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Review

Aspects of gene polymorphisms in cerebral infarction: inflammatory cytokines

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Abstract. During the last decade, a growing corpus of evidence has indicated an important role of inflammatory cytokines in the pathogenesis of cerebral lesion following stroke. Recent data suggest that genetics may in turn contribute to modulating the effects of inflammatory cytokines on cerebral infarction (CI). This paper reviews the physiologic characteristics of major inflammatory cytokines and recent research developments related to cell biology and pathobiology in CI. In particular, this review focuses on the genetic aspects of inflammatory cytokines and their implications in CI.

Key words. Inflammatory cytokines; pathogenesis; stroke; cerebral infarction; gene polymorphisms.

Introduction

Ischemic stroke is a complex vascular and metabolic process resulting in neuronal death that evolves with time. Occlusion of a cerebral artery results in a markedly ischemic core surrounded by a moderately hypoperfused region known as the penumbra. The penumbra, which represents the target for a given therapeutic intervention, is a region at risk; it can evolve to infarction or towards viability. A number of mechanisms have been proposed to explain the progression of the penumbra to infarction. These include formation of vasoconstrictor elements that can worsen the ischemia-associated hypoperfusion, generation of cytotoxic substances (e.g. excitatory amino acids, calcium, free radicals), occurrence of spreading depression and induction of deleterious proteins [1, 2]. Also, infiltration of leukocytes early in the ischemic region and development of brain edema characterize ischemia-induced inflammation [3, 4]. Data accumulated over the last 10 years have led to the popular hypothesis that neutrophils and other inflammatory cells play a prominent role in the neuropathology of cerebral ischemia. Moreover, brain-resident cells (e.g. astrocytes, microglia and endothelial cells) become activated in response to the ischemic injury. Much of this inflammatory response appears to be mediated by interleukins, a multifunctional subclass of cytokines. Proinflammatory cytokines, tumour necrosis factor-alpha (TNFα) and interleukin-1 beta (IL-1β) initiate an inflammatory reaction and induce expression of other cytokines and inflammatory mediators [5]. In physiological conditions, the expression of cytokines is very low. However, these cytokines are up-regulated in the brain after injury.

Recent attention has focused on the inflammatory component of atherogenesis and acute ischemia [6, 7]. Indeed, atherosclerosis is now described as an inflammatory disease [6], and flared plaque inflammation is considered

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a cause of intimal erosion and rupture and therefore of acute ischemia [7]. Studies in vitro and in experimental animals are supported by the clinical finding of increased inflammatory markers in patients with chronic stable angina, severe unstable angina, and acute myocardial infarction and by the predictive value of such markers for subsequent coronary events [7]. Most cerebrovascular disease is also related to atherosclerosis of the cerebral arteries. Furthermore, the common and major pathological changes in cerebrovascular disease are atherosclerosis and thrombogenesis in the artery.

Inflammatory cytokines play an important role in the etiology of cerebral infarction (CI), and these genes are under strong genetic control. Because genetic traits contribute significantly to CI, a number of studies have now addressed the hypothesis that variations in the genetics of the inflammatory system may increase the risk of the disease. Differences in the genetic regulation of inflammatory processes might explain why some people but not others develop the disease and why some develop a greater inflammatory response than others [7]. A growing list of polymorphisms is associated with stroke in humans [8]. However, the association between the gene polymorphisms of inflammatory cytokines and CI has been less studied.

The present review focuses on the physiologic characteristics of major inflammatory cytokines and recent research developments related to cell biology and pathobiology in CI. In particular, this review focuses on the genetic aspects of inflammatory cytokines and their implications in CI.

IL-1

The IL-1 family currently consists of three genes that encode for three distinct proteins with high structural homologies. Interleukin-1 alpha (IL-1α) and IL-1β bind to receptors and induce an intracellular signal; however, the third member of the family, IL-1 receptor antagonist (IL-1ra) binds to the same receptors as IL-1α and IL-1β but does not induce any intracellular signal. IL-1ra acts as an inhibitor of IL-1 activity [9]. IL-1β represents the major secreted molecule and the more predominant form in the brain, whereas IL-1α is produced in a membrane-associated form [9].

IL-1 is a proinflammatory cytokine that has been identified as an important mediator of neurodegeneration induced by experimental cerebral ischemia (stroke) or excitatory or traumatic brain injury in rodents [10, 11]. Both IL-1 ligands (IL-1α and IL-1β) are produced rapidly in the brains of rodents exposed to cerebral ischemia [12–14], and recombinant IL-1β administered intra-cerebroventricularly, or directly into the brain, enhances ischemic and other forms of injury [10, 15–17]. Conversely, blocking IL-1 actions, by administration of the naturally occurring and selective IL-1ra, markedly reduces neuronal loss and inflammation induced by a variety of experimental brain insults [18–21].

Although there is compelling evidence to implicate IL-1β in ischemia-induced brain damage, the mechanisms and mediators of IL-1 actions are unknown, though several hypotheses have been proposed. IL-1β has pleiotropic actions. It exerts direct effects on neurotransmission, glia and endothelial cell stimulates, the synthesis of some growth factors, neuropeptides and other cytokines, such as tumour growth factor-beta (TGF-β), IL-10 and IL-4, which are anti-inflammatory and anticoagulant cytokines. It also induces others, TNFα, IL-6, IL-8, IL-2 or interferon-γ (IFN-γ), which are proinflammatory cytokines [22]. Therefore, the net effects of IL-1β on ischemic cell damage, whether beneficial or detrimental, may depend on a wide range of interacting factors including timing and duration, as well as the level and location of IL-1β expression in the ischemic brain.

Polymorphisms of the IL-1 cluster

The genes for IL-1α, IL-1β, and IL-1ra, located in a cluster on human chromosome 2, have several polymorphisms [9]. IL-1α has a base-exchange polymorphism at position –889 in the promoter region [23]. IL-1β has two base-exchange polymorphisms, at position –511 in the promoter region and at position +3953 in exon 5 [24, 25]. The IL-1ra gene has a penta-allelic polymorphic site in intron 2, containing variable numbers of an 86-bp identical tandem repeat (VNTR) [26]. These polymorphisms of IL-1β and IL-1ra affect on the level of cytokine production in vitro [27–30]. In addition, certain combinations of the IL-1ra and IL-1β loci regulate the plasma levels of IL-1ra [31], which would imply allelic co-operation of these genes in the immune and inflammatory responses.

Despite the extensive evidence implicating IL-1 in ischemic brain damage, little information is available in the literature concerning the effect of IL-1 polymorphisms in cerebral ischemia. Um et al. [32] reported a significant increase for the IL-1α (–889) allele 2 in CI patients compared with controls. In addition, they also reported that the IL-1α (–889) allele 2 carriers increased the relative risk for CI in subjects without the IL-1ra allele 2 [33]. However, the authors found no significant association between IL-1β (+3953) polymorphism and CI [33]. These same risk-enhancing polymorphisms have been previously associated with other inflammatory conditions. IL-1α is markedly overexpressed in the brains of patients with Alzheimer's disease (AD). Several groups have reported that polymorphisms in the promoter regions of IL-1β and IL-1α increase the risk of AD [34–37], but these findings were not confirmed by others [38–41]. This controversy might be in part due to the difference in

ethnic background between populations. Unlike Dutch [42] and Finnish subjects [43], in a population of Korean origin, few cases bore the IL-1α-889 A2/A2 genotype (0.8% instead of 10.0 and 8% found in Dutch and Finnish subject respectively) [44].

In case of ischemic stroke patients, several groups have reported that no significant association was seen for the IL-1β (-511) polymorphism [45–47]. Zee et al. [48] reported that the IL-1α (–889) was not associated with the susceptibility to ischemic stroke. In contrast, Seripa et al. [45] reported that ischemic stroke survivors that carry the IL-1 ra allele 1 showed a strong association with the disease with respect to age-matched controls (odds ratio, OR = 3.905) and healthy Italians (OR = 3.256). Lee et al. [46] also screened the IL-1ra polymorphism and found a significant association with ischemic stroke.

The mechanism by which the IL-1 gene polymorphisms might influence inflammation is probably related to different IL-1 synthesis, secretion and activity. Cerebral ischemia initiates an inflammatory response in the brain and periphery. Increased levels of proinflammatory cytokines in cerebrospinal fluid (CSF) and/or in serum of acute ischemic stroke patients correlate with brain infarct volume and stroke severity and may have a predictive value for stroke outcome. The effect of genetic polymorphisms on cytokine production has been investigated mainly in circulating level. For instance, Dominici et al. [49] reported that the A2A2 genotype significantly increased the transcriptional activity of the IL-1α –889 gene with respect to the A1A1 genotype, and that a slight increase of the IL-1α messenger RNA (mRNA) and protein levels was also observed in the plasma. Also the allele 2 of IL-1ra and IL-1β (+3953) is functionally linked to increased IL-1ra [28, 50] and IL-1β [27] production in plasma, respectively. On the one hand, brain-resident cells localized within the ischemic region rapidly synthesize cytokines, proteins involved in cellular communication. The cytokines become important mediators of endothelial-leukocyte interactions leading to the influx of hematogenous inflammatory cells into the brain ischemic region. According to functional analysis of allele polymorphism, the IL-1α (–889) A2A2 genotype significantly increased the transcriptional activity of the IL-1α gene with respect to the A1A1 genotype in human pancreatic cell line [49]. Therefore, cytokines in the circulating blood as well as cytokines generated locally in the cerebral circulation are important in stroke, and then genetic polymorphisms may be of relevance not only for systemic but also for local cytokine generation.

On the other hand, thus far, attempts to assign allelic functional properties, i.e. to determine the effect of each IL1ra allele on IL-1ra synthesis and release, have often yielded conflicting results. Studies using peripheral blood monocytes have shown allele 2 of IL-1ra to be associated with enhanced IL-1ra release [27, 51]. Meanwhile, studies on epithelial/endothelial cells have either shown allele IL-1ra allele 1 to be more anti-inflammatory or have altogether failed to demonstrate any allelic effect on IL-1ra expression [52, 53]. Some studies suggest that the IL-1β/IL-1ra ratio is critical in determining the severity of the inflammatory reaction. Ishihara et al. [54] reported that the total amount of IL-1 (IL-1α + IL-1β)/IL-1ra ratio was correlated with the severity of periodontitis, but IL-1ra itself was not significant. Therefore, further studies are needed to classify the relationship between IL-1 polymorphisms and their production levels in CI patients.

TNF

TNF is a member of a family of peptide signaling molecules that exert their biological activity by interacting with high-affinity receptors [55]. Human TNFα is synthesized as a membrane-bound polypeptide precursor (26 kDa). It undergoes proteolytic cleavage to yield the soluble form (17 kDa), which forms the active trimer polypeptide. This proinflammatory cytokine is produced upon stimulation by monocytes, macrophages, T and B lymphocytes, neutrophils and mast cells. In addition, ischemic or traumatic brain injury induces synthesis of a TNFα precursor peptide, pro-TNFα, activates proteolytic enzymes that hydrolyze membrane-bound pro-TNFα, and release soluble TNFα into extracellular space.

CI is a multifactorial disease caused by the interactions of several genetic and environmental factors, and is pathologically based on atherosclerosis. Atherosclerosis can be now viewed as an inflammatory process [6, 56–58]. Inflammatory mediators not only can contribute to atheroma formation but may also be involved in the rapid evolution of the atheromatous injury, leading to rupture of the plaque and intraluminal thrombosis [6]. TNFα is a macrophage- and lymphocyte-derived immune mediator that regulates the inflammatory response, modulates growth and cellular differentiation, and activates blood coagulation [59]. In general, increased TNFα plasma levels and activity are also associated with increased production of other interleukins [56]. Previous studies using in situ hybridization techniques showed increased levels of TNFα messenger RNA in the atherosclerotic plaque of symptomatic patients [60]. These data pointed to a local rise in the expression of this inflammatory mediator, which may therefore contribute to arterial thrombosis.

The proinflammatory cytokine TNFβ (or lymphotoxin α, LTα) is also a key mediator in the initiation of a local vascular inflammatory response. Its action is characterized by the stimulation of adhesion molecule production, thrombogenesis, smooth muscle proliferation, platelet activation and release of vasoactive agents [6, 56–58]. Hence, expression of this cytokine may contribute to the initiation and progression of atheromatous plaques.

TNF polymorphisms

The TNFα and the TNFβ genes are both located on the short arm of chromosome 6 between the class I and class II regions of the HLA complex. A striking feature of the entire HLA complex is a high degree of genetic variation. A number of polymorphisms have also been described for the TNF locus. A dimorphism with potential functional relevance is a guanine-to-adenosine transition at base pair –308 in the promoter region (termed the A allele) [61]. The A allele has been shown to be associated with increased TNFα expression after in vitro stimulation [62–64]. Therefore this genetic variation might result in an altered TNFα expression and thereby affect susceptibility and clinical severity of inflammatory diseases. Indeed, the A allele of TNFα –308 is associated with a sevenfold increased risk for cerebral complications of malaria [65] and with a worse prognosis and longer disease duration in dermatitis herpetiformis [66, 67].

A polymorphism in the TNFβ was also reported: an A$\rightarrow$G transition at nucleotide position +252, in the first intron of the gene (termed the G allele). The presence of guanine at this position defines the mutant allele known as TNFβ (5.5 kb), whereas adenine defines the wild-type allele, TNFβ (10.5 kb). The mutant allele results in significantly increased production of TNFβ in in vitro-stimulated mononuclear cells, related to increased gene transcription [68].

Um et al. [69] reported that a significant increase was found for the TNFα A allele in controls compared with CI patients. They showed that the TNFα GG genotype increased the relative risk for CI in subjects with TNFβ AA genotype [70]. According to their results, compared to controls, the frequency of the TNFα AA genotype was decreased, whereas that of TNFβ AA was increased in CI patients. The former implies an association with resistance, while the latter suggests an association with susceptibility to the disease. This result was consistent with those reported by Lee et al. [46]. In addition, several studies reported that the TNFα A allele might reduce the risk of diseases related with proinflammatory cytokines [71, 72]. The mechanism by which the TNFα gene polymorphisms might have a protective role for CI is probably related to a different TNFα synthesis, secretion and activity. TNFα is a cytokine; however, TNF appears to have several functions, making its role difficult to understand. Moreover, not all investigators [73–75] concur regarding what these functions may be, because TNF not only is involved in neuroprotection but also plays a role in cell death. In addition, Wilson et al. [63] reported that the AA genotype significantly increased the transcriptional activity of the TNFα-308 gene with respect to the GG genotype, and that a slight increase of the protein levels was also observed in the plasma. Therefore, G allele carriers are regarded to be low producers of TNFα, whereas A allele carriers are high producers of TNFα. The increased TNFα in A allele carriers might induce neuro-protective effects, particularly in conditions of excitotoxic death, and thereby affect the susceptibility of CI. The TNFβ G allele has been associated with a higher TNFβ response at both the mRNA and the protein levels [76]. However, some studies have found that the TNFβ A allele leads to a higher TNFα secretory capacity than the TNFα G allele [77], and higher plasma TNFα levels [78], whereas others could not confirm this observation [76]. Based on a study by Um et al. [70], TNFβ AA was increased in CI patients, while TNFβ GG did not show a significant difference between CI and controls. Considering these results, the TNFβ A allele may influence high spontaneous and induced TNFα release, and consequently it may confer susceptibility to CI. This indicates that the TNFα and TNFβ polymorphisms could independently affect susceptibility/resistance to CI. However, relevance of the levels of this cytokine to susceptibility/resistance to CI is not clear.

IL-6

IL-6 is a pleiotropic cytokine that plays a pathogenic role in certain acute and chronic cerebral disorders. It is produced by several cell types, including fibroblasts, monocytes, adipocytes and endothelial cells [79]. IL-6 gene expression is also increased in animal models of ischemia/reperfusion brain injury [80]. In humans, IL-6 participates in the acute phase response that follows cerebral ischemia, and there is an association between high plasma levels of IL-6 and early neurological worsening after stroke [81] and progression of lacunar infarction [82].

IL-6 polymorphism

Fishman et al. [83] identified a G/C dimorphism at nucleotide –174 within the promoter of the IL-6 gene on chromosome 7. The more frequent G allele (when compared with C) is associated with higher plasma concentrations of IL-6 among healthy subjects and with enhanced secretion of IL-6 by reporter gene constructs in response to IL-1 and to lipopolysaccharide (LPS) [84]. Revilla et al. [85] reported that the prevalence of CC genotype and the frequency of C allele were statistically significantly higher in patients with lacunar stroke than in asymptomatic controls. Also, Greisenegger et al. [86] evaluated the IL-6 (–174) polymorphism in 214 patients with ischemic stroke or transient ischemic attack and in 214 control subjects. The variant was associated with severe stroke in young patients with acute cerebrovascular events. These results were constant with those reported by several groups [87, 88].

Other inflammatory molecules

In addition to inflammatory cytokines, the level of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) has been also elevated after experimental brain ischemia [89–91]. Clinical studies have reported increased level of adhesion molecules in the peripheral blood and CSF of patients with ischemic stroke [92–94]. Therefore, several groups investigated the association between ischemic stroke and polymorphisms of genes encoding prototypical inflammatory molecules, such as ICAM-1, C-reactive protein (CRP), macrophage migration inhibitory factor (MIF), E-selectin (E-sel), macrophage chemoattractant protein-1 (MCP-1) and matrix metalloproteinase-3 (MMP-3) [87, 88, 95]. Flex et al. [87] reported that MCP-1GG, ICAM-1 EE, E-sel AA, and MMP-3 5A5A genotypes were significantly and independently associated with stroke history. Di Napoli et al. [95] and Pola et al. [88] also reported that CRP and ICAM-1 are markers of increased risk in ischemic stroke.

Cytokine levels

Cytokines, which promote emigration of leukocytes from the vascular lumen into the brain tissue, are produced at the site of incipient CI. Some cytokines are regulators of inflammatory and immune response and are produced by stimulated macrophage and T cells [96, 97]. Early gene expression of inflammatory cytokines has been reported in the brain following global and focal CI [98]. After reperfusion with transient cerebral ischemia, an inflammatory reaction is observed in the ischemic core and penumbra areas. An inflammatory reaction associated with ischemia and reperfusion contributes to the development of brain cell injury resulting from stroke. It has long been recognized that an inflammatory response involves leukocyte activation, chemotaxis, intracellular adhesion and secretion of cytokines including Th1/Th2 cytokines (IFN- γ, IL-2, TNFα, IL-1, IL-4, IL-6, IL-8 and IL-10) and transforming growth factor-β1 (TGF-β1).

IFN-γ has important immunoregulatory roles and enhances both antigen specific and non-specific immune responses through actions on monocytes and macrophages [99, 100]. As previously described, IL-1 has an important role in the development of brain damage following cerebral ischemia [21]. The mechanisms underlying IL-1 actions in stroke have not been clearly elucidated, and it seems likely that its effects are mediated through stimulation and inhibition of a wide range of pathophysiological processes. IL-2 cytokine (also known as T cell growth factor) has multiple immunoregulatory functions and biological properties. IL-2, together with other factors and in conjunction with antigens, mitogens or anti-immunoglobulin antibodies, controls B cell proliferation and differentiation into antibody-producing plasma cells [101]. Natural killer (NK) and lymphokine-activated killer cells, monocytes and macrophages all have the ability to respond to IL-2 with increased activity or proliferation [102, 103]. IL-4, an inflammatory cytokine, is a pleiotropic cytokine derived primarily from Th2 lymphocytes and mast cells. Described originally as a B cell growth factor, IL-4 subsequently has been found to influence activities of diverse cell types, including T lymphocytes, monocytes, endothelial cells and fibroblasts [104–108]. IL-6 is a multifunctional regulator of immune and inflammatory processes that has a range of biologic activities, including important roles in the development of plasma cells and stimulation of the production of acute phase response protein by hepatocytes [109]. IL-6 has also been described in a variety of neuropathologies, including brain injury [110]. IL-8 is a potent activator and chemoattractant of neutrophils and mediates their migration to local inflammation. Brain tissue levels of IL-8 were increased significantly by reperfusion after a transient ischemia [111]. TNFα levels in brain tissue, CSF and plasma have been found to be elevated in several central nervous system (CNS) disorders, including AD, multiple sclerosis, Parkinson's disease, meningococcal meningitis, and human immunodificiency virus (HIV) infection [112]. Although cytokines were identified primarily by their role in the immune response, increasing evidence suggests that these mediators may also play an important role in establishing and maintaining the normal homeostatic environment of the CNS, in addition to functioning as intercellular signals that orchestrate the response of the CNS in injury. After injury to the CNS, these cytokines have been implicated in a wide range of pathologic processes.

The involvement of inflammatory cytokines in ischemic brain injury has been investigated intensively, preclinically and clinically, in recent years. However, although accumulating evidence suggests that inflammatory-mediated damage plays a role in brain ischemia, it remain unclear whether inflammation also intervenes in CI. In clinical studies, Jeong et al. [113] reported that serum IL-2 levels, but not IFN-γ, decreased significantly in the patients with CI compared with controls. In contrast they also reported that a significant increase of TNFα [114], IL-4 [115], IL-6 [115] and immunoglobulin E (IgE) [116] serum levels was observed in the patients with CI (table 1).

Recent studies have investigated the effect of Korean medicines on IL-4 and IL-10 production in humans from lipopolysaccharides (LPS) or phytohaemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs) of CI patients. Seogak Jihwang-Tang and Jeodang-Tang inhibited IL-4 production, and increased IL-10 production [117, 118]. An anti-inflammatory cytokine, IL-10, significantly inhibited the production of other proinflammatory mediators, such as reactive oxygen intermediates, reactive

Table 1. Serum levels of cytokines from CI patients.

Cytokines	Subjects without CI	Subjects with CI	Study
IFN-γ, pg/ml	140.5 ± 80.6	133.6 ± 44.2	Jeong et al. [97]
IL-2, pg/ml	186.9 ± 45.9	135.8 ± 57.5*	Jeong et al. [97]
IL-4, pg/ml	101.7 ± 23.2	364.7 ± 58.8*	Shin et al. [99]
IL-6, pg/ml	68.7 ± 54.6	455.2 ± 158.3*	Shin et al. [99]
TNFα, pg/ml	23.88 ± 5.55	225 ± 10.2*	Jeong et al. [98]
IgE, pg/ml	87.0 ± 61.5	1988.7 ± 1422.7*	Kim et al. [100]

Serum levels of cytokines among the clinical groups were compared using the Mann-Whitney's *u* test; a value of $P < 0.01$ was accepted as statistically significant. Values of cytokines are given in the text as mean ± standard deviation (SD). *$P < 0.01$, significantly different from subjects without CI.

nitrogen intermediates and prostanglandins in monocyte/-macrophages [119]. The presence of an inflammatory response in the pathophysiology of acute brain ischemia is relatively well established, but less is known about the anti-inflammatory mechanisms. Perini et al. [120] reported that stroke patients displayed significantly lowered IL-10 serum levels, and these indicate that anti-inflammatory response is down regulated in acute stroke patients. Spera et al. [121] also reported that IL-10 reduces rat brain injury following focal stroke. A significant increase of IL-10 in PBMCs treated with Korean medicine is implicative. However, further study is necessary to clarify the mechanism of cytokine regulation for the effects of the medicines in patients with CI. Cytokines are likely to act as important signaling molecules directing processes of inflammatory reaction, tissue repair and functional reorganization following CI. Therapeutic agents and strategies are devised to either increase the production of anti-inflammatory cytokines or inhibit the production of inflammatory cytokines. One consequence of such studies should be the development of new medicines as a regulator of cytokines to enhance recovery from CI.

Future directions

An extensive body of literature has been developed over the past 10 years that is commonly interpreted as supporting the hypothesis that inflammatory cytokines play a prominent role in cerebral ischemia. Despite the extensive evidences implicating cytokines in ischemic brain damage, little information is available in the literature concerning the effect of inflammatory cytokines polymorphisms in cerebral ischemia. Therefore, this review focused on the genetic aspects of inflammatory cytokines and their implications in CI.

Several of the reviewed polymorphisms have been found to be functional, that is, to have direct effects on gene transcription and protein function. However, although allele A may clearly be associated with protein function or concentrations, and protein concentrations with disease, it still may not be possible to relate a given genetic variant to disease, as an individual polymorphism contributes only a fraction to the entire heritable variance in protein concentrations [122]. Additionally, the small contribution of a single novel polymorphism to the overall risk of a multifactorial disorder such as CI may be obscured by the presence of one or more dominant classical risk factors [122]. Prospectively designed studies and the analysis of haplotyes may overcome some of the limitations of population association study [123]. Another way to confront these limits is through careful phenotypic characterization of patients, since a new genetic factor is more likely to emerge within homogeneous groups of patients in whom it has a similar role. Identifying such homogeneous groups may be difficult and may require rigorous control not only of age, sex, race and ethnic grouping but also of clinical features and biochemical markers linked to specific pathogenetic mechanisms.

The relation between the inflammatory cytokine gene polymorphisms and CI, which has been the main breakthrough of genetics in this area, remains an attractive but at the same time highly controversial hypothesis. A different role of this trait in different subgroups of CI patients, as well as a different strength of association depending on the interaction with different environmental and other genetic factors, is probable.

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