (for an extensive analysis of “old” data, refer to [29]). Consistent replication in different populations has been argued as strong evidence of a true association. However, the genetics of AS is both complex and intriguing and may alter according to sex and age group. The lack of replication may not necessarily imply a false

association, but might simply point to the need for more studies in certain populations or more detailed study of the function of a particular gene, taking into account different gene environment interactions. It is already accepted that the cardiovascular/ atherosclerosis-related phenotype is strongly affected by life-style and environmental factors and by complex epistatic and pleiotropic effects in several genes. So, small contribution of a single novel polymorphism to the overall risk of multifactorial diseases, such as CVD and MI, might be obscured by the presence of one or more dominant classical risk factors [29].

However, some interesting data have recently been obtained, such as those derived by meta-analysis. Meta-analysis provides a mean to quantitatively synthesize association data across studies of the same genetic variant. Thus, the use of meta-analyses has recently become an important part of genetic research mainly to reconcile previously conducted studies that gave inconsistent results [30].

A recent meta-analysis assessed the association between Asp299Gly (+896A>G; rs4986790) polymorphism in TLR-4 gene and AS risk. This missense polymorphism alters the extracellular domain of TLR-4, so it attenuates the TLR-4 signaling pathway and diminishes the inflammatory response to Gram-negative pathogens. In particular, it may influence inflammatory responses and the risk of major inflammatory age-related diseases by affecting the production of inflammatory mediators. To clarify and confirm the biological effects of +896A>G SNP and its role in the pathophysiology of age-related diseases and longevity, we recently assessed the levels of IL-6, TNF-α, IL-10 and eicosanoids after in vitro stimulation with lipopolysaccaride (LPS) of whole blood samples from 50 young healthy Sicilians, screened for the presence of this SNP. Both pro-inflammatory cytokines and eicosanoids were significantly lower in carriers bearing the TLR-4 polymorphism, whereas the anti-inflammatory IL-10 values were higher. So, the TLR-4 receptor, activated by pathogens or endogenous molecules, would have the role of hub in the inflammatory responses involved in the pathophysiology of AS [31, 32].

A total of 15 case–control studies with 9,989 cases and 6,746 controls were included in the meta-analysis, and no statistically significant association was found between the A allele and the AG/GG genotype and the risk of AS by random effects model [33]. On the other hand, other studies have reported an association in specific subgroups [34] (Table 1). Further studies estimating the effect of gene-gene and gene-environment interactions may eventually provide a better comprehensive understanding, although the relatively low prevalence of the 896G allele is a potential limitation in association studies [34].

Other inflammatory molecules seem to have a crucial role in AS. Among these, monocyte differentiation antigen CD14 is considered an important cell-activating mediator of inflammatory responses that may result in AS, CHD, thrombus formation, and MI. A common -260C>T polymorphism in the promoter of the CD14 gene, the trans-membrane receptor of LPS, has been inconsistently associated with CHD. This C>T SNP, in position −260 (rs259190) of the promoter, has been shown to increase transcriptional activity associated with enhanced expression of CD14 on monocytes [35]. A recent meta-analysis of 28 studies involving a total of 13,335 CHD cases and 7,979 controls for -260C>T of the CD14 gene evaluated the effect of CD14 on genetic susceptibility for CHD. An overall random effect of 1.24 odds ratio (ORs) was found for the T allele. Significant results were also observed using dominant or recessive genetic model. After stratification by ethnicity, significant results were found in East Asians, but not in Caucasians and other ethnic populations in all genetic models. Thus, the results of this meta-analysis suggest that the CD14 -260C>T polymorphism is a risk factor of CHD, especially in East Asians [36].

Other relevant data analyzed the association of genetic variants in genes coding for cytokines, adhesion molecules and chemokines. Concerning adhesion molecules, the ICAM-1 gene -469E>K polymorphism, known to influence molecule serum levels [37], has been reported to be associated with CHD, but results are conflicting. A systematic review and meta-analysis of the published studies were performed to gain a clearer understanding of this association. The pooled result showed that the ICAM-1 gene -469E>K polymorphism was significantly associated with an increased risk of CHD. Subgroup analysis supported the results in Asian and Caucasian populations [38].

Several studies have also reported apparently conflicting findings about the effects of TNF-α and IL-6 SNPs, such as TNF-α -308G>A and IL-6 -174G>C promoter SNPs on CHD susceptibility. A systematic review and meta-analysis recently investigated the association between the TNF-α -308G>A gene variant, responsible for an increased cytokine production [39], and CHD predisposition in 24 studies providing data for 9,921 cases and 7,944 controls. Pooled analysis based on ORs adjusted by CHD risk factors showed that carrying the TNF-α gene A variant conferred a 1.5-fold increased risk of developing CHD in Caucasian population [40]

Another recent meta-analysis evaluated the IL-6 -174 G>C promoter SNP, responsible for different production of this cytokine [30], in relation to CHD risk, including 6,434 participants of the Rotterdam Study. Analyses on the relation between genotypes and CHD were performed using Cox proportional hazards tests, and the association between genotype and plasma levels of IL-6 and CRP was investigated. All of the analyses were adjusted for age, sex, and common cardiovascular risk factors. A meta-analysis was performed, using a random effects model. No association between genotype and the risk of CHD was observed. The

Table 1 Data from our investigations in a Sicilian population

Genes	Alleles of SNPs or genetic variants	Centenarians	Young controls (< 55 years)	MI patients(< 55 years)	*Pp* -value
TLR-4 [47]	+896A>G[1](Asp299Gly; rs4986790)	NN=55 males	N=127 males	N=105 males	< 0.001
CCR5 [48]	WT>Δ32[2](rs333)	N=123 males	N=136 males	N=133 males	=0.00006
Cox-2 [49]	−765G>C[3](rs20417)	N=96 males	N=170 males	N=140 males	=0.000007
5-LO[49]	−1078G>A[4](rs2115819)21C>T	N=96 males	N=170 males	N=140 males	=0.00003
		N=96 males	N=170 males	N=140 males	=0.001
FLAP [49]	−336G>A[5]	N=96 male	N=170 males	N=140 males	=0.0007
Cx37 [50]	−1019C>T[6]	N=56 males	N=196 males	N=97 males	=0.0035
IL-10 [52]	−1082G>A[7](rs1800896)	N=52 males	N=110 males	N=90 males	=0.0003
α1AT [53]	342G>L [8]	N=143	N=255	N=127	=0.0000001
MEFV [54]	694 M>V[9]	N=68	N=196	N=121	=0.003

[1] This missense polymorphism alters the extracellular domain of TLR-4, so it attenuates the TLR4 signaling pathway and diminishes the inflammatory response to Gram-negative pathogens. In particular, it may influence inflammatory responses and the risk of major inflammatory age-related diseases , such as AS, by affecting the production of inflammatory mediators (see text)

[2] A non functional allele, resulting from a 32-bp deletion in exon 4, determines a loss of expression of functional CCR5 receptor. So, this genetic variant may have a protective role against AMI as consequence of an attenuated inflammatory response that should determine a slower progression of atherosclerotic lesion among CCR5Δ32 carriers (ref. in 48)

[3] Located within a putative binding site for the transcription factor Sp1, associated with a different transcription of gene (ref. in 49)

[4] SNPs in promoter region and exon-1 of 5-LO gene, respectively, able to modify the gene transcription or the putative protein (ref. in 49)

[5] It has been claimed to be functional, modifying gene transcription or modifying the putative protein derived from gene translation (ref. in 49)

[6] This SNP causes a shift from proline to serine at amino acid 319. In a mouse model of atherosclerosis, the mouse Cx37 protein was shown to be atheroprotective by properly regulating leukocyte recruitment, namely one of the first inflammatory steps in atherosclerotic process (Ref. in 50, 51)

[7] SNP in the IL-10 proximal gene region (considered potential target for transcription regulating factors) involved in genetic control of IL-10 production, even if contrasting literature data have been reported. In particular, the homozygous -1082GG genotype seems to be associated with higher IL-10 production respect to G>A heterozygous and AA homozygous genotypes. Furthermore, this SNP seems to be functionally relevant. It has been demonstrated that −1082 A carriers (low producers) seem likely develop a major number of chronic inflammatory diseases (ref. in 39, 52)

[8] It results in a severe protein deficiency that is characterised in the homozygote state by levels of plasma concentrations that are lower by 84 % when compared with levels in MM individuals and in the MZ heterozygote state, by intermediate levels that are lower by 17 %. It has been suggested that α1AT deficiency could lead to less cleaved fragments of α1AT (i.e. the pro-inflammatory peptide C-36) of α1AT in atherosclerotic plaques, and thereby reduce AS inflammatory process (ref. in 53)

[9] This mutation in the pyrin gene is liable to lead to leukocyte survival otherwise designed to follow the apoptotic pathway, increasing the inflammatory response (ref. in 54)

polymorphism was not associated with IL-6 levels, but the C-allele was associated with higher CRP levels. Thus, this meta-analysis did not show a significant association between the genotype and the risk of CHD [41].

Monocyte chemoattractant protein-1 (MCP-1) and its receptor C-C chemokine receptor (CCR) type 2 have also been implicated in promoting AS. Several studies have investigated the association between variants of the MCP-1 gene or CCR2 gene and risk of CVD, but results are inconsistent. A meta-analysis of 20 publications, including 24 studies on two genetic variants—2518A>G in the MCP-1 and 64 V>I in the CCR2—with a total of 9,844 patients with CVD and 11,821 controls, has recently been performed. The results suggest that MCP -2518A>G SNP, known to increase MCP-1 production [42] shows association with an increased risk of CVD in Caucasians, likely due to publication bias and insufficient sample size. The CCR2 64 V>I, affecting stability of the CCR2A isoform [43], has not been found to be associated with CVD [44].

An alternative approach to studing potential susceptibility genes for cardiovascular diseases has been followed by our group, using centenarians as healthy controls. On the other hand, the major feature characterizing centenarian offspring is the significant reduction of CVD prevalence. Thus, alleles associated to CVD susceptibility would not be included in the genetic background favoring longevity [45]. This kind of approach has been named “positive biology”. Rather than making diseases the central focus of research, positive biology seeks to understand the causes of positive phenotypes, trying to explain the biological mechanisms of health and wellbeing [46]. In these studies, performed in a very homogeneous sample of young Sicilian patients and age-matched controls, we have investigated the eventual association of some functional SNPs of pro-inflammatory genotypes with MI. The results described in Table 1 clearly show that the pro-inflammatory SNPs of TLR-4, CCR5, cyclo-oxygenase(COX)-2, 5-lypo-oxygenase(LO), lipo-oxygenase-activating protein(FLAP), Connexin 37 (CX37),

Table 2 CHD and MI sSusceptibility (GWAS) (genes related to inflammation are in bold/italics)

BAND	GENE REGION(S)	REFERENCE
1p13.3	PSCR1, CELSR2, SORT1, MYBPHL	[59–62]
1p32.2	PPAP2B	[70]
1p32.3	PCSK9	[62]
1q41	MIA 3	[59–62]
2q33	WDR12, ALSC2R13	[62]
2q36	IRS1	[71]
3q22.3	MRAS	[63]
6p21.3	***HLA***	[58]
6p21.31	ANKS1A	[70]
6q23.2	TCF21	[70]
6p24	PHACTR1	[62]
6q26-27	SLC22A3, LPAL2, LPA	[64, 72]
7q32	KLF14	[71]
7q32.2	ZC3HC1	[70]
8p22	NAT2	[71]
9p21	CDKN2A, CDKN2B, ANRIL, MTAP	[59, 60, 62, 65–67]
9q34.2	***AB0***	[59, 70, 73]
10q11.	***CXCL12>SDF-1***	[59, 60, 69]
10q24.32	CYP17A1, CNNM2, NT5C2	[70]
11q23.3	ZNF259, APOA5, A4, C3, A1	[70]
12q24	***SH2B3***	[57, 68]
12q24.3	HNF1A, C12orf43	[63]
13q34	COL4A1, COL4A2	[70]
14q32.2	HHIPL1	[70]
15q25.1	ADAMTS7	[70, 73]
17p11.2	RASD1, SMCR3, PEMT	[70]
17p13.3	SMG6, SRR	[70]
17q21.32	UBE2Z, GIP, ATP5G1, SNF8	[70]
19p13.2	LDLR	[62, 63]
21q22	SLC5A3, MRPS6, KCNE2	[62]

IL-10, α1anti-trypsin(AT) and pyrin (MEFV) are overrepresented in patients when compared to centenarians. Reciprocally, centenarians possess genetic factors that modulate ageing processes and are protective for CVD [47–54].

Genome-Wide Association Study

Genome wide association study (GWAS) consists in a scanning of whole genome analyzing markers to find variants associated with the trait of interest using a case–control study [55•]. It is important to note that the finding of common genetic variants with low allelic frequency across studies is consistently difficult because of the multitude of data to analyze. Population admixture may produce possible false positives due to different genetic backgrounds among ethnic groups is another problem. A family-based association study and the analysis of geographically isolated population could permit to improve the detection of true positive genetic association loci, in particular those of modest size.

GWAS is a useful tool for the identification of AS associated alleles with frequency above 5 % [55•]. Each, taken singularly, has a moderate or null effect, but borrowing a concept from pharmacology, it is possible to speculate that more alleles have, en bloc, a synergic rather than an additional effect. It means a potentiated effect respect to the sum of alleles.

With GWAS approach of CVD patients, several loci have reached a genome wide significant level not always confirmed in different studies (Table 2) [57–73]. The locus 9p21 (in particular carrying cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B) and ANRIL was identified as the strongest genetic susceptibility locus for CHD and MI. This was consistently associated in different studies and in meta-analysis, and this datum was replicated among different ethnic groups [59, 60, 62, 65–67].

Concerning inflammatory genes, C-X-C motif ligand 12 (CXCL12), SH2B adaptor protein 3 (SH2B3), a novel Human

Leukocyte Antigen (HLA) locus and the AB0 locus were identified as significantly associated to CHD.

CXCL12 is a gene encoding for a chemokine involved in vascular repair and remodeling. It is expressed in atherosclerotic lesion, and its effects on platelets could promote atherothrombosis [56]. It was demonstrated that SNPs rs1746048 and rs501120, in a region 80 kb downstream of the CXCL12 gene (on chr 10q11), are associated with higher CXCL12 plasma levels and higher CXCL12 mRNAs [60, 69, 71].

SH2B3 encodes for an adaptor protein and promotes a pro-inflammatory state in human vascular endothelial cells, where it is expressed. Its SNP rs3184504, whose biological significance is unknown, determines a missense mutation and it has been identified as possible driver in coronary arteries plaque formation and in MI in different populations, including Europeans [57].

Human Major Histocompatibility Complex (HLA) plays an important role in inflammation and T cell responses, closely related to CHD and in particular to the progression of AS. An association between a novel locus HLA, 6p21.3, and CHD was found in a well-selected European population. In particular, the SNP rs3869109 located in an intragenic region between HLA-C and HCG27 (HLA complex group 27), although the biological basis of this association is not clear, has reached a genome-wide significance level [58].

A recent GWAS conducted on level of inflammatory markers in a Sardinian population highlighted the association between CVD and AB0 locus. The association was established between this locus and IL-6. Subjects homozygous for G allele in rs657152 SNP, corresponding to blood type 0 carriers, showed higher IL-6 circulating levels respect to non-0 carriers, although the reason in unknown, reinforcing a relevant involvement of blood group antigens in inflammatory process [74]. Indeed, a previous GWAS demonstrated that a variant in ABO genes can explain the variation in soluble E-selectin levels [75]. On the whole, these GWAS might clarify the relation between ABO and CVD observed in GWAS [59, 70, 73] and in classic case control studies [73].

Conclusions

In this paper, we reviewed data in the literature, comparing the gene frequencies between patients and controls of pro-inflammatory gene polymorphisms potentially related to AS and its complications. Inflammation represents a key process involved in the development of the diseases. A large number of epidemiologic studies have identified significant associations between the levels of circulating inflammatory biomarkers and AS and its complications. However, circulating inflammatory biomarker levels fluctuate in response to acute infection or tissue damage, which may result in measurement error because of high intra-individual variation.

In contrast, the study of genetic variants associated with inflammatory molecules may offer a more accurate picture of inflammatory status. On the other hand, they might be unaffected by common, environmental confounding factors. The characterization of genetic determinants may also provide further insight into the biological mechanisms regulating inflammation and their role in pathophysiology of AS.

In the last years, the number of genetic studies demonstrating associations between different pro-inflammatory gene polymorphisms and AS and CVD has particularly increased. However, divergent results exist across population groups, different sample size studies, and between the types and severity of CVD and age of subjects.

As a consequence, as discussed, the use of meta-analyses has recently become an important part of genetic research, mainly to reconcile previously conducted studies having inconsistent results.

Today, with the coming of non-biased techniques (i.e. GWA studies) and the sophisticate genetic analyses, which enable assessment of multiple genetic variants, a large number of novel genetic associations of genes coding for the inflammatory molecules with AS and its complications have been identified. In addition, they have provided the basis for subsequent research to assess the causality of these relationships.

However, the high throughput genetic analyses that allow the simultaneous risk assessment of multiple genetic variants with sophisticated biostatistical programs are going to allow for the identification of "genetic signatures" for CVD. Dissecting out the influence of pro-inflammatory genes within the complex pathophysiology of AS and CVD will help to provide a more complete risk assessment and complement known classical cardiovascular risk factors. The detection of a risk profile will potentially allow both the early identification of individuals susceptible to disease and the possible discovery of potential targets for drug or lifestyle modification, i.e. it will open the door to personalized medicine.

However, a note of caution should be added, that since the polymorphisms involved are fairly common in the general population, there is a strong likelihood that any given individual will inherit one or more of the high-risk alleles: the occurrence of the disease is likely to depend on interaction between different high-risk alleles, exposure to pathogens, environmental factors, and lifestyle choices.

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Conflicts of Interest Egle Incalcaterra declares that she has no conflicts of interest.

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- • Of importance
- •• Of major importance

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