Genes, inflammation, and age-related diseases

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Chapter 1

General introduction

A dramatic increase in mean and maximal life span, coupled with a significant reduction in early mortality has lead to a large increase in the number of elderly people in modern societies. Progression of age is associated with a reduction of the response to environmental stimuli and, in general, is associated with an increased predisposition to illness and death. The high incidence of death due to infections, cardiovascular disease, and cancer underlies a common feature in these pathologies that is represented by dysregulation of both systemic and innate immunity (1). Ageing is accompanied by a chronic low-grade inflammation state clearly showed by 2-4-fold increase in serum levels of pro-inflammatory mediators (2). This pro-inflammatory state, interacting with the genetic background, potentially triggers the onset of age-related inflammatory diseases like atherosclerosis, dementia, and cancer (1). Genetic epidemiology is an important tool to investigate the association between innate immunity and age-related diseases.

Inflammation and cardiovascular disease

Atherosclerosis, a progressive disease characterized by the accumulation of lipid and fibrous elements in the large arteries, is the most important contributor of cardiovascular disease (3). Advances in medical science have established a fundamental role for inflammation in mediating all stages of atherosclerosis from initiation through progression (4-6). For example, macrophages and blood leucocytes, mediators of host defenses and inflammation, are found in the earliest lesions of atherosclerosis. Macrophage-derived foam cells and leucocytes drive lesion progression by secreting inflammatory stimuli (7).

Inflammatory stimuli, like the pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor-alpha (TNFα), are implicated in the development of atherosclerosis (6;8). IL-6 stimulates endothelial activation, vascular smooth muscle cell proliferation and leucocyte recruitment, all of which lead to plaque growth or instability. Major effects of TNFα on the cardiovascular system include increased expression of adhesion molecules, release of endothelial cytokines and nitric oxide, and enhanced vascular permeability. Another pro-inflammatory cytokine is lymphotoxin-alpha (LTA), also known as tumor necrosis factor beta (TNFβ), which activates a cytokine cascade

by inducing IL-1 (9;10). LTA is expressed in atherosclerotic lesions and induces the expression of a number of molecules involved in atherogenesis (11;12). Moreover, atherosclerotic lesions in LTA knock-out mice are significantly smaller compared to LTA wild-type mice (12).

Besides these pro-inflammatory cytokines, anti-inflammatory cytokines like IL-10 may also play a role in the development of atherosclerosis (13). In IL-10 knockout mice, the absence of IL-10 leads to a marked increase in susceptibility of atherosclerosis (14). Furthermore, after occlusion of the middle cerebral artery, brain infarcts in IL-10 knockout mice are 30% larger compared to wild-type mice (15). However, the contribution of IL-10 to the modulation of the atherosclerotic process in humans remains largely to be elucidated.

Inflammation and cognitive function

Inflammation plays also an important role in the development of cognitive decline and dementia in old age (16). There is abundant evidence that inflammatory mechanisms contribute to cognitive impairment via cytokine-mediated interactions (16). Animal models expressing high levels of pro-inflammatory cytokines in the brain suffer from neurodegeneration (17). Furthermore, up-regulation of pro-inflammatory cytokines in tissue cultures leads to microglial activation and neuronal damage (18) and moreover, several markers of inflammation have been found in and around senile plaques in the brain (19).

The interleukin-1 signaling pathway is likely to have a prominent role in the development of cognitive decline and dementia (20-23). For example, in rodents peripheral administration of interleukin-1beta (IL-1β) induces various cognitive-behavioral effects (20). Furthermore, expression of IL-1β is increased in patients with Alzheimer's disease (22). One of the possible mechanisms by which IL-1β acts on cognitive function is by binding to IL-1 type-1 receptors which are abundantly expressed in the hippocampus (21), the area of the brain that has a critical role in memory and learning.

It has been shown that patients with sporadic Alzheimer's disease have lower IL-10 serum levels compared to healthy controls (24;25). Various studies have also investigated the association between genetic variation in the IL-10 gene and Alzheimer's disease (24;26-30). The majority of the studies found that the prevalences of variant alleles of IL-10 promoter polymorphisms, determinants of low IL-10 production capacity, were increased in patients with Alzheimer's disease compared to healthy controls (24;26;27;30). This indicates that besides pro-inflammatory processes also anti-inflammatory processes play a role in cognitive function and dementia in old age.

Inflammation and cancer

Cancer is now generally accepted as an age-related disease. In fact, incidence and mortality rates of most cancers increase consistently with age up to 90 years. Inflammatory responses are also thought to be critical in many aspects of promoting the growth and spread of cancers (31). Various studies support the hypothesis that inflammatory stimuli, like the pro-inflammatory cytokines IL-6, IL-1β, and TNFα, are involved in cancer pathogenesis (32-35). Moreover, elevated levels of various cytokines, like IL-1, IL-6, TNFα, fibroblast growth factor (FGF), and transforming growth factor (TGF) have been found in blood, urine, and ascites of cancer patients, suggesting that these cytokines are involved in incidence and growth and spread of cancer (36).

A recent study showed that cell lines of Lewis lung carcinoma had an increased production of the pro-inflammatory cytokines IL-6 and TNFα through activation of the Toll-like receptor (TLR) family members TLR2 and TLR6 (37). Moreover, pro-inflammatory cytokines are also involved in promoting tumor cell adhesion in metastatic sites which then activate local normal cells to produce tumor growth factors (36). Distant-site metastases are the leading cause of cancer-associated mortality. Furthermore, animal studies have suggested a role for pro-inflammatory cytokines in the generation of cancer-associated cachexia, which is the most important cause of morbidity among cancer patients (33;38-40).

Genetic risk factors

Various studies have reported only moderate associations between inflammatory markers and cognitive decline (20;23;41). Therefore, systemic markers are unlikely to be useful as risk predictors for cognitive decline (42). On the contrary, genetic variation in inflammatory genes is more likely to be a good marker for risk prediction, especially since associations between genetic variation and cognitive function are assumed to be unconfounded (43). Moreover, uncertainty exists whether levels of cytokines are risk factors for cognitive decline or whether they are a consequence of cognitive decline. Functional polymorphisms determine the level of cytokine plasma levels, therefore genetic variation can be used as useful marker to overcome this problem of reverse causality.

The innate production capacity of inflammatory markers has been shown to be under tight genetic control. An extended twin study found that over 50% of the variance in production capacity of cytokines is explained by genetic factors (44). IL-1 receptor antagonist (IL-1Ra) and TNFα had the lowest heritability (53%), IL-6 and IL-10 had a heritability of 57 and 62% respectively, whereas IL-1β had the highest heritability (86%). The gene encoding for interleukin-1β-converting enzyme (ICE) is likely to be one of the main genes influencing IL-1β. ICE mediates the cleavage of the inactive precursor of IL-1β into the biologically active form (45). Inhibition of ICE decreases the age-related increase in IL-1β levels (45). Genetic variation in the ICE gene is likely to be functional since patients with the 5352AA genotype in the ICE gene have an increased risk of developing restenosis after percutaneous coronary intervention, a process where inflammation plays a major role (46). Genotypic variation in the IL-10 gene is associated with significantly lower IL-10 responsiveness upon stimulation with bacterial lipopolysaccharide (LPS) (47). Since genetic variation in the promoter region of the IL-10 gene influences the production levels of IL-10, this variation can also be related to cardiovascular disease and/or cognitive function.

Other studies have also shown that genetic variation in genes involved in inflammatory processes are associated with vascular disease, like myocardial infarction (MI) and stroke (11;48-52). For

example, reports have appeared on the association between the LTA C804A polymorphism and the severity of atherosclerosis in patients with coronary artery disease (11). This indicates that genetic variation in genes involved in inflammation can lead to an increased risk for cardiovascular disease.

Epigenetics and ageing

While the relationship between inflammation and ageing has been widely investigated, other possible mechanisms influencing the progress of ageing and age-related diseases are not well understood. One of these mechanisms is epigenetics, the study of heritable changes in gene expression that occurs without a change in the sequence of DNA (53-56). The best known epigenetic modifications are DNA methylation and post-transcriptional histone modifications, including methylation, acetylation, ubiquitylation and phosphorylation. An increased DNA methylation will lead to less gene expression, whereas increased histone acetylation will lead to more gene expression. Recently it has been shown that aged organisms have modified epigenetic features. The best understood epigenetic modification in relation to age-related diseases is that of DNA methylation in relation with cancer (53). In cancer cells it is well established that DNA methylation patterns are abnormal. More specifically, a global state of hypomethylation is observed, along with a hypermethylation state in many gene promoters. In various malignancies an increased promoter methylation of the adhesion molecule E-cadherin, an important tumor suppressor, is observed. As a result, the hypermethylation state leads to the loss of expression of E-cadherin resulting in a predisposition to develop cancer.

Moreover, epigenetic processes modulate gene expression patterns and have profound effects on the cellular repertoire of expressed genes. Therefore, epigenetic regulators involved in histone acetylating and deacetylating activities can play an important role in extracellular matrix formation, inflammation, and proliferation, processes involved in cardiovascular pathologies such as atherosclerosis and restenosis (57;58). Another argument that epigenetics could play a role in the pathology of cardiovascular disease comes from the Dutch Hunger Winter Study. Subjects who were prenatally exposed to a period of famine during World War II had 5.2% less DNA methylation

of the IGF2 gene compared to their unexposed sibling (59). Furthermore, subjects exposed to famine during gestation had a higher cumulative incidence of coronary artery disease and an earlier onset of coronary heart disease compared to unexposed subjects (60). These two findings together indicate that changes in epigenetic information can play a role in the pathology of complex diseases like cardiovascular disease. Hence, epigenetics can help to explain the relationship between an individual's genetic background, the environment, ageing, and disease. Therefore, active investigations continue into potential functional relationships between epigenetic changes and disease pathology of age-related diseases.

Mendelian Randomization

One of the aims of observational research is to identify causes of disease. However, observational research has had several high-profile failures when risk factors of disease that were identified in observational studies were later shown to be non-causal in randomised controlled trials. Reasons for such failures include confounding or disturbance by reverse causality. Genetic epidemiology can contribute to establishing the causal nature of environmentally modifiable risk factors, through the application of Mendelian randomization approaches (43;61;62). Or in other words, Mendelian randomization provides an alternative way of dealing with the problem of causal inference that is inherent to observational studies (63).

This new genetic epidemiological tool is based on Mendel's law that inheritance of one trait is independent of inheritance of other traits. This means that the association between a genetically determined phenotypic trait and a disease is not susceptible to reverse causality or confounding, provided that the presence of the genotype that causes the trait does not influence the subject's lifestyle or environment. This condition will usually be fulfilled as long as subjects are unaware of their genotype.

There are three key assumptions for a genetic variant to be met before they can be used in Mendelian randomization (63). First, the genetic variant is associated with the exposure of interest. Second, the genetic variant is unrelated to all confounding factors and third, there is no effect of the

genetic variant on the outcome or any other mediated effect other than through the exposure of interest. These assumptions are graphically illustrated in figure 1.

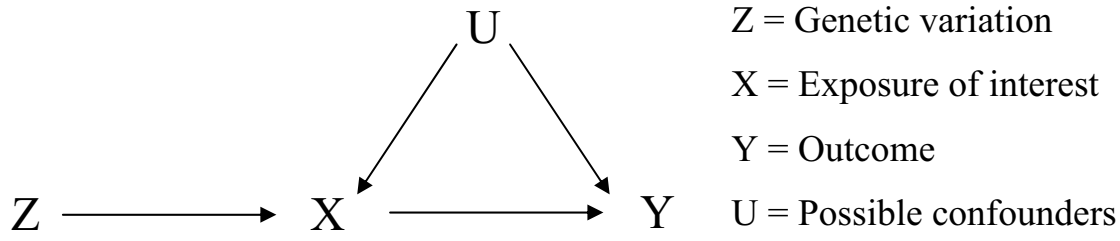

Figure 1: Graphical representation of the Mendelian randomization concept.

In 1986 Katan was the first to propose to investigate the causality of cholesterol in the relation with cancer by making use of genetic variation in the Apolipoprotein E (ApoE) gene (64). Observational studies had shown a relationship between low plasma cholesterol levels and an increased risk for cancer. However, randomized clinical trials with cholesterol lowering medications have not shown an increased risk for cancer (65). The results of the observational studies could be the result of confounding or reverse causality since cholesterol levels could also be lowered by the presence of latent tumours. The ApoE gene is known to affect plasma cholesterol levels, with rising cholesterol levels from isoform E2 to E3 to E4. Katan's idea was that individuals carrying the ApoE2 will naturally have lower plasma cholesterol levels from birth and that if naturally low cholesterol favours tumour growth, carriers of the ApoE2+ genotype should have an increased risk of cancer (64). This proposal constituted a prime example of what would later be named Mendelian randomization (66;67).

General objective

The general objective of this thesis was to investigate associations between variants in genes involved in inflammation and epigenetics and age-related diseased at old age to get more insights in the patho-physiological mechanisms involved in age-related diseases like cardiovascular disease,

dementia, and cancer. For all analyses we used data of the participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).

The aim of PROSPER was to determine whether therapy with pravastatin, a cholesterol-lowering medication, would have a beneficial effect on cardiovascular disease risk in an elderly population. The PROSPER study was a multicenter, double-blind, placebo-controlled trial of pravastatin against placebo in 70-82 year old men and women with either pre-existing vascular disease or at elevated risk of such disease due to smoking, hypertension or diabetes. Between December 1997 and May 1999, subjects were screened and enrolled in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). A total number of 5804 subjects were randomly assigned to pravastatin or placebo. The trial concluded that pravastatin given for three years reduces the risk of coronary heart disease in the elderly. However, no beneficial effect of pravastatin was found on occurrence of stroke and cognitive performance. Therefore, cholesterol-lowering treatment with pravastatin is also recommended in the elderly for preventing coronary heart disease.

Aim of this thesis

First we present five studies investigating the association between genetic variation in inflammation related genes and age-related diseases like cardiovascular disease, cognitive decline, and cancer. In chapter 2 and 3 we assessed the relation between genetic variation in pro- and anti-inflammatory genes and cardiovascular disease. We first assessed the association between LTA and the risk of clinical stroke (chapter 2) and then the association between four promoter polymorphisms in the anti-inflammatory IL-10 gene and cardiovascular disease (chapter 3). In chapter 4 and 5 the results of the association between genetic variation in inflammatory genes and cognitive function are shown. We investigated the association between genetic variation in the ICE gene and cognitive function in chapter 4 and the relation between genetic variation in the anti-inflammatory IL-10 gene and cognitive function in chapter 5. In chapter 6 we describe the relation between pro-inflammatory cytokines, measured by systemic levels and innate production capacity, and cancer incidence and cancer death.

Second, we describe two studies investigating the relation between genetic variation in genes involved in epigenetics and age-related diseases. In chapter 7, we investigated the relation of the CREB gene, a histone acetyltransferase (HAT) involved in epigenetic control, with cognitive function in old age. Chapter 8 describes a study investigating the relation between the PCAF gene, a transcriptional co-activator with intrinsic HAT-activity, and cardiovascular outcomes in three different study populations, the PROSPER study, the WOSCOPS study and the GENDER study.

Third, we introduce an innovative approach to investigate the relation between a phenotype and outcome independent of confounding and reverse causality by using genetic variation, which is called Mendelian randomization. In chapter 9 we investigated whether low cholesterol is a risk factor for cancer by using the ApoE genotype, a typical example of a Mendelian randomization study. Finally, the main conclusions are summarized and discussed in chapter 10.

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