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Cardiovascular Effects of Short-term Exposure to Air Pollution

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Exploring potential pathways and susceptible subgroups

Petter Ljungman

Karolinska Institutet

From
the Department of Clinical Science and Education, Södersjukhuset
Karolinska Institutet, Stockholm, Sweden

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To my Elsa, Stella and Lisa

Nothing shocks me, I’m a scientist!

Harrison Ford as Indiana Jones

ABSTRACT

Cardiovascular disease is the most common cause of mortality globally, with a number of contributing risk factors. Ambient air pollution represents a universally present risk factor with a substantial preventive potential. Although the relationship between exposure to air pollution and all-cause mortality, or even cardiovascular mortality, has been relatively well established, the details of how, when, who and even how much remain unclear.

This thesis focuses on understanding associations between short-term exposure to air pollution and cardiovascular disease, with special attention to ischemic heart disease and ventricular arrhythmias.

In the first paper we conducted a case-crossover study with 660 first-time myocardial infarctions in Stockholm, interviewed shortly after diagnosis to determine time of disease onset. Air pollution levels 2 and 24 hours preceding the time of onset were compared in the same individual with 3-4 equally long control periods without myocardial infarction matched by time of day and day of week within the same calendar month. No associations were found between air pollution levels and myocardial infarction in the main analyses or in subgroup analyses.

Atherosclerosis is an inflammatory disease leading to a number of cardiac disorders including myocardial infarction. Low-grade inflammation, measured by slight increases in blood markers such as interleukin-6 (IL-6), has been associated with poorer prognosis for heart disease and mortality. In a multi-center study involving 6 European cities and 1003 myocardial infarction survivors we conducted repeated (5.8 in average) blood sampling for inflammatory markers including IL-6, and genotyped for single nucleotide polymorphisms (SNPs) in inflammatory genes. In the second paper results are presented for associations between interleukin-6 (*IL6*) genotypes and mean IL-6 plasma levels using mixed model analyses with random intercepts. Four SNPs showed associations with IL-6 levels with eg 6.3% (95% confidence interval 1.7-11.2%) increased IL-6 per minor allele of an *IL6* SNP labeled rs1800795. In the third paper we analyzed the effect modification of selected SNPs in the *IL6* gene and the fibrinogen alpha- (*FGA*) and beta-chain (*FGB*) genes on the IL-6 response to preceding air pollution levels. We found associations demonstrating gene-environment interaction effects for an *IL6* SNP and a *FGB* SNP. Strongest effects were found when patients with the minor alleles of *FGB* rs1800790 were exposed to carbon monoxide in the 6-11 hour exposure-window prior to sampling. In homozygotes for this allele a 10% (4.6-16%) increased mean IL-6 was observed per 0.64 mg/m^3 increase of pollutant.

In the fourth paper we studied the relationship between air pollution levels 2 and 24 hours preceding ventricular arrhythmias in 211 patients with implantable cardioverter defibrillators in Stockholm and Gothenburg using a similar methodology as in Paper I. We found associations for ventricular arrhythmias and 2 hour exposure to particulate pollution indicating an odds ratio of 1.31 (1.00-1.72) per 13.2 $\mu g/m^3$ PM_{10}. Interaction analyses showed strongest effects for events occurring closer to the monitoring station, outdoors and in Gothenburg.

Conclusions: Air pollution exposure was not associated with the onset of myocardial infarction in our sample. Four strongly correlated polymorphisms in the *IL6* gene were associated with plasma IL-6 levels implicating that variants in the *IL6* gene affect the inflammatory status of myocardial infarction survivors and potentially their future prognosis. The air pollution effect on IL-6 levels was modified by variations in the *IL6* and *FGB* genes. This suggests that if inflammation is a pathway for the air pollution effect on heart disease, these genetic variations may lead to some people being more susceptible to air pollution than others. Particulate air pollution was associated with ventricular arrhythmias already after 2 hours of exposure giving further support to the hypotheses that air pollution may contribute to dysfunctional autonomic regulation of the electrical conduction of the heart and be an important alternative explanation for the cardiovascular mortality seen in association with air pollution.

LIST OF PUBLICATIONS

I. Berglind N, **Ljungman P**, Möller J, Hallqvist J, Nyberg N, Rosenqvist M, Pershagen G, Bellander T. Air Pollution Exposure - A Trigger for Myocardial Infarction? *Submitted*.

II. **Ljungman P**, Bellander T, Nyberg F, Lampa E, Jacquemin B, Kolz M, Lanki T, Mitropoulos J, Müller M, Picciotto S, Pistelli R, Rückerl R, Koenig W, Peters A. DNA variants, plasma levels and variability of Interleukin-6 in myocardial infarction survivors: Results from the AIRGENE study. *Thrombosis Research ISSN 0049-3848 (Print) Available online. doi:10.1016/j.thromres.2008.10.009*

III. **Ljungman P**, Bellander T, Schneider A, Breitner S, Forastiere F, Hampel R, Illig T, Jacquemin B, Katsouyanni K, von Klot S, Koenig W, Lanki T, Nyberg F, Pekkanen J, Pistelli R, Pitsavos C, Rosenqvist M, Sunyer J, Peters A. Modification of the Interleukin-6 Response to Air Pollution by Interleukin-6 and Fibrinogen Polymorphisms. *Environmental Health Perspectives. Accepted for publication.*

IV. **Ljungman P**, Berglind N, Holmgren C, Gadler F, Edvardsson N, Pershagen G, Rosenqvist M, Sjögren B, Bellander T. Rapid Effects of Air Pollution on Ventricular Arrhythmias. *European Heart Journal 29(23):2894-2901*.

CONTENTS

LIST OF ABBREVIATIONS

AIRGENE	Air Pollution and Inflammatory Response in Myocardial Infarction Survivors study
ALVA	Air Pollution and Life-threatening Ventricular Arrhythmias study
APHEA2	Air Pollution and Health: A European Approach 2 study
BMI	body mass index
C	cytosine
CI	confidence interval
CO	carbon monoxide
CRP	C-reactive protein
DNA	deoxyribonucleic acid
FGA	fibrinogen alpha chain gene
FGB	fibrinogen beta chain gene
G	guanine
H4	interleukin-6 gene haplotype number 4 in the AIRGENE study
HNO_3	nitric acid
HRV	heart rate variability
ICAM-1	intercellular adhesion molecule 1
ICD	implantable cardioverter defibrillator
IL-6	interleukin-6 protein
IL6	interleukin-6 gene
IQR	interquartile range
LD	linkage disequilibrium
MI	myocardial infarction
NMMAPS	National Morbidity and Mortality of Air Pollution study
NDIR	non-dispersive infra-red detector
NO	nitrogen oxide
NO_2	nitrogen dioxide
NOx	nitrous oxides
SO_2	sulphur dioxide
O_3	ozone
OR	odds ratio
RNA	ribonucleic acid
PM	particle matter
$PM_{0.1}$	PM with aerodynamic diameter <=0.1 µm, “ultrafine particles”
$PM_{2.5}$	PM with aerodynamic diameter <= 2.5 µm, “fine particles”
$PM_{2.5-10}$	PM with aerodynamic diameter 2.5-10 µm, “coarse particles”
PM_{10}	PM with aerodynamic diameter <=10 µm
PNC	particle number concentration, ultra-fine particles
SHEEP	Stockholm Heart Epidemiology Program
SNP	single nucleotide polymorphism
TEOM	tapered element oscillating microbalance
USA	United States of America
US	United States
VCAM-1	vascular cell adhesion molecule 1
vWF	von Willebrand factor

1 INTRODUCTION

Heart disease has for decades been a leading killer. Globally, 17.5 million people died of cardiovascular disease in 2005 (7.6 million in coronary heart disease, 5.7 million in stroke), representing 30% of all deaths [1]. Incidence continues to increase in the developing world with increased longevity, urbanization and lifestyle changes [1]. In Sweden the age-standardized incidence for coronary heart disease in 2006 was estimated to 661 per 100,000 inhabitants [2]. This corresponds to 17,660 deaths in coronary heart disease during 2006 compared to for example 6,772 deaths for gastrointestinal cancer, the most common group of tumor-related deaths [3]. Cardiovascular disease has a multi-factorial aetiology and although it comprises many different diseases, many share the same risk factors. Well-established risk factors include gender, age, heredity, smoking, blood lipids, low physical activity, obesity, diabetes and high blood-pressure [4]. Some risk factors are modifiable, such as smoking and blood lipids while others are not (age, gender, heredity).

Air pollution has increasingly been recognized as a risk factor for cardiovascular disease during the last decade and has been afforded place in some of today's textbooks in cardiology [5]. As a risk factor, air pollution needs to be considered in a different light than traditional risk factors (Figure 1). Traditional risk factors, although in some cases demonstrating large relative risk increases, exert their influence on a limited proportion of the population. Air pollution is a continuous exposure affecting the whole population albeit at different levels depending on geographical location and variation in pollution levels over time (temporal variation). Coupled with the high incidence and mortality of cardiovascular disease this means that even small relative risk increases incurred by air pollution can have a large public health impact since these increases affect a very large population at risk.

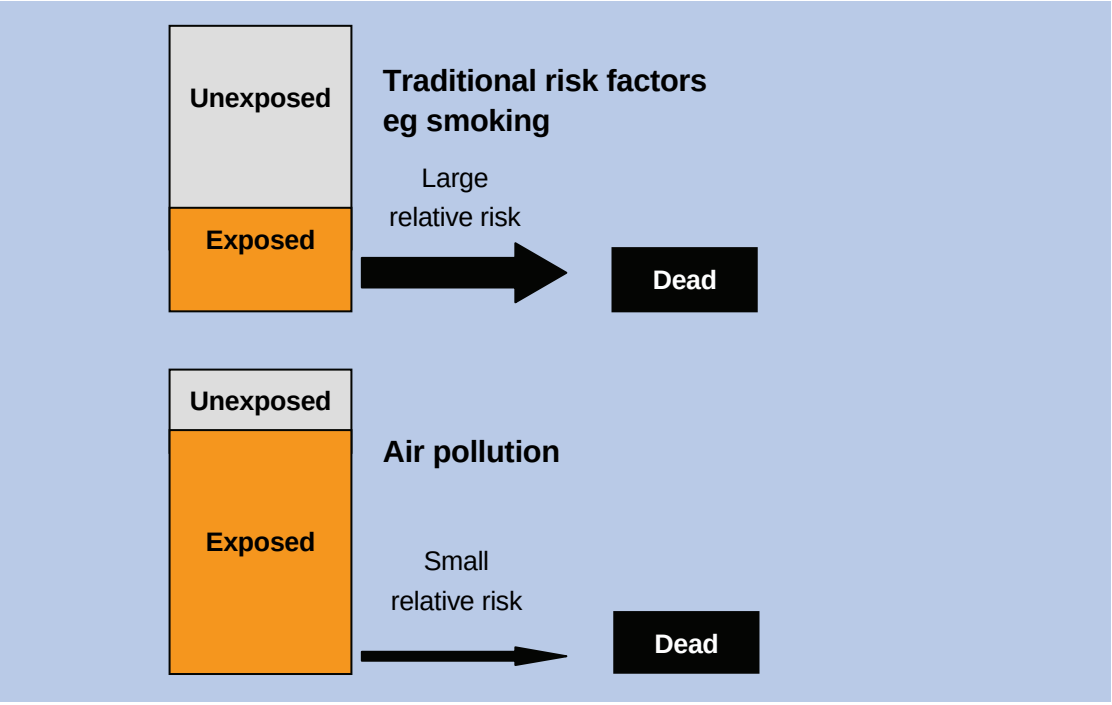


Figure 1. Relative versus absolute risk

Health effects of air pollution exposure became clearly visible early in the last century when mortality and morbidity increased sharply in conjunction with episodes of extremely high levels of air pollution over a period of days or weeks eg during the Meuse Valley Fog in Belgium 1930 [6]. But it wasn't until The London Fog episode of December 1952 which exposed the Greater London Area to extremely high pollutions levels during four days that detailed analyses of morbidity and mortality could be made of the 3000-4000 immediate deaths which in the successive months increased to around 12,000 deaths [7,8]. It became apparent that although the relative increase in morbidity and mortality for heart disease was lower than for pulmonary diseases the absolute number of deaths or hospital admissions attributable to cardiovascular disease presented a larger public health impact.

1.1 CHARACTERISTICS OF AIR POLLUTION

Air pollution contains gases as well as liquid and solid particles suspended in air. At any given moment air pollution contains different mixtures of gases and particles depending on geography, emission sources, wind speed and direction, temperature, ultra-violet radiation levels, and relative humidity. Since pollutants are correlated with each other due to similar sources and dispersion patterns, health effects seen when investigating one pollutant maybe attributable to another pollutant or possibly a conjunction of pollutants.

1.1.1 Gases

Carbon monoxide (CO), sulphur dioxide (SO_2) nitric oxide (NO) and to a lesser extent nitrogen dioxide (NO_2) are primary pollutants, directly emitted from fuel combustion. The proportion of SO_2 in air has in many places decreased dramatically since the extraction of sulphur from oil and the decreased use of coal. Nitric oxide has also been reduced since the introduction of cleaning techniques in power plants and catalytic converters in petrol cars. Secondary gas pollutants include ozone (O_3) and NO_2. Ozone is formed by NO_2 and volatile organic compounds (Figure 2), from combustion as well as natural sources, in the presence of sunlight and hot weather, whereas NO_2 is formed by oxidation of NO (thus leading to a consumption of O_3 locally). Dispersions of CO, SO_2, and nitrogen oxide species (NO, NO_2 and others, abbreviated NOx) remain within a vicinity of their emissions whereas O_3 is more broadly spread. Nitrogen dioxide and SO_2 can also form particles by further oxidation into nitric acid (HNO_3) and sulphuric acid respectively.

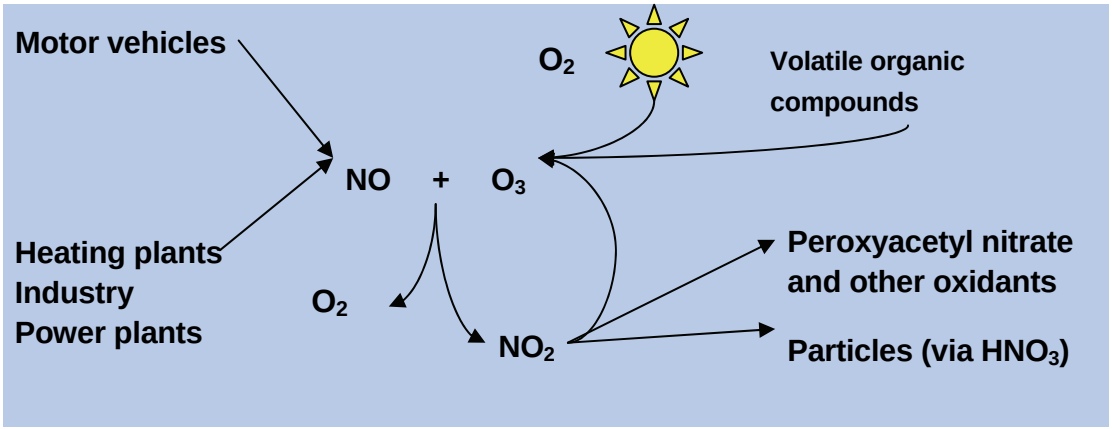


Figure 2. Relationship between primary and secondary nitrogen oxides

1.1.2 Particles

Particles represent a heterogeneous group of pollutants with varying sources, modes of dispersion, and final deposition in the human body. Classification according to diameter size provides information concerning these differences. Ambient particulate matter equal to or less than 10 micrometers in diameter (PM_{10}) comprises all particles commonly considered in health effects research (Figure 3).

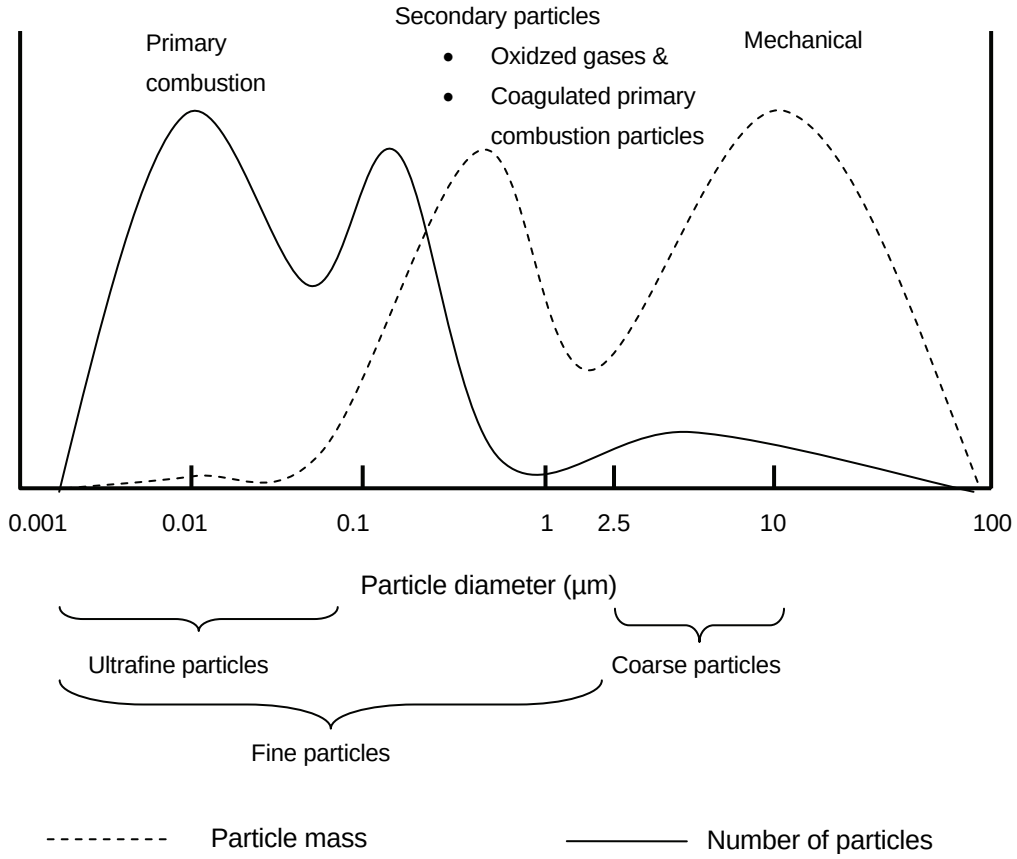


Figure 3. Conceptual illustration of trimodal distributions by number and mass of anthropogenic particles suspended in air. Larger particles have more mass but are much fewer in number per cubic meter of measured air and ultra-fine particles have a negligible contribution to mass concentrations but constitute a large proportion of the number of particles. Specific proportions vary across time and space.

Particles with the largest diameter, commonly called the coarse fraction or $PM_{2.5\text{-}10}$ are mainly derived from crustal materials such as salts, soil, organic or inorganic fragments in dust or from for example road and vehicle wear. These particles are generally dispersed in a limited geographic area and deposited in the upper bronchial tree of the lungs.

Fine particles ($PM_{2.5}$) are predominantly emitted from combustion processes of fossil fuels and have a high carbon content. Inorganic particles are formed by oxidation of NO_2 and SO_2. The larger particles of this fraction can be transported in air for several hundreds of kilometres. $PM_{2.5}$ are deposited in the outer reaches of the bronchial tree.

Ultra-fine particles ($PM_{0.1}$), often measured using particle number concentration (PNC), are also emitted from combustion engines and especially diesel engines. These particles are dispersed in a limited geographical area but over time will coalesce together into larger particles (thus become categorized as $PM_{2.5}$). Ultra-fine particles will penetrate deep into lungs reaching the alveoli and possibly crossing over into the bloodstream. They have the greatest surface area per mass unit of the different particles and are considered to be the most reactive species. All particle fractions have varying degrees of chemical compounds including metals, inorganic ions, biological fragments including endotoxins and spores, organic and elemental carbon, nitrates, sulphates and chlorides.

1.1.3 Ambient versus personal exposures

In ascertaining air pollution exposure, personal exposure measurements in the study subject's breathing zone area offers the most accuracy. There are several limitations, however, such as high cost, required compliance in wearing the instruments continuously, selection bias of subjects both willing and physically able to participate and incur certain restrictions to activities, lack of accurate portable instruments, and other logistical challenges. The relative risks of health effects for air pollution are small, requiring a very large sampling population, in practice making personal exposure studies unsuitable except in attempts to validate ambient exposure measurement. Instead strategically situated fixed air pollution monitors with continuous ambient measurements are generally used. This use relies on the assumption that changes in measured ambient levels (rather than absolute levels) correspond well to changes in personal exposure within a given area. This assumption appears to be most valid for ambient $PM_{2.5}$ measurements [9] and can depend on the location of the study.

1.2 HEALTH EFFECTS OF AIR POLLUTION

1.2.1 Life-expectancy and mortality after long-term exposure

The mortality effect of air pollution is largely attributable to cardiovascular disease [10]. Cardiovascular mortality[10-20] and morbidity [21-24] effects of long-term exposure to air pollution have been shown in several studies both for particulates and gaseous pollutants. Exposure to relatively low concentrations of air pollution as in Europe has been calculated to reduce life-expectancy by little more than one year [25] using studies on long-term exposure. By comparison hypertension reduces life expectancy by 2.7 years when comparing severe hypertensive (15% of sample) to normotensive subjects in a Finnish population [26] and obesity (body mass index [BMI] $\geq$ 30 kg/m^2) reduces life-expectancy by 0.8 years in a US population [27], both well-known cardiovascular risk factors. In Sweden particulate anthropogenic air pollution transported over long distances have been estimated to be responsible for 3,500 deaths annually (corresponding to reduction of life-expectancy by up to 7 months) and air pollution from local sources 1800 annually (2-3 months reduced life-expectancy) [28,29]. A recently published study found that the reduction in air pollution levels in the USA was associated with an increased life-expectancy and that approximately 15% of the overall increase in life-expectancy was attributable to air pollution decreases [30].

1.2.2 Mortality after short-term exposure

More than 100 published studies of short-term exposure to air pollution have reported results on mortality in cities from all inhabited continents [31]. Many of these have studied associations in single cities and some have been incorporated in meta-analyses [32,33] which give a better understanding, and possibility to adjust, for the variability in effect estimates. Multi-city studies with the advantage of less selection and publication bias have also been performed. In the National Morbidity and Mortality of Air Pollution Study (NMMAPS), conducted in the largest 88 US cities, exposure to a 10 μg/m³ increase of ambient PM_{10} on the preceding day was associated with a 0.31% (posterior standard error 0.09) increased combined cardiovascular and respiratory mortality [34]. In the Air Pollution and Health: A European Approach 2 (APHEA2) study, in 29 European cities comprising 49 million inhabitants, a 10 μg/m³ increase of ambient PM_{10} on the same and preceding day increased cardiovascular mortality by 0.76% (95% confidence interval [CI] 0.47-1.05%) [35]. A recently published study [36] conducted in cities in Europe (n=22), USA (n=90) and Canada (n=12) all-cause mortality increased by 0.29-0.84% per 10 μg/m³ increase of ambient PM_{10} on the preceding day. Largest risks were seen in Canada in the elderly population (1.00% 95% CI 0.25-1.80%).

1.2.3 Potential pathways

Several pathways, possibly interdependent, have been proposed on the basis of results from animal and human experiments as well as observational studies (Figure 4).

1.2.3.1 Inflammation

The progression and prognosis of atherosclerosis and ischemic heart disease has been associated with low to medium grade inflammation [37-39]. Consequently inflammation has been a hypothesized effect of air pollution exposure.

Experimental animal model studies mimicking long-term exposure have demonstrated vascular inflammation, altered vascular tone and increased atherosclerosis in ApoE-/- mice [40]; myocardial inflammation and fibrosis in rats [41]; and progression of atherosclerosis in rabbits [42]. Short-term exposure to particulate air pollution has shown in canines increased neutrophils, lymphocytes and macrophages in bronchoalveolar lavage and peripheral blood [43] as well as exacerbated myocardial ischemia [44]; in rats decreased coronary flow and depressed myocardial contractile response [45] in hearts infused with ultra-fine particles; in hamsters increased neutrophils and histamine in bronchoalveolar lavage and rapid platelet activation in peripheral blood [46,47]; and in ApoE-/- mice endothelial dysfunction [48].

Experimental studies on humans using exposure chambers have shown increased inflammatory activity in airways [49,50], increased markers of inflammation in peripheral blood [49,51,52] and endothelial dysfunction [52-54] after air pollution exposure. Van Eeden et al performed a series of both animal and human exposure studies demonstrating both effects of long-term and short-term exposure to particulate air pollution on pulmonary and systemic inflammation, bone marrow stimulation and the progression of and destabilization of atherosclerotic plaques [55].

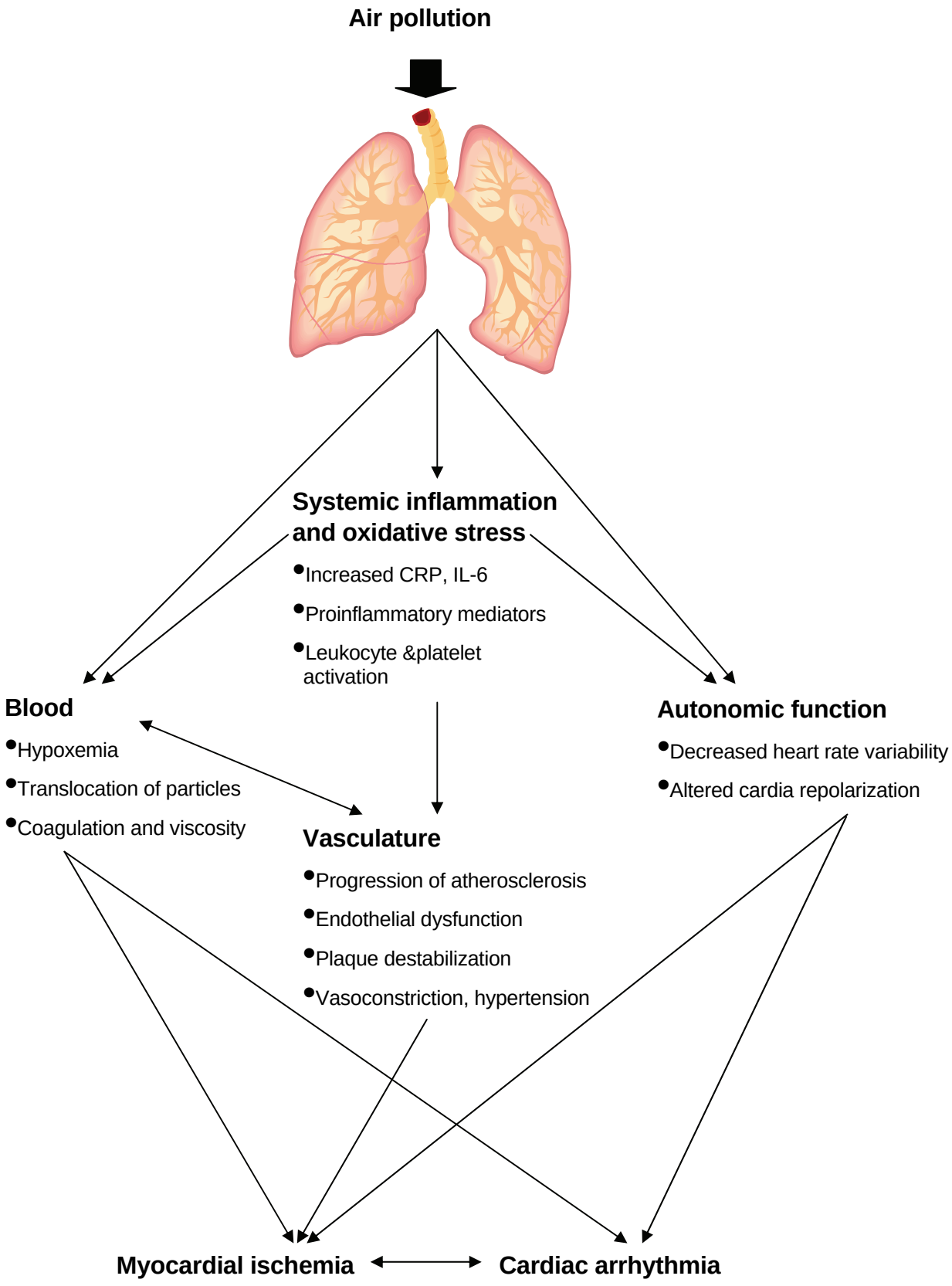


Figure 4. Potential pathways of cardiac health effects of air pollution exposure. Adapted from Pope and Dockery, J Air & Waste Management Association 2006.

In observational studies, long-term exposure to particulate matter has been associated with an increased carotid intima media thickness [56], increased levels of fibrinogen, platelets and white blood cells [57], and chronic pulmonary inflammation [58]. Short-term exposure to air pollution has been associated with increased levels of C-reactive protein (CRP) [59-62] , interleukin-6 (IL-6) [60,63] , fibrinogen [63,64], plasma viscosity [65], soluble intercellular adhesion molecule 1 (ICAM-1) [66], vascular cell adhesion molecule 1 (VCAM-1) [66], von Willebrand factor (vWF) [66], markers of oxidative stress [67], and subclinical pulmonary inflammation measured by fractional concentrations of exhaled nitric oxide [68,69]. Altered vascular tone mediated through endothelial dysfunction has also been associated with particulate air pollution exposure in an observational study [70].

Since inflammation plays a role in ischemic heart disease [71] characterized by angina pectoris, myocardial infarction and in some cases congestive heart failure or hypertension, associations with these clinical endpoints could support inflammation as a pathway for the air pollution effect. Long-term exposure to air pollution has been associated with incidence [21-24] and mortality in myocardial infarction [10,17,22-24]. ST-segment depressions have been associated with diesel exhaust in a controlled human exposure study [72] and with ambient particulates in observational studies [73-75]. Hospitalizations [76-95] and mortality [35,86,96-100] due to ischemic heart disease and heart failure have also been associated with air pollution. Air pollution has also been associated with increased blood pressure in a controlled experiment [101] and in an observational study [102].

1.2.3.2 Autonomic dysfunction

In addition to the evidence presented for inflammation as a pathway of air pollution effect, several studies have shown arrhythmogenic associations with air pollution, suggesting a possible direct effect on autonomic regulation of the heart possibly via reflexes invoked in the airways [103], by direct action [45,104] of translocated particles on the myocardium or via inflammation [105]. Heart rate variability (HRV) is a well-defined measure of cardiac autonomic function and studies have shown that decreases in HRV are strong predictors of mortality [106,107]. Many air pollution studies examining HRV have demonstrated associations indicating increased heart rate and decreased HRV both in controlled experiments [108] and in observational studies [109-112]. Disturbances in cardiac repolarization [113], increased atrial fibrillation [114], ventricular tachyarrhythmias [115-117] and out-of-hospital cardiac arrest [118] have all been associated with short-term exposure to air pollution.

1.2.3.3 Other pathways- Translocation and hypoxemia

Some experimental studies have indicated the possibility for ultrafine particles to cross over from the alveoli into the bloodstream [119-121], with possible cardiotoxic and vascular effects [45,104], although others have not been able to reproduce these results [122-124]. Air pollution has also been hypothesized to cause hypoxemia via lung damage but studies exploring this have shown mixed results [125-127].

1.2.4 Susceptible subgroups

The observation of differing effects of air pollution in various subpopulations can be helpful in understanding both the mechanisms of action and public health impact. The concept of susceptibility implies a modification of the air pollution effect and can cast a wide net of possible variables including individual medical characteristics, socio-economic status, geography, meteorology, housing characteristics or activity-based factors. I have chosen to focus on individual characteristics with a conceivable impact on health status.

1.2.4.1 Elderly

Elderly populations, often defined as older than 65 years of age, have been studied with and without a parallel comparison to younger populations. Associations with long-term exposure to air pollution in panels of elderly have been seen for all-cause non-traumatic mortality [18,128], cardiovascular mortality [17,18], cardiovascular morbidity [17,128], and cerebrovascular events [17]. Stronger associations with increased age were seen for myocardial infarction mortality [21] and carotid intima media thickness [56], although a higher relative risk for myocardial infarction mortality in younger subjects was seen in one long-term study [129]. Short-term exposure has also been associated with all-cause non-traumatic mortality [130] in an elderly sample and increased relative risks in older compared to younger age groups were seen for myocardial infarction mortality [86], and out-of-hospital coronary death [100]. Pertinent to a possible inflammatory pathway effect, short-term exposure to air pollution have been associated with increased levels of inflammatory markers [60,61,68,69], ST-segment depression [74,75], hospitalization for cardiovascular disease [78,131,132], ischemic heart disease [78], myocardial infarction [85,92], and congestive heart failure [78,133] in elderly samples. Comparing age-groups within samples, older subjects have shown greater relative risks for hospitalization for cardiovascular disease [76,79,80], ischemic heart disease [76,79,80,82], myocardial infarction [79,86,91], and congestive heart failure [79]. Associations between air pollution and autonomic dysfunction as a function of decreased HRV [108,110-112] or supraventricular arrhythmias [134] have been observed in elderly sample populations and finally, in support of hypoxemic effect, decreased oxygen saturation [125-127] has also been demonstrated in this group. Measures of particle matter, primarily $PM_{2.5}$, have overwhelmingly shown the most consistent results for all these associations.

These associations in elderly seen for many potential pathways could be a function of several factors. Elderly are more likely to have had longer exposures and several co morbidities that could conceivably interact to make them more susceptible to a low grade risk factor such as air pollution compared to younger individuals. Older populations have different social patterns including more time spent indoors and less physical activity that could affect their exposure to air pollution (in which case, however; we would expect to see weaker associations in studies using fixed site monitors that are better suited to reflect exposure outdoors). Finally the elderly have by virtue of age an increased baseline risk for disease and death, particularly in degenerative processes such as cardiovascular disease. In analogy with Figure 1 this indicates a higher absolute risk for each relative risk increase so even at relative risks similar to those of younger individuals elderly may contribute to a larger public health impact [135].

1.2.4.2 Women

The impact of air pollution on cardiovascular effects by gender has been explored to some degree with different results for different endpoints.

In studies of long-term exposure, the largest study restricted to women, the Women's Health initiative [17], comprised ca 66 000 post-menopausal women demonstrating associations with cerebrovascular and cardiovascular morbidity as well as a remarkably high (76%) increase of cardiovascular mortality with a 10 μg/m³ $PM_{2.5}$. A German cohort [15] including ca 5000 women 50-59 years old showed similar effect estimates of cardiopulmonary mortality for an interquartile range (IQR, a unit reflecting the difference between the air pollution level at the 25th percentile and the level at the 75th percentile) increase in NO_2 (57% increased risk) or PM_{10} (34% increased risk). Larger estimates were seen when using 50m distance to major roads as a proxy for air pollution exposure. In Künzli et al's study of carotid intima media thickening [56], a measure of atherosclerosis, a stronger association was seen for women compared to men, and in particular for older women. This possibly points to a post-menopausal effect although specific hormonal status was not determined.

Short-term exposure studies have shown more mixed results. In younger individuals (20-33 years old) a study using personal monitors showed stronger associations for women than men in measures of oxidative stress in students [67], whereas ambient concentrations of SO_2 and CO were more strongly association to increased plasma viscosity in men than women in a random population-based sample of younger individuals (25-64 years old) [65]. Furthermore one study [82] saw stronger associations for men compared to women between short-term exposure to CO and NO_2 and hospitalization for ischemic heart disease.

Based on the limited number of studies addressing the impact of air pollution on women compared to men, inconclusive evidence suggests that air pollution may have a greater effect on older, perhaps post-menopausal, women compared to men.

1.2.4.3 Diabetics

Some studies indicate that diabetics have an increased risk for cardiovascular mortality similar to a post-myocardial infarction patient [136]. This subgroup has not as of yet been greatly studied with regard to air pollution exposure. Finkelstein et al [14] demonstrated the highest mortality rate-advancements for diabetes compared to patients with chronic ischemic heart disease or chronic obstructive pulmonary disease in conjunction with living near a major road. In studies investigating inflammatory response in association with short-term air pollution exposure [60,66,131,137], diabetics have shown susceptibility. Type II diabetics have also showed stronger associations to vascular dysfunction in conjunction with air pollution exposure [70]. In the largest short-term exposure study investigating diabetics [77] hospitalization for dysrrhythmias, peripheral arterial disease and cerebrovascular disease was greater for patients with diabetes exposed to air pollution than for non-diabetics.

1.2.4.4 Pre-existing cardiovascular disease

In studies of long-term exposure to air pollution and mortality, associations have been seen in patients with pre-existing ischemic heart disease [14,128] or heart failure [138]. Interestingly, exposure to air pollution seems to predispose individuals more to fatal than non-fatal myocardial infarction both for long-term exposure [22-24] and short-term exposure [94].

Furthermore, in studies of short-term exposure to air pollution, patients with prior ischemic heart disease have shown increased inflammation [62,63], ST-depression [73,74] and myocardial infarction [83,90,91,93,94].

Patients with implantable cardioverter defibrillators (ICDs) due to previous ventricular arrhythmias, heart failure and ischemic heart disease, have shown an increased risk for new ventricular arrhythmias in association with short-term air pollution exposure [115-117,139]. Hospitalization for cardiovascular [132] or ischemic heart disease [81,91] in conjunction with air pollution exposure was also increased in individuals with prior arrhythmic diagnoses.

1.2.4.5 Genetic subgroups

In the wake of the growing evidence implicating the inflammatory process in atherosclerotic disease, hypotheses have been generated that genetic variation in inflammatory genes might modify the extent of inflammation and thereby affect the risk of future cardiovascular disease. The most common form of variation in genes is known as a single nucleotide polymorphism (SNP) and involves an exchange in one building block or nucleotide at a specific position within the genetic sequence (Figure 5). Since each individual has two strands or alleles of deoxyribonucleic acid (DNA) an individual may have one copy of each of the possible nucleotides (heterozygote) or two copies of either one (homozygote). Although many differences in DNA code will not affect the function of the DNA, some may lead to alterations in protein synthesis and thereby involve physiological changes. SNPs located in the promoter region may affect the rate of transcription of DNA into ribonucleic acid (RNA) and final translation into protein. SNPs in exons might affect the final translation into different amino acids (the building blocks of proteins). The functional effects of SNPs located in introns are difficult to ascribe but may have unforeseen regulatory effects. Each gene codes for a specific protein and only one DNA strand is read or transcribed at a time. Therefore combinations of SNPs along one strand, known as haplotypes, may better reflect functional genetic variation. Haplotypes have been mapped in the human genome and are inherited with different frequencies in larger combinations known as haplotype blocks.

Polymorphisms of the interleukin-6 gene (*IL6*) coding for one of the key cytokines involved in the inflammatory response, IL-6, have been studied in attempt to determine whether variations in the gene are associated with plasma levels [140-153] and/or clinical outcomes [140-143,150-152,154-166]. Most attention has been on the SNP located in the promoter region of the gene (rs1800795 or -174 G/C) [144-151,153,158,159,161-163,165] but the results are inconclusive. Building on the evidence for an inflammatory effect of air pollution, several potential gene-environment interactions have been studied in relation

to lung disease and air pollution [167] whereas very few have been studied for cardiovascular disease. Air pollution associations with HRV have been shown to be enhanced in subjects with genetic variations coupled with oxidative stress repair [168-170]. No previous studies have examined the interaction of genetic variants of inflammatory genes and air pollution exposure on the inflammatory response.

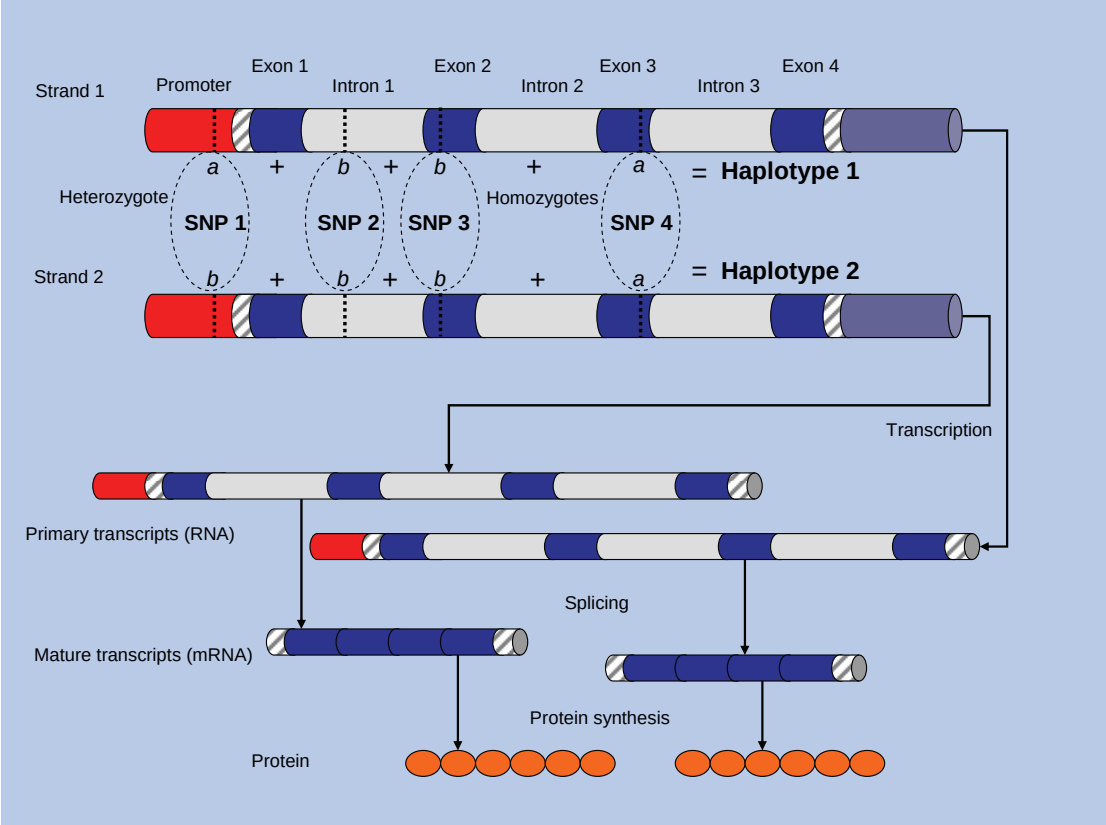


Figure 5. Conceptual illustration of genetic polymorphisms

1.2.4.6 Other pre-disposing factors

Exposure to air pollution has been associated to progression [171] and exacerbations [172] of chronic obstructive lung disease. Chronic obstructive lung disease is also a risk factor for cardiovascular disease [173,174] and this has been observed in some studies [77,85,100] investigating the short-term exposure associations with air pollution. Individuals using statin [66,105] or betablocker [73] medication have shown decreased cardiovascular associations although without randomization to therapy the associations observed are prone to bias [56].

1.3 RATIONALE FOR RESEARCH FOCUS

Although studies investigating the effects of long-term exposure to air pollution can better address the impact on public health since the dimension of time to death is included, disadvantages include high expense and difficulty of controlling for confounders such as smoking, socio-economic status and other patient characteristics. Studies investigating effects of short-term exposure to air pollution are more feasible and can easier explore populations at particular risk or specific outcomes which in turn can give insight into the mechanisms of effect.

One theory for the cardiovascular mortality seen in association air pollution exposure is the induction of the major cardiovascular killer myocardial infarction. Although many time-series studies have seen that daily counts of hospital admissions for ischemic heart disease increase as air pollution levels are increased, few studies with mixed results have specifically studied myocardial infarction and been able to adjust for other cardiovascular risk factors. Several studies have proposed inflammation as a possible pathway of effect, but most focus has been on CRP and fibrinogen, with little investigation on a central player in the inflammatory cascade, IL-6. Mixed results and very few studies using repeated measurements have been presented for the effect of *IL6* polymorphisms on plasma levels of IL-6. In addition, little is known about how genetic polymorphisms of the *IL6* gene or other inflammatory genes can affect an inflammatory response to air pollution exposure. Finally, several studies have explored air pollution effects on markers of cardiac autonomic dysfunction although few have directly studied life-threatening ventricular arrhythmias. Studies of ventricular arrhythmias have presented differing results and used slightly different exposure classifications.

2 AIMS

2.1 GENERAL AIMS

To explore effects of short-term exposure to air pollution on cardiovascular disease focusing on inflammation and autonomic dysfunction as potential pathways and ischemic heart disease, genetic subgroups and arrhythmic disease as susceptible subgroups.

2.2 SPECIFIC AIMS

I. Does very short-term exposure to moderate air pollution levels trigger myocardial infarction in individuals without prior infarction?

II. Which single nucleotide polymorphisms (SNPs) of *IL6* are associated with higher IL-6 plasma levels in myocardial infarction survivors?

III. Do variations in inflammatory genes affect the IL-6 response to air pollution?

IV. Does air pollution trigger ventricular arrhythmias in patients with pre-existing heart disease and during which time-course?

3 MATERIALS AND METHODS

The papers contributing to this thesis are based on three study projects: 1. The Swedish Onset study, a case-crossover study nested in a large population based case-control study of causes of myocardial infarction — the Stockholm Heart Epidemiology Program (SHEEP); 2. The Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group (AIRGENE) study, a longitudinal panel study; and 3. The Air Pollution and Life-threatening Ventricular Arrhythmias (ALVA) study, a case-crossover study of high-risk patients.

3.1 THE SWEDISH ONSET STUDY

Paper I made use of the Swedish Onset Study and included first-time myocardial infarction cases, recruited from April 1993 to December 1994 from all 10 emergency hospitals in Stockholm County. The study base included all Swedish citizens aged 45-70 years who had not had a previous myocardial infarction and were living in Stockholm County. Cases were included at the time of disease incidence and were interviewed during their hospital stay or shortly afterwards concerning detailed information on all episodes of pain (clock time, type, duration, etc.) and other symptoms and circumstances during the 4 days before the myocardial infarction to determine the precise time of disease onset. The interviewers were also instructed to distinguish between premonitory symptoms of disease onset and symptoms of ordinary angina pectoris. Comprehensive information on common cardiovascular disease risk factors was available from the postal questionnaire of the SHEEP Study that was delivered after the Onset interview. The response rate of the questionnaire was 91 percent among cases already interviewed in the Onset study. Out of the 1,489 cases in the study area during the study period, 699 patients were interviewed for the case-crossover study. After exclusion of patients with unreliable information on time of onset or with a high percentage of missing or clearly inaccurate answers, 660 cases remained for analysis.

3.2 THE AIRGENE STUDY

Papers II & III are based on results from the AIRGENE study, a multi-center longitudinal study of myocardial infarction survivors in six European cities in 2003-2004. A total of 1003 subjects in Athens, Greece; Augsburg, Germany; Barcelona, Spain; Helsinki, Finland; Rome, Italy; and Stockholm, Sweden between 35-80 years of age who had experienced a myocardial infarction (MI) between 4 months and 6 years before start of the study were recruited through population based MI registries or from administrative databases of hospital admissions. Patients with MI or interventional procedures <3 months before the beginning of the study or with chronic recurring inflammatory diseases such as Crohn's disease were excluded. Patients were invited to participate in 6-8 clinical visits for interview and blood sampling scheduled every 4-6 weeks on the same weekday and at the same time of the day to minimize the impact of weekly and circadian variation in biological processes and air pollution levels. Patients were characterized with respect to their cardiovascular risk profile at baseline and at each follow-up visit a short questionnaire was administered regarding smoking behaviour, time of last meal and a seven-day recall of medication intake. Participants

were genotyped for 114 SNPs in 13 inflammatory genes and at each visit measurements of plasma IL-6, high-sensitive CRP, and fibrinogen were obtained. The average number of visits per patient was 5.8 resulting in 5,813 plasma samples. Previous results have been published for the outcome variables CRP and fibrinogen. Papers II & III have focussed on IL-6 as a measure of inflammation.

3.3 THE ALVA STUDY

Paper IV includes patients recruited during 2001-2006 at Sahlgrenska University Hospital, Gothenburg; Karolinska University Hospital, Stockholm; and Stockholm South General Hospital, Stockholm who previously received implantable cardioverter defibrillators (ICDs) or were implanted with ICDs during the course of the study period. The patients were asked to contact the clinic within three days after sensing an arrhythmia being treated by their ICD. Information from the ICD concerning time and date of arrhythmia, type of arrhythmia, therapy administered and any change in programming was downloaded and documented. All intracardiac electrograms were reviewed by an electrophysiologist and only ventricular tachycardias and ventricular fibrillations were accepted. Patients were interviewed concerning symptoms and possible triggering activities for both the 2-hour and 24-hour period preceding the ICD discharge as well as the location indoors or outdoors and address at time of event in order to calculate the distance from the monitor. A total of 211 patients were enrolled, 99 from Gothenburg and 112 from Stockholm, experiencing all together 114 ventricular arrhythmias.

3.4 AIR POLLUTION EXPOSURE ASSESSMENT

For Papers I, III and IV air pollution data were collected retrospectively from fixed roof-top monitors in each city using hourly mean levels aggregated to create pre-defined exposure-windows of interest. The monitors are maintained by local authorities and placed in order to give representative exposure metrics of urban background levels. For Paper I we collected data from measurements of PM_{10}, NO_2, CO, and O_3; for Paper III PM_{10}, $PM_{2.5}$, PNC, CO, and NO_2; and for Paper IV PM_{10}, $PM_{2.5}$ (for Stockholm only), and NO_2. PM_{10} and $PM_{2.5}$ measurements were collected using a tapered element oscillating microbalance (TEOM). PNC was measured using particle condensation counters TSI model 3022. CO levels were obtained using a non-dispersive infra-red (NDIR) detector to measure the compound's absorption of infra-red light. Nitrogen dioxide was measured using a chemical luminescence method. O_3 was measured from rurally fixed site monitors reflecting regional background levels based on O_3 absorption of ultraviolet light. Meteorological variables such as air temperature, pressure and relative humidity were obtained from the same monitoring stations and used for confounding control.

3.5 STATISTICAL METHODS

In Papers I and IV we made use of a case-crossover design [175] where a time period immediately preceding the time of the event is considered the case period and other similar time periods when no events occurred are considered control periods for that subject. In this manner, each case serves as its own control and covariates that are constant within a subject during the studied time period are adjusted for by design. The

association is then analyzed using a conditional logistic regression model. Time-stratified control period selection was performed by matching control periods to the case period on time of day, day of week, calendar month, and year within each subject, resulting in three or four control periods for each case period [176].

In Paper II we analyzed the repeated measurements of plasma IL-6 using a random intercept model. We allowed for the different genotype groups to have their own between- and within- individual variability estimating separate variance components for each genotype. Best guess haplotypes were constructed using the available *IL6* SNPs and analyzed using the most common haplotype as the reference. Haplotype blocks were defined as regions with high linkage disequilibrium (LD) according to Lewontin's D'>0.8 between consecutive SNPs. Haplotype estimation was performed using the expectation-maximum algorithm [177] with SNPs entered in the gene-specific reading direction. Haplotype probabilities were estimated for each city separately to avoid bias resulting from population stratification.

In Paper III city-specific analyses were conducted using additive mixed models adjusting for patient characteristics, time trend and weather to assess the impact of air pollutants on plasma IL-6. City-specific estimates were pooled using meta-analysis methodology. We selected for gene-environment analyses three *IL6* SNPs showing associations with IL-6 in Paper II. Associations have been seen between IL-6 levels and fibrinogen levels [178] and previous analyses in our study population [179] have shown evidence of modification of the effect of fibrinogen polymorphisms on fibrinogen levels by levels of IL-6. Therefore one SNP each from the fibrinogen alpha (*FGA*) and beta chain gene (*FGB*), which in previous analyses of this sample were associated with fibrinogen levels [179], were also selected.

Confidence intervals (95%) were computed to quantify random error.

4 RESULTS

4.1 PAPER I: AIR POLLUTION AND MYOCARDIAL INFARCTION

All of the 660 studied first-time myocardial infarctions were non-fatal, the vast majority of cases males and 46 percent current smokers. Monitoring of PM_{10} did not begin until March 1994 and was thus only available for approximately half of the study period (corresponding to 342 cases). The most highly correlated pollutants were CO and NO_2 (r=0.74 for 24-hour means)

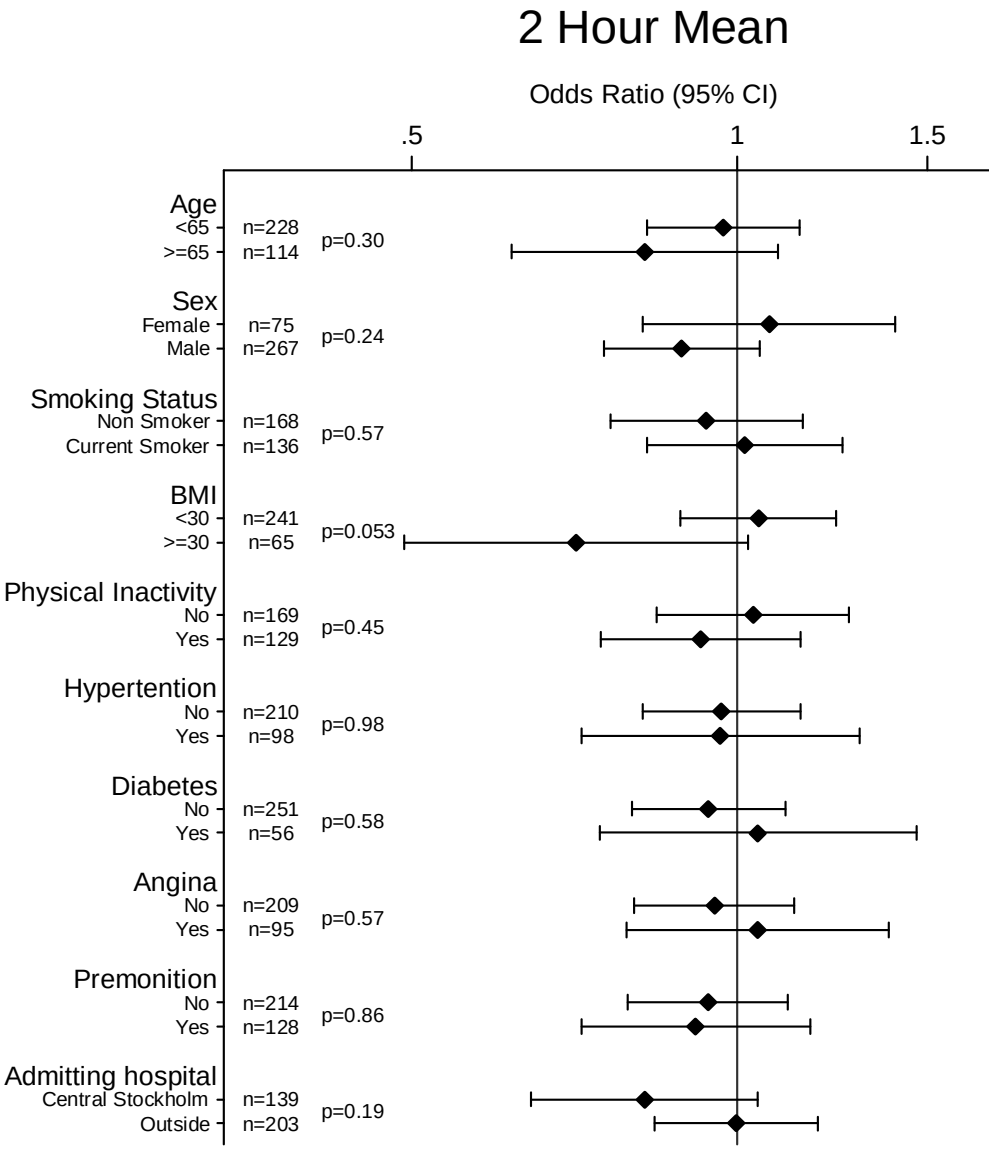


Figure 6. Odds ratios of myocardial infarction for an interquartile range increase in 2 h moving averages of PM_{10} in different subgroups (P-values for interaction). Number of observations for each subgroup with complete data on analyzed covariate and air pollution indicated by n

No associations were observed between the risk for onset of myocardial infarction and 2-hour air pollution exposure with estimated odds ratios (OR) for an IQR increase ranging from 0.93 (95% CI 0.81-1.08) for PM_{10} to 1.02 (95% CI 0.85-1.23) for

O_3. Furthermore, no associations were seen for any of the pollutants using 24-hour averages and no evidence of susceptible subgroups was found (Figure 6).

4.2 PAPER II: *IL6* POLYMORPHISMS AND PLASMA IL-6

Of the total 1003 participants, 919 with 5349 observations were successfully genotyped for all *IL6* SNPs and had complete data on potential confounder variables. Due to successful imputation, 946 individuals with 5520 observations had complete haplotype and confounder variable data. The overall mean IL-6 level in the total population was 2.31 pg/ml (standard deviation 1.68), ranging from 1.99 in Stockholm to 2.83 in Barcelona.

4.2.1 Genotype influence on IL-6 levels

After adjusting for possible confounders, using mixed modelling analyses assuming an additive genetic model we found significant associations between genotype and mean plasma IL-6 levels in each of 4 different SNPs separately (rs1800795, rs2069832, rs1554606, rs2069845) (Figure 7). For these SNPs the minor allele was associated with a higher individual IL-6 level. SNPs rs1800795 and rs2069832 were in strong linkage disequilibrium (LD) (r^2 0.99) as were rs1554606 and rs2069845 (r^2 0.99) and between the 2 pairs the LD was still strong (r^2 0.88-0.89). The minor alleles of a fifth *IL6* variant, the SNP rs2069840, showed a non-significant negative association with individual IL-6 level. This SNP was not in strong LD with the other four SNPs (r^2 0.2-0.4).

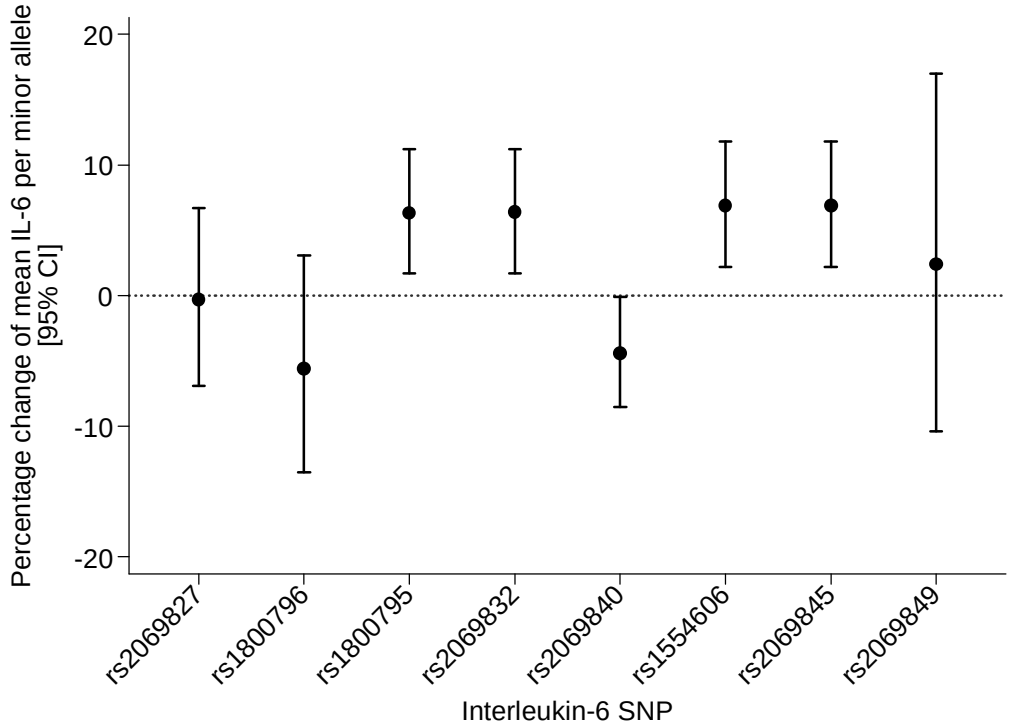


Figure 7. Association of interleukin-6 gene SNPs with mean concentrations of plasma IL-6 comparing heterozygotes to the common homozygote after adjustment for confounders

4.2.2 Genotype influence on IL-6 variability

The group of subjects with genotypes associated with higher IL-6 levels demonstrated higher variability on the log scale between individuals. A weaker inverse pattern was seen for within individual variability over time, where subjects with genotypes associated with higher mean IL-6 levels showed less or similar within individual variability compared to other genotypes. Overall variability was estimated using the model with the same variables as the fully adjusted analysis model but excluding the genetic variables. In comparison to this, only between-individual and not within-individual variability determines overall variability when including genetic variables.

4.2.3 Haplotype influence on IL-6 levels

We identified 5 haplotypes with a frequency of at least 5%. Three major haplotypes were observed, with overall frequencies ranging from 19% to 31%. The haplotype incorporating in essence all earlier associations seen in single SNP analyses (H4) was associated with 9% (95% CI 3.5-14.8) higher IL-6 values compared to the reference (the most common haplotype).

4.2.4 Haplotype influence on IL-6 variability

Variability of IL-6 between individuals with at least one copy of H4 was higher than in individuals without any H4 haplotype. This is in concordance with the SNP results. Variability within individuals with H4 revealed less clear patterns with a slight tendency towards higher variability in IL-6 over time in individuals having 2 copies of H4.

4.3 PAPER III: AIR POLLUTION, GENES AND IL-6 RESPONSE

The consecutive 24-hour mean levels of CO ranged from 0.29 mg/m^3 in Stockholm to 1.48 mg/m^3 in Athens. The city-specific interquartile range varied from 0.08 in Helsinki to 0.73 in Athens.

Less than 5 % of the subjects were homozygotes for the minor allele of *FGB* rs1800790 whereas 17 % were homozygotes for minor allele of *IL6* rs2069832. Genotype frequencies differed between the cities for all SNPs (χ^2-test, $p<0.03$) displaying a north-south gradient with the minor alleles of *IL6* rs2069832 and *IL6* rs2069845 having higher frequencies in the northern cities whereas the minor alleles of *IL6* rs2069840 had higher frequencies in the southern cities. The fibrinogen SNPs did not show such a pattern.

The minor allele of the fibrinogen alpha chain SNP *FGA* rs2070011 showed non-significant association to increased plasma levels of IL-6 whereas *FGB* rs1800790 polymorphism did not show any clear association to plasma IL-6.

4.3.1 Air pollution and IL-6 response.

We saw weak associations between air pollutants and IL-6 levels after 24 hours of exposure ranging from 0.6% to 1.7% increase in mean IL-6 ($PM_{2.5}$ and NO_2 respectively) per interquartile range increase of pollutant, statistically significant only for NO_2. Slightly stronger associations were seen in the second 6-hour exposure-

window (6-11 hours) preceding the blood sampling for CO, 2.2% (95% CI 0.3-4.2%), and PNC, 1.8% (95% CI 0.2-3.5%), and 12-17 hours of exposure for PNC 2.6% (95% CI 0.8-4.5%).

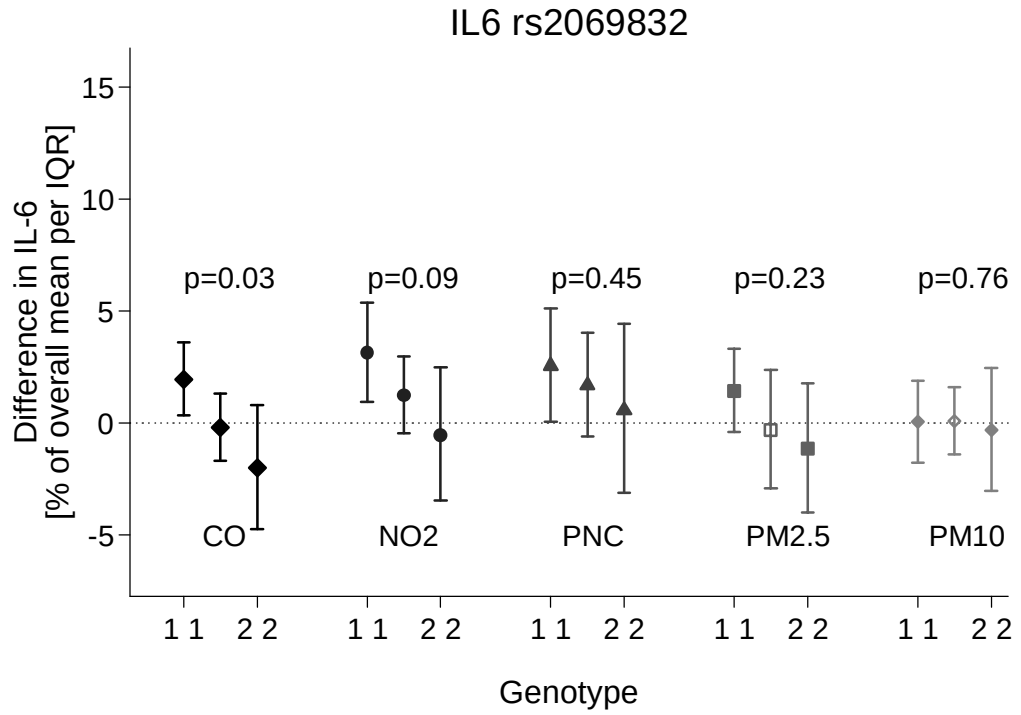


Figure 8. Modification of plasma IL-6 response to 24-hour air pollution exposure by *IL6* rs2069832. Hollow symbols indicate heterogeneity of city-specific estimates with a p-value <0.1, estimates derived by random effects analyses.

4.3.2 Genotype modification of IL-6 response

4.3.2.1 24-hour exposure-windows

Patterns of effect modification of the IL-6 response to air pollution were seen for all three *IL6* polymorphisms in relation to CO but only for *IL6* rs2069832 was a similar pattern seen for NO_2. Although less clear interaction effects, the pattern of modification is also seen for PNC and $PM_{2.5}$ (Figure 8). Major allelic genotypes of *IL6* SNPs showed stronger responses than minor allelic genotypes.

Compared to the *IL6* SNPs, subjects with the homozygote minor allele genotype of *FGB* rs1800790 showed both a larger and clearer effect modification for the IL-6 response to increased CO (Figure 9). The overall effect of a 0.8% increase in IL-6 per 0.34 mg/m^3 increase of CO in the preceding 0-24 hours seemed to be confined to individuals carrying the minor allele of *FGB* rs1800790. In the 4% of the study sample that were homozygous with this allele, the corresponding CO effect on IL-6 was a 4.5% increase. Similar magnitudes of effect modification were seen for $PM_{2.5,}$ PM_{10} and NO_2, but the effect modification pattern was not statistically significant for PM_{10} and NO_2. The *FGA* SNP did not modify the response to air pollution.

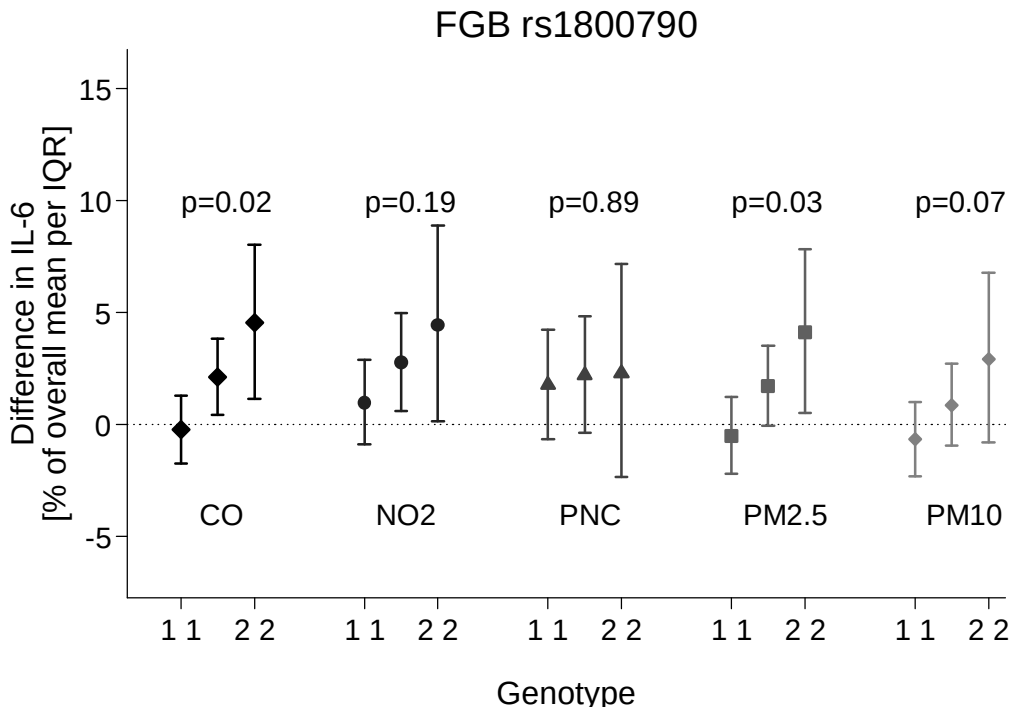


Figure 9. Modification of plasma IL-6 response to 24-hour air pollution exposure by *FGB* rs1800790.

4.3.2.2 6-hour exposure-windows

Analyses of 6-hour exposure-windows during the 24 hours immediately preceding blood sampling showed the clearest effect modification of the IL-6 response to CO

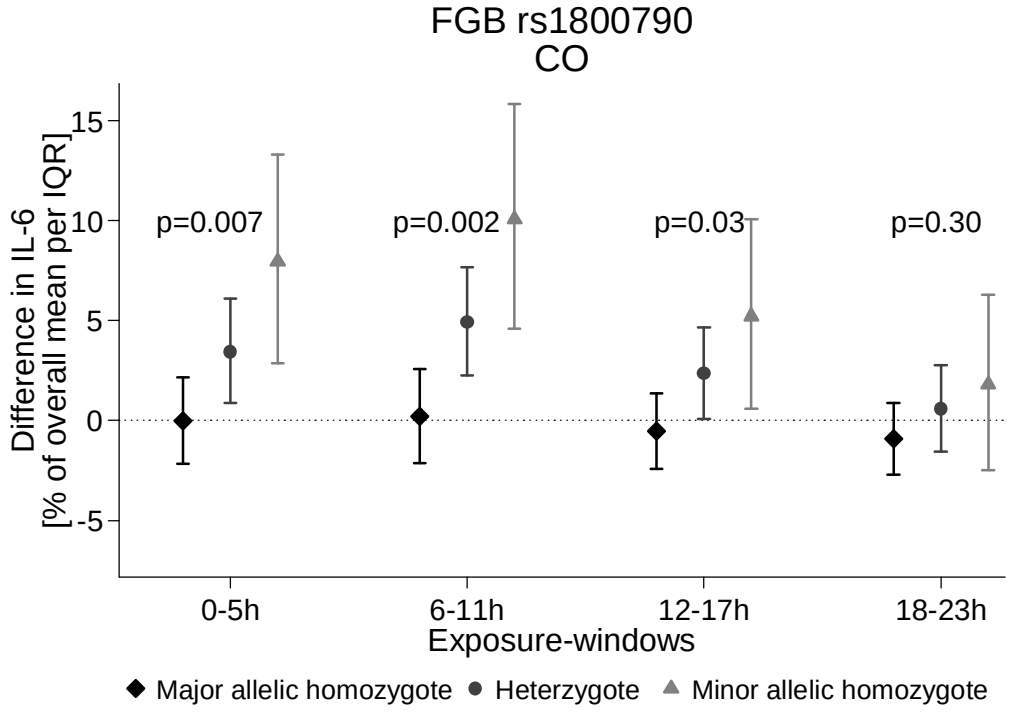


Figure 10. Modification of plasma IL-6 response to 6-hour CO exposure windows by *FGB* rs1800790.

within the three preceding 6-hour windows. Greatest effect modification was seen for *FGB* rs1800790 for increased CO during the 6-11 hour exposure-window preceding sampling (Figure 10). The overall effect of a 2.2% increase in IL-6 per 0.64 mg/m^3 increase of CO in this time-window seemed again to be confined to individuals carrying the minor allele of *FGB* rs1800790, with a 10% (95% CI 4.6%-16%) increase in homozygotes (4% of study sample). In homozygotes for the major allele of *IL6* rs2069832 (36% of the study sample) the corresponding increase was 3.6% (95% CI 1.0%-6.2%). Differences in the NO_2 and $PM_{2.5}$ effects across genetic subgroups for different 6-hour exposure-windows were similar but weaker.

4.4 PAPER IV: AIR POLLUTION AND VENTRICULAR ARRHYTHMIAS

Seventy-three out of 211 included patients had 114 ventricular arrhythmias during an average follow-up time of 33 months. Primary shock therapy was administered for a total of 58 events, whereas primary anti-tachycardia pacing (ATP) was administered for 17 events. Both shock and ATP was administered for 39 events. The most common arrhythmia in our study was ventricular tachycardia (76%). Fifty-seven of the ventricular events occurred at home (50%) and 5 at work (4%). Five of the ventricular events (4%) were preceded by angina symtoms with a duration of approximately an hour for 2 of them. Pre-existing ischemic heart disease was the most common cardiovascular condition of the study patients and only 18 percent had normal ejection fraction. Most patients had dual chamber ICDs and approximately half had experienced discharges prior to the study period. Patients had a mix of anti-arrhythmic medication. Gothenburg generally showed higher air pollution levels than Stockholm, in particular for NO_2 and PM_{10}.

4.4.1 Associations for 2-hour moving averages

The risk of ventricular arrhythmias was associated with increased levels of air

Table 1. Odds ratios of ventricular tachyarrhythmia for an interquartile range increase in air pollutants in patients with implantable cardioverter defibrillators.

Pollutant	Moving average	IQR*	No. subjects	No. events	OR†	95% CI‡
PM_{10}	2-hour	13.2	65	101	1.31	(1.00 – 1.72)
(μg/m^3)	24-hour	10.3	67	106	1.24	(0.87 – 1.76)
$PM_{2.5}$	2-hour	7.5	33	49	1.23	(0.84 – 1.80)
(μg/m^3)	24-hour	5.2	35	53	1.28	(0.90 – 1.84)
NO_2	2-hour	15.3	69	109	1.09	(0.84 – 1.42)
(μg/m^3)	24-hour	11.0	70	110	1.07	(0.81 – 1.42)

* Interquartile range for joint distribution of pollutants in Stockholm and Gothenburg used in case-crossover analyses except for $PM_{2.5}$ where only data from Stockholm are presented

† Odds ratio

‡ Confidence interval

pollution in the preceding 2-hour exposure period. The strongest association was seen for PM_{10}. NO_2 showed a positive association although weaker in effect. $PM_{2.5}$, available only to Stockholm, also showed associations similar in magnitude to PM_{10} arrhythmias although the 95% confidence interval included unity (Table 1).

Ventricular arrhythmias showed a stronger association with PM_{10} in Gothenburg than in Stockholm (Figure 11). Ventricular arrhythmias occurring within the median distance from the air pollution monitor (15 km in Gothenburg [range 2-172 km] and 10 km in Stockholm [range 1-65km]) demonstrated an OR of 1.76 (95% CI 1.18-2.61) for an IQR increase in PM_{10} whereas events further away showed no association. No such patterns were seen for NO_2 exposure. PM_{10} tended to be stronger associated with arrhythmias in patients spending the preceding 2 hours outdoors. Ventricular arrhythmias in patients with more than two interventions during the study period tended to show a stronger association with PM_{10} than patients with only one or two interventions.

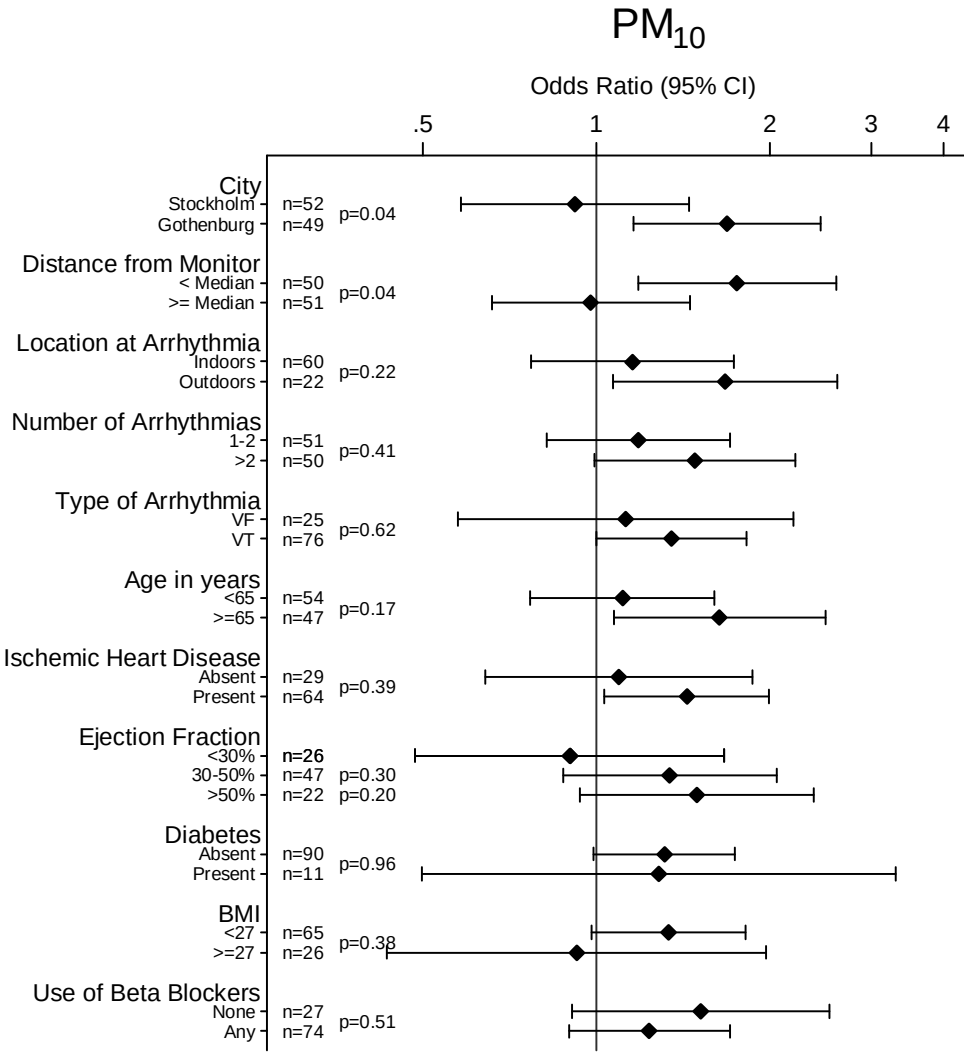


Figure 11. Odds ratios of ventricular arrhythmia for an interquartile range increase in 2 hour moving averages of PM_{10} in different subgroups (*P*-values for interaction). For variables distance from monitor and location at arrhythmia observation data available only for case periods

NO_2 demonstrated associations in the same direction as for PM_{10} but generally with weaker estimates and less clear patterns across groups with the exception of the difference in effect of being outdoors or indoors at time of event that showed a p-value for interaction of 0.04.

4.4.2 Associations for 24-hour moving averages

Positive associations were seen between increases in PM_{10}, NO_2 and $PM_{2.5}$ for 24-hour means and the onset of ventricular arrhythmias but all included unity (Table 1). The point estimates for PM_{10} and $PM_{2.5}$ were similar to that for the 2-hour mean.

Compared to the 2-hour moving average analysis, the 24-hour moving average tended to show less difference in effects between study centres. Events closest to the air pollution monitor were strongly associated with increased PM_{10} and events farther away showed no associations. For the 24-hour moving averages no consistent pattern was seen for patients with more frequent arrhythmias during the study.

Increased PM_{10} and NO_2 were strongly associated with ventricular index arrhythmias, which occurred prior to implantation of the device. For NO_2 there was a difference in effect between the events collected from the ICDs and the index events.

Age, ejection fraction, pre-existing ischemic heart disease, anti-arrhythmic medication did not show any associations.

5 DISCUSSION

Air pollution has been associated with mortality, including cardiovascular mortality, and both observational and experimental studies support this. The details of how, when, who, and even how much remain unclear. The focus of this thesis is on associations with short-term exposure with special attention to aspects of ischemic heart disease and ventricular arrhythmias.

5.1 AIR POLLUTION AND ISCHEMIC HEART DISEASE

5.1.1 Triggering of myocardial infarction (Paper I)

5.1.1.1 Are subjects with no prior myocardial infarction sensitive to the effects of air pollution in triggering non-fatal myocardial infarction?

Our study showed no associations for air pollutants measured and triggering of myocardial infarction in relation to 2-hour and 24-hour preceding pollutant concentrations. Nor did we find any associations in subgroup analyses. Based on these findings we could not find support for the hypothesis that short-term increases of air pollution trigger myocardial infarction, in contrast with a similar study from Boston [87].

However, a number of issues need to be considered. Our study differed from the previous four studies specifically investigating air pollution as a trigger for myocardial infarction. Our subjects were exclusively first-time myocardial infarctions. This could imply a lower susceptibility to cardiovascular effects from air pollution in the general population compared to subjects with more progressive atherosclerotic disease with previous major ischemic events occluding larger coronary vessels or providing myocardial substrates for ventricular arrhythmias. In the Boston study [87] roughly a third of the subjects had previously experienced a myocardial infarction, perhaps providing support to this hypothesis, but this proportion was similar in the Seattle study [88] where no associations were found. One study focusing on fatal coronary events in Rome [100] found associations with air pollution and other studies have seen associations restricted to, or stronger for, fatal myocardial infarctions [22-24,94,180]. However, none of our study subjects experienced fatal complications. It may therefore be possible that patients without previous ischemic disease do not constitute a susceptible subgroup to short-term air pollution exposure in relation to non-fatal myocardial infarctions.

The previous air pollution studies designed to investigate the triggering of myocardial infarction have shown associations with increased $PM_{2.5}$ 2 and 24 hours preceding the event in Boston (N=772) [87] and with time in traffic as a proxy for traffic-related air pollution 1 hour preceding the event in Augsburg (N=691) [181]. However, when studying the association in the same Augsburg sample population using measurements from central monitors, no association was found [83]. In addition, in a larger study of myocardial infarctions in Seattle (N=5793) [88] no association with fine particulate pollution was found. In our study, the Boston study and the Augsburg study, detailed interviews with each subject immediately followed their myocardial infarction, while the Seattle study relied on information collected from a myocardial infarction registry. The interviews performed in the Boston study, like our study, enabled a very accurate determination of the time of onset of the myocardial infarction, including

information about premonitory symptoms. This increases the accuracy of temporal exposure classification.

We had PM_{10} exposure data for only approximately 340 cases of myocardial infarction and no data for $PM_{2.5}$, giving us less power to uncover associations with particulate pollution. This is important because particulate air pollution, especially $PM_{2.5}$, has been most consistently associated with cardiovascular effects. In addition, although we had accurate data on time of myocardial infarction and individual-level patient characteristics, the use of city-center roof-top air pollution monitors meant that for any given moment all subjects were allocated with the same level of air pollution exposure. This does not distinguish our study from most other time-series or case-crossover studies of air pollution but contributes to non-differential exposure misclassification which attenuates estimates of true associations.

Taken together, these five studies do not convincingly show a triggering of myocardial infarction in relation to short-term exposure to air pollution. However, a large amount of observational studies using records of hospital admissions have seen associations with air pollution [79,84-86,89-95,99] in spite that they used less detailed data concerning time and diagnosis of myocardial infarction and individual-level data, including premonitory symptoms and co morbidities. Transient ST-segment depression, an indication of myocardial ischemia, has been seen in conjunction with particulate pollution exposure in elderly [75], including those with heart disease, and for patients with coronary heart disease in both an observational [73,74] and in a double-blind randomized crossover study in a controlled exposure chamber [72]. Recently a controlled experimental study on healthy humans showed increased thrombus formation and platelet activation after exposure to dilute diesel exhaust [182].

In summary the results from our study do not support the hypothesis that general levels (urban background) of air pollution are strongly associated with the risk of myocardial infarction in the general population. But they do not disprove the hypothesis that myocardial infarction may be involved in the cardiovascular effects of short-term exposure to air pollution.

5.1.2 Inflammation and genetic interaction (Paper II & III)

5.1.2.1 Do IL6 polymorphisms affect IL-6 levels, constituting an increased risk for cardiovascular events via inflammation in certain subgroups? (Paper II)

After correcting for multiple testing we found associations between four *IL6* SNPs and mean IL-6 levels which were corroborated by similar associations for the haplotype incorporating the positively associated alleles in the separate SNP analyses. This would suggest that some *IL6* polymorphisms including rs1800795, rs2069832, rs1554606 and rs2069845, do indeed affect IL-6 levels in myocardial infarction survivors.

Comparing the effect of the minor homozygote of eg rs1554606 on IL-6 levels (14.2% increased IL-6; 95% CI 4.5-24.9%) with a 5 kg/m^2 increase of BMI in the same model we found roughly a similar effect (16.1% increased IL-6; 95% CI 12.0-20.5%),

which is interesting, considering that adipose tissue has been postulated to contribute to one third of all circulating IL-6 [183]

Several studies have investigated associations between *IL6* polymorphisms and IL-6 levels in different populations and the results are conflicting. Studies have showed increased IL-6 levels for the cytosine (C) allele [142,144-146,150,184] of the SNP rs1800795, located in the promoter region, but also for the guanine (G) allele [147-149,185-187] in the same SNP although some studies including a meta-analysis [140] showed no effect of the SNP on IL-6 levels [143,151,156,188]. One study has shown increased IL-6 levels in heterozygotes of rs1800796 [141] (also located in the promoter region) in patients experiencing subsequent cardiovascular events.

The results for associations of IL6 polymorphisms and clinical cardiovascular outcomes are also inconclusive. Studies have shown increased risk for cardiovascular disease in subjects with the C allele [144,150] and the G allele of rs1800795 [147,148,151] as well as no increased risk for either allele [140-143,156,188].

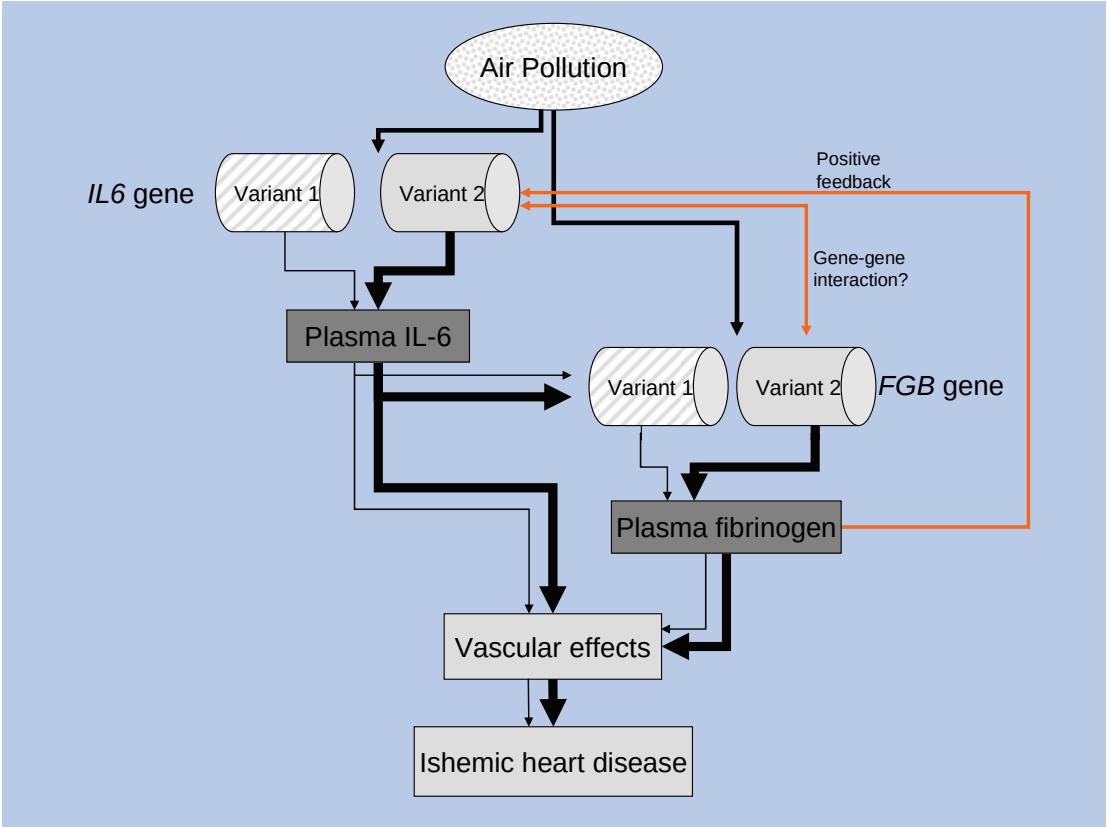
IL-6 is a recognized prognostic risk factor for future cardiovascular events in both healthy individuals and in patients with heart disease [38,189]. Studies investigating patients with previous heart disease[190] have seen increased mortality in unstable coronary disease comparing levels above and below 5 pg/ml after 12 months of follow-up, higher than our mean levels. However, the levels of IL-6 seen for all genotypes in our study fall within the top quartile of the study performed by Ridker et al [191] on healthy men (>2.28 pg/ml) who had a relative risk of future myocardial infarction of 2.3 compared to the bottom quartile (<1.04 pg/ml), suggesting that our whole cohort is at increased risk of cardiac events. However, whether the differences in IL-6 levels observed between genotypes, although significantly different, actually are large enough to confer a discernable difference in risk of cardiac events is unknown. The relationship between *IL6* genotype, IL-6 plasma levels and cardiovascular events is therefore still unclear.

5.1.2.2 Can variations in genes involved in potential pathways of cardiovascular effects increase susceptibility to air pollution? (Paper III)

We hypothesized that air pollution exposure stimulates a systemic low-grade inflammatory response, particularly in a population with previous myocardial infarction (thus with increased risk for re-infarction), and that this response is different across groups with variants of inflammatory genes (Figure 12).

In our study we saw weak associations between air pollutants and IL-6 levels after 24 hours of exposure and slightly stronger associations with the 6-11 hour or 12-17 hour exposure-windows. Interaction analyses between selected *IL6, FGA*, and *FGB* SNPs, however, showed patterns of effect modification primarily for *IL6* rs2069832 and *FGB* rs1800790 with the strongest effects seen for the *FGB* rs1800790 in relation to increased CO in the first three 6-hour exposure-windows preceding blood sampling. For the *IL6* SNP this rapid effect fits well with the expected rise of IL-6 after stimulus given the cytokine's short half-life[192] of IL-6.

FGB rs1800790 however, is a polymorphism in a gene coding for fibrinogen whose production is generally stimulated by IL-6 levels which are upstream in the inflammatory response. There are, however, studies demonstrating that fibrinogen may invoke a positive feedback mechanism further increasing the production of IL-6 [193,194] and that variation in the fibrinogen genes can affect the levels of IL-6 [195]. Since the fibrinogen protein has a half-life yielding expected increases within 5 days of stimulus [196], the effects of this positive feedback mechanism would be expected after the increase of fibrinogen level, ie after 5 days from the stimulus. Therefore the association observed would have to be explained by something more direct, for example gene-gene interaction where specific combinations of variations across several genes rather than within one gene result in greater transcription. This implies a theory of cross-talk between genes which goes beyond the scope of this thesis.



Figur 12. Gene-environment interaction. Levels of IL-6 are increased more by air pollution depending on the variant of interleukin-6 gene or fibrinogen gene potentially leading to greater risk of ischemic heart disease.

For *FGB* rs1800790 the results suggested that the weak overall effect of air pollution on IL-6 levels, seen before subdividing according to genotypes, appeared to be an averaging of the confined effect seen in individuals with the risk allele. In other words only individuals with minor alleles for *FGB* rs1800790 were susceptible to air pollution by generating an inflammatory response.

The *IL6* SNP alleles associated with lower mean plasma IL-6 in Paper II (major alleles) were associated with greater IL-6 response to air pollution in Paper III. This indicates that although their baseline levels of IL-6 were lower than other genotypes' levels they responded more readily to the extrinsic stimuli of air pollution. This is puzzling and was unexpected. The variability analyses of Paper II indicated a weak

tendency for increased within variability of IL-6 over time for the major alleles compared to minor alleles which might support a greater responsiveness to external stimuli for the major alleles.

Very few studies have investigated gene-environment effects of air pollution on heart disease. Peters et al reported similar effect modification of the fibrinogen response to 5 day increases in PM_{10} [197] in the same sample. Previous studies investigating associations between oxidative stress as a pathway for the effect of air pollution exposure on heart rate variability have also seen greater air pollution effects in subjects with different gene polymorphisms [105,168-170]. These studies open the way for understanding possible intermediary pathways of air pollution effects and suggest susceptible subgroups according to genetic makeup.

Overall our results give further support to hypothesized inflammatory effect of air pollution and imply that the genetic variant of *IL6* or *FGB* gene that a person carries may influence the person's inflammatory response to air pollution exposure.

5.2 AIR POLLUTION AND ARRHYTHMIAS

5.2.1 Triggering of ventricular arrhythmias (Paper IV)

5.2.1.1 Can the rapid effect of air pollution on ventricular arrhythmias shed light on a potential pathway?

In Paper IV we found an increased risk for ventricular arrhythmias after a 2 hour increase in PM_{10}. This association was strengthened when considering events occurring closer to the air pollution monitor and those occurring outdoors, both subgroups having better exposure classification than their complements. In addition, although changes in air pollution levels over a large area may be characterized by central monitors, in a specific place, the absolute levels from which they change will not be characterized well by a roof-top monitor. Therefore temporal changes in personal exposure in individuals experiencing events further away from the city-center (where the monitor is located) will probably include increases from lower levels of air pollution due to lower traffic activity. Events occurring outdoors may also have an interaction effect with greater physical activity [198], although this hypothesis could not be tested. Gothenburg showed a stronger effect than Stockholm perhaps due to a greater proportion of the $PM_{2.5}$ fraction in PM_{10} compared to Stockholm where we know road-wear debris has a large contribution to the coarse fraction of PM_{10} [199].

The associations, seen already after 2 hours of exposure, point to a rapid mechanism of effect. Ventricular arrhythmias occur primarily after initiation of a re-entry tachycardia sparked by a premature impulse in a myocardium containing scar tissue from previous myocardial infarctions or cardiomyopathy. In less than 10% of cases [200], tachycardia is caused by abnormal automatic acceleration of spontaneous depolarization (phase 4 of the action potential) usually due to metabolic causes such as acute myocardial ischemia, hypoxemia, hypokalemia, hypomagnesemia, acid-base disorders, high sympathetic tone and use of sympathomimetic medication. A third possibility for induction of ventricular arrhythmias is by so-called triggered activity whereby specific tachycardias such as torsades de pointes and digitalis-induced

arrhythmias are triggered by early afterdepolarizations of sufficient magnitude to generate another action potential. In our study, however, a majority of patients had structural cardiovascular disease making re-entry tachycardia (arrhythmias arising in tissue with different conduction times due to injury) the most plausible form of ventricular arrhythmia in all but two cases where prolonged angina symtoms preceding the intervention could have induced abnormal automatic ventricular tachycardia.

The initiation of the premature impulse leading to a re-entry tachycardia may conceivably be secondary to inflammation or disturbances of the parasympathetic and sympathetic balance controlling the heart rhythm. Exposure to air pollution measured using central monitors (2 hours of exposure) [87], time in traffic (1 hour of exposure) [181] and a controlled human exposure-chamber study (<1 hour of diesel exhaust exposure) [72] have been associated with myocardial infarction or ST-depression indicating that an inflammatory/ischemic effect of air pollution can be rapid. However, only 5 of 114 ventricular arrhythmias in our study were preceded by symptoms of angina giving less support to an ischemic triggering of ventricular arrhythmias by air pollution, although not ruling out subclinical ischemia. Alternatively, air pollution has been associated with changes in autonomic function measured by HRV with indications that this effect is mediated through oxidative stress [105,168-170] but dysrhythmias might be elicited via direct stimulation of autonomic reflexes in the airways [201] and repolarization changes in association with ambient particle exposure [113] suggest such a route. Such a reflexive effect on the electrical conduction of the heart would presumably represent a very rapid effect of air pollution.

In summary the results from our study could not give firm support for which pathway air pollution might exert its effect on ventricular arrhythmias but it points to the possibility of a reflexive effect.

5.2.1.2 Which patients are at particular risk of developing ventricular arrhythmias in response to air pollution exposure?

For Paper IV we recruited ICD patients in Gothenburg and Stockholm. These patients are at a particularly high risk of ventricular arrhythmias and the results from our study as well as those from Boston [115-117] and St Louis [139] indicate that this may be a susceptible group for the arrhythmogenic effects of air pollution. However, there are indirect indications of a wider group of people being affected with ventricular arrhythmias from air pollution. A population-based study from Indianapolis showed associations between preceding 1 hour increases in $PM_{2.5}$ and out-of-hospital cardiac arrest [118] and it has been reported that approximately 60-70% of all out-of-hospital cardiac arrests present with ventricular fibrillation or ventricular tachycardia [202]. In Europe around 275 000 cases of out-of-hospital cardiac arrests are treated by emergency health services each year with a survival rate between 10-20% [203]. Therefore, if air pollution does, as indicated, trigger ventricular arrhythmias, potentially a very large population is at risk and in particular patients with pre-existing myocardial disease.

5.3 OVERALL DISCUSSION

The studies included in this thesis can be seen as a series of investigations examining the effects of air pollution along the "cardiovascular continuum" [204] from health to death in cardiovascular causes (Figure 13). Although the study sample in Paper I (ONSET) included a minority of diabetics, hypertensive patients, current smokers and angina patients, none of the subjects had previously experienced a myocardial infarction. Relative to the subjects included in Papers II-IV they had had a lower burden of cardiovascular disease and seem to have a lower susceptibility to short-term effects of air pollution. This generally falls in line with the previous onset studies and studies showing weaker associations in non-fatal infarctions. Studies of chronic effects of air pollution implicating atherosclerotic disease suggest that air pollution also induces systemic inflammation in subjects without previous myocardial infarction. Despite this, in the absence of other simultaneous major triggers, perhaps greater air pollution exposure is required to trigger the first myocardial infarction. An indication of this can be inferred by Peters et al Augsburg studies, where no effect on the triggering of myocardial infarction was seen in association with fluctuations in urban background levels but where very strong associations were seen for individuals in exposed in traffic with presumably higher levels of pollution and a high proportion of fresh ultra-fine combustion particles.

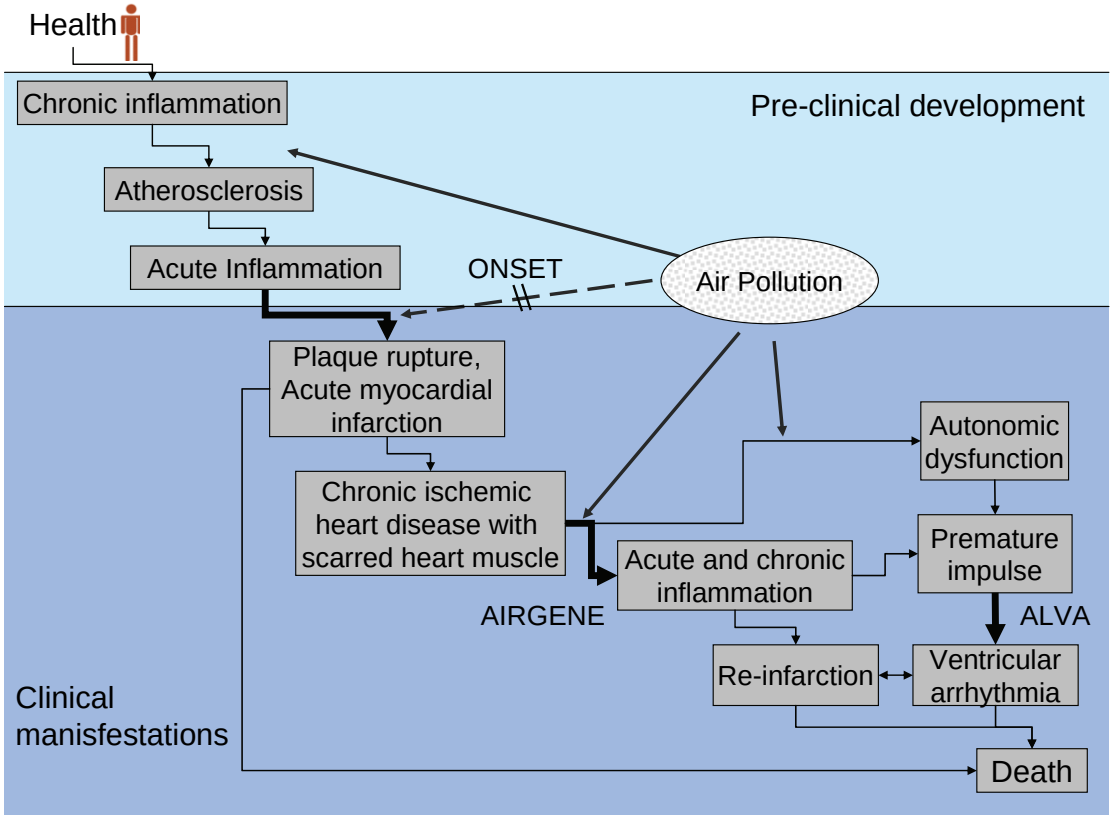


Figure 13. Air pollution effects on stages of cardiovascular disease. Bold arrows indicate steps studied in this thesis. The dashed arrow indicates the lack of association seen in Paper II.

At a later stage in the progression of cardiovascular disease we found that air pollution seems to induce inflammation in subjects with certain genetic makeup. It remains to be seen whether this, with future air pollution exposure, predisposes them to

progress faster through further stages of cardiovascular disease. In subjects with pre-existing heart disease, generally increased levels of air pollution in the urban background were sufficient to trigger ventricular arrhythmias. These patients had established re-entry circuits in fibrous scar tissue and require only an untimely premature impulse to initiate a life-threatening ventricular arrhythmia. Taken together, this implies the necessity of a greater burden of insult for triggering a first-time myocardial infarction than for triggering ventricular arrhythmias in scarred myocardium which may explain why a risk factor such as air pollution with a low relative risk shows discrepancies in effect depending on the individual's susceptibility.

6 CONCLUSIONS

I. We found no support that 2 hour or 24 hour mean levels of air pollution in Stockholm contributed to triggering of a first-time non-fatal myocardial infarction.

II. Two pairs (rs1800795, rs2069832 and rs1554606, rs2069845) of interleukin-6 gene single nucleotide polymorphisms, highly correlated within and between pairs, and the haplotype incorporating the associated alleles in each of these four single nucleotide polymorphisms, were associated with increased plasma interleukin-6 levels in myocardial infarction survivors.

III. Subjects with a major allele of the interleukin-6 gene single nucleotide polymorphism rs2069832 or a minor allele of the fibrinogen beta chain gene single nucleotide polymorphism rs1800790 showed stronger interleukin-6 responses to increased air pollution levels.

IV. Increased levels of particle matter less than 10 μm in diameter increased the risk of ventricular arrhythmias already within 2 hours of exposure.

7 FUTURE RESEARCH

Based on the knowledge gained and the questions raised from this research, several paths of research are of interest.

Paper I unexpectedly did not support the hypothesis that air pollution can trigger myocardial infarction. This could be due to a number of methodological issues but could also reflect that the sample studied, namely first-time non-fatal myocardial infarctions, is indeed less susceptible to air pollution exposure. A large study comparing individuals with and without pre-existing cardiovascular disease might shed light on this question.

The differences in plasma IL-6 levels by genotype seen in Paper II intrinsically raise the questions as to whether this effect can be seen in another sample population and if it leads to differences in clinical outcomes.

In Paper III the gene-environment interactions seen for the *IL6* and *FGB* SNPs need to be replicated in another population, preferably with similar air pollution exposure. This is particularly of interest for the associations seen for the fibrinogen gene since the association with IL-6 levels preceded the expected rise of plasma fibrinogen, indicating modification independent of fibrinogen levels.

Given the increased risk for ventricular arrhythmias seen in Paper IV within 2 hours of exposure to air pollution, perhaps especially outdoors, it would be of interest to study how exposure affects the risk for out-of-hospital cardiac arrest. Out-of-hospital cardiac arrest has a very high mortality rate and is generally caused by ventricular tachyarrhythmias, presumably beginning with a tachycardia and degenerating into fibrillation. This could be one of the major mechanisms for cardiovascular mortality of air pollution considering that the evidence for triggering myocardial infarction is somewhat inconclusive. There are very few studies addressing this research topic. A comparison of associations in Gothenburg and Stockholm, including $PM_{2.5}$ measurements, might help explain the differences of effect seen for both cities.

8 POPULAR SCIENCE SUMMARY IN SWEDISH

Hjärtkärlsjukdom är den vanligaste dödsorsaken i världen. Ett antal riskfaktorer har identifierats för dess uppkomst och prognos. Luftföroreningar har de senaste dryga tio åren uppmärksammats allt mer som en riskfaktor för hjärtkärlsjukdom som en väldigt stor andel av befolkningen utsätts för och som också kan minskas genom effektiv reglering. Tidigare studier i Sverige har visat att luftföroreningsexponering bidrar till ca 5000 årliga dödsfall i Sverige och för 290 årliga inläggningar för hjärtkärlsjukdom i Stockholms län. Kunskapsläget idag visar ett ganska entydigt förhållande mellan luftföroreningsexponering och dödlighet, men frågorna hur, när, vem och hur mycket är fortfarande inte helt besvarade.

I den här avhandlingen har jag koncentrerat mig på sambander mellan hälsoeffekter och kortvarig exponering för luftföroreningar, med särskilt fokus på hjärtsjukdom pga nedsatt syresättning till hjärtmuskeln (ischemisk hjärtsjukdom) och pga allvarliga rytmstörningar till hjärtat.

Luftföroreningsexponering har här uppskattats, i likhet med andra studier, med hjälp av fasta mätstationer, strategiskt utplacerade ovan tak höjd i centrala delar av respektive stad. Mätningarna representerar sk urban bakgrundshalter för hela geografiska området som studeras. Det betyder att alla i tex Stockholm blir tilldelade samma exponeringshalt vid ett och samma tillfälle och därmed gör vi jämförelser av halter mellan olika *tidpunkter* snarare än mellan olika områden i staden. Detta vilar på antagandet att förändringarna i halterna sker ungefär samtidigt från ena området till andra området även om det kan vara från eller till olika absoluta nivåer beroende på trafiktäthet och bebyggelse. Genom att vi mäter bara förändringar i taknivå och bortser ifrån gatunivåns mera lokala halter samt att luftföroreningar sprids ganska jämnt över städer kan detta antagandet anses rimligt. De mätfel vi introducerar med ett sådant förfarande bidrar att försvaga vår möjlighet att upptäcka samband snarare än förstärka den.

Delarbete I.Tidigare studier har visat blandade resultat vad gäller samband mellan luftföroreningsexponering och insjuknande i hjärtinfarkt. Vi ville i första delarbetet se om ett sådant samband kunde ses i Stockholm och om det kunde ske efter en mycket korttids exponering. I första arbetet samlade vi information hos 660 patienter som fått sin första hjärtinfarkt i Stockholm. Genom intervjuer fastställde vi starttiden för hjärtinfarkten och en del kring omständigheterna kring händelsen. Sedan uppskattade vi deras exponering för luftföroreningshalter 2 och 24 timmar innan starttiden med hjälp av fortlöpande mätningar vid en i Stockholm centralt belägen mätstation. Vi såg inga samband mellan luftföroreningsexponering och risken för insjuknande i ickedödlig förstagångs hjärtinfarkt. Att luftföroreningar har en roll i risken för utvecklandet av hjärtinfarkt finns det dock forskning som talar för men det kan hända att vi inte såg ett samband pga att vi hade för liten studie, inte kunde undersöka den typ av partiklar (”fina partiklar”) som väcker störst misstanke för hälsoeffekter i form av hjärtsjukdom, eller att luftföroreningar har en begränsad roll för riskökningen hos tidigare relativt sett friska hjärtinfarktspatienter, dvs som är tidigare hjärtfriska före sin första hjärtinfarkt vilken dessutom inte är av dödlig karaktär.

Delarbete II+III Åderförkalkning har visat sig vara en inflammatorisk process som leder till flera hjärtsjukdomar, bl a hjärtinfarkt. Tecken på låggradig inflammation, mätt genom små ökningar i inflammationssubstanser såsom interleukin-6 (IL-6), har

förknippats med ökad risk för hjärtsjukdom och död. IL-6 bildningen i olika celler sker efter en signal att läsa av mallen för dess byggstenar, dvs interleukin-6 genen (*IL6*). Även om vi alla har ungefär samma uppsättning av gener så skiljer de sig en aning på vissa platser i molekylen. Dessa skillnader kan innebära olika sjukdomsrisk och olika känslighet för yttre påverkan. I delarbete II och III önskade vi undersöka om 1. Skillnader i *IL6* genen ger olika halter av IL-6 i blodet. 2. Om skillnader i *IL6* genen påverkar individens känslighet för luftföroreningar mät som inflammationssvar (IL-6 halt i blodet). I 6 Europeiska städer samlade vi in drygt 5,000 blodprovsmätningar av IL-6 hos ca 1000 patienter som överlevt hjärtinfarkt. Variationer i deras IL6 gen och genen för en närbesläktad inflammationssubstans, fibrinogen, bestämdes. I delarbete II fann vi resultat som talar för att halten av IL-6 i blodet bestämdes av variationer på fyra speciella platser i *IL6* genen som oftast ärvs ihop. I delarbete III såg vi att variationen i framförallt en av dessa platser i *IL6* genen samt på en plats i en fibrinogengen påverkade hur mycket patienterna reagerade med förhöjd IL-6 nivå i blodet vid en ökning av luftföroreningsexponering. Dessa data talar för att luftföroreningar verkar genom inflammation och att vissa personer pga sin genetiska uppsättning får mera inflammation av luftföroreningar än andra.

Delarbete IV De första 3 delarbeten undersökte hur luftföroreningsexponering påverkar hjärtsjukdom som uppstår efter dålig syretillförsel till hjärtat pga åderförkalkning. Det sista arbetet undersökte istället hur luftföroreningar kan påverka elektriciteten i hjärtat. Störningar i elektriska impulsöverledningen genom hjärtats kammarmuskulatur kan snabbt bidra till hjärtat slutar att slå helt. Människor som återfått sin hjärtrytm och därefter överlevt får numera en apparat (ICD) inopererad. Den är lite större än en pacemakerdosa med elektroder placerade i hjärtat så att de kan känna av om liknande störningar återinträffar och därefter tillföra en elstöt direkt till hjärtat som kan starta om en ny normal impulsöverledning. Dessa apparater kan avläsas och information hämtas avseende tid och typ av rytmstörning som skett och vilken elektrisk terapi som givits. Vi följde ca 200 personer i Göteborg och Stockholm som fått dessa apparater och registrerade när och vilken typ av händelse som inträffat. Sedan har vi jämfört hos samma individ luftföroreningsexponeringen 2 och 24 timmar innan händelsen med lika långa perioder då ingen händelse inträffat för samma klockslag, samma veckodagar i samma kalender månad (tex mellan kl 9-11, alla fredagar i maj om händelse inträffat kl 11 en fredag i maj). Vi fann att ökningar av partikulära luftföroreningar under 2 timmar ökade risken för att få livshotande rytmstörning till hjärtat som, utan en ICD apparat, kan leda till död. Risken var störst för de som bodde i Göteborg, de händelser som skedde mera centralt i städerna och de händelser som skedde utomhus.

Sammanfattning Vi har mera stöd för att luftföroreningar verkar genom ett inflammationssvar som i sig är behäftat med risk för hjärtsjukdom och att de även troligen ger elektriska störningar i hjärtat som kan bidra till plötslig död, en av viktigaste och relativt vanliga följderna av tidigare hjärtsjukdom. Effekterna av luftföroreningar kan vara mera uttalade i grupper med viss genetik, som tidigare haft hjärtinfarkt eller som tidigare genomlevt en livshotande rytmstörning till hjärtat. Detta kan ha till följd att när regleringar för luftföreningar bestäms måste man beakta att effekten inte är likt fördelad i hela populationen utan kan vara allvarligare hos vissa individer. Dessutom kan det i förlängningen bidra till råd till patienter som tex att man närmaste tiden efter en hjärtinfarkt, då risken är störst för plötslig död, bör undvika att vistas i starkt förorenade miljöer.

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Robin Hood, Disney

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