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Novel risk factors for atrial fibrillation in an urban population

Adamsson Eryd, Samuel

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Abstract

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This thesis is based on four epidemiological papers. Paper I-II included subjects from the Malmö Preventive Project (n=22 444, aged 26-61 years), followed during a mean follow-up time of 25 years. Paper III-IV included subjects from the Malmö Diet and Cancer Study (n=30 477, aged 44-74 years), followed during mean follow-up of 13.6 and 15.3 years respectively. Cases of incident AF were retrieved by linkage with the Swedish Hospital Discharge Register and the Swedish Cause of Death Register. Participants in Paper I-II underwent measurements of inflammation-sensitive proteins (ISPs) and genotyping of polymorphisms in the ceruloplasmin gene (*CP*). Red blood cells distribution width (RDW) was measured in participants of Paper III. Carotid intima-media thickness (IMT) was measured in participants of Paper IV. All subjects were without history of AF, myocardial infarction and heart failure at study entry.

A score of five ISPs (ceruloplasmin, fibrinogen, haptoglobin, orosomucoid and α_1-antitrypsin) were significantly associated with incidence of AF. Ceruloplasmin was independently associated with incidence of AF. Genetic polymorphisms in the promoter of the ceruloplasmin gene were associated with elevated plasma levels of ceruloplasmin. One of these polymorphisms was also associated with incidence of AF, suggesting a causal relationship between ceruloplasmin and AF. RDW and carotid IMT were both independently associated with incidence of AF.

In conclusion, this thesis shows that ceruloplasmin, RDW and carotid IMT are all factors that may predict future events of AF in the general population. Further studies are needed to elucidate the causal pathway between ceruloplasmin and AF.

Key words
atrial fibrillation, ceruloplasmin, epidemiology, genetic polymorphisms, intima-media thickness, plasma proteins, red blood cell distribution width, risk factors

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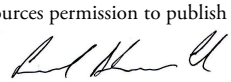
Signature  Date DECEMBER 10, 2014

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"Life can only be understood backwards; but it must be lived forwards."
Søren Kierkegaard

Contents

List of papers

This doctoral thesis is based on the following original papers. The papers are reproduced with permission from the publishers.

Paper I: Adamsson Eryd S, Smith JG, Melander O, Hedblad B, Engström G. Inflammation-sensitive proteins and risk of atrial fibrillation: a population-based cohort study. European Journal of Epidemiology. 2011;26(6):449-455.

Paper II: Adamsson Eryd S, Sjögren M, Smith JG, Nilsson PM, Melander O, Hedblad B, Engström G. Ceruloplasmin and atrial fibrillation: evidence of causality from a population-based Mendelian randomization study. Journal of Internal Medicine. 2014;275(2):164-171.

Paper III: Adamsson Eryd S, Borné Y, Melander O, Persson M, Smith JG, Hedblad B, Engström G. Red blood cell distribution width is associated with incidence of atrial fibrillation. Journal of Internal Medicine. 2014;275(1):84-92.

Paper IV: Adamsson Eryd S, Östling G, Rosvall M, Persson M, Smith JG, Melander O, Hedblad B, Engström G. Carotid intima-media thickness is associated with incidence of hospitalized atrial fibrillation. Atherosclerosis. 2014;233(2):673-678.

Abstract

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In conclusion, this thesis shows that ceruloplasmin, RDW and carotid IMT are all factors that may predict future events of AF in the general population. Further studies are needed to elucidate the causal pathway between ceruloplasmin and AF.

Sammanfattning (in Swedish)

Förmaksflimmer är den vanligaste rytmrubbningen i hjärtat. Man beräknar att 1-2% av befolkningen har förmaksflimmer. Förmaksflimmer blir vanligare med stigande ålder. Bland personer äldre än 75 år har 5-10% förmaksflimmer. Var fjärde person över 40 år riskerar att drabbas av förmaksflimmer någon gång i livet. Förmaksflimmer ger bl.a. en ökad risk för blodproppar och stroke, och är även förknippat med en ökad risk för förtidig död. Förutom ålder finns det flera andra faktorer som kan påverka risken att drabbas av förmaksflimmer. En ökad kunskap om dessa riskfaktorer skulle kunna bidra till att man snabbare kan identifiera individer med förhöjd risk, samt leda till nya behandlingsmetoder. Låggradig inflammation, storleksvariationen hos röda blodkroppar, och begynnande åderförkalkning har tidigare visats leda till en ökad risk för andra hjärt-kärlsjukdomar såsom hjärtinfarkt, hjärtsvikt och stroke. Det är dock oklart om dessa faktorer även leder till en ökad risk för förmaksflimmer.

Detta avhandlingsprojekt är baserat på insamlade uppgifter och material från två stora befolkningsstudier i Malmö; Malmö Förebyggande Medicin och Malmö Kost Cancer-studien. Malmö Förebyggande Medicin startade 1974 som ett screeningprogram i syfte att förebygga hjärtsjukdom. Mellan 1974 och 1992 undersöktes totalt 22444 män i åldrarna 26-61 och 10902 kvinnor i åldrarna 28-58. Samtliga deltagare lämnade blodprov och genomgick en fysisk undersökning där man mätte blodtryck, längd och vikt. Deltagarna fyllde även i ett frågeformulär som behandlade livsstil och socioekonomiska faktorer. Mängden inflammatoriska proteiner i blodet uppmättes hos ett slumpmässigt urval av de manliga deltagarna (ca 30 %). Komplett information om dessa inflammationsproteiner fanns tillgängligt för totalt 6193 män, vilka utgjorde studiepopulationen i delarbete I. En del av dessa individer genomgick en återundersökning inklusive ett DNA-prov några år senare och de individerna utgjorde studiepopulationen i delarbete II.

Malmö Kost Cancer-studien designades under tidigt 80-tal i det primära syftet att studera sambandet mellan kostvanor och cancer. Alla män födda mellan 1923 och 1945 samt alla kvinnor födda mellan 1923 och 1950 boende i Malmö erbjöds att delta. Totalt 30477 individer undersöktes mellan åren 1991 och 1996. Deltagarna lämnade blodprov, besvarade ett frågeformulär och genomgick mätning av blodtryck, längd och vikt. Blodproverna analyserades och sparades i en biobank. Information om storleksvariationen hos röda blodkroppar fanns tillgängligt för 27124 individer och dessa inkluderades i delarbete III. Under perioden oktober 1991 och februari 1994 erbjöds hälften av deltagarna i Malmö Kost Cancer-studien att genomgå en

ultraljudsundersökning av halskärlen i syfte att studera halspulsåderns epidemiologi. Av de 6103 individer som valde att delta återkom 5540 för provtagning under fastande omständigheter. Komplett information från blodprov och frågeformulär fanns tillgängligt för 4846 av dessa individer, vilka inkluderades i delarbete IV.

Med hjälp av slutenvårdsregistret och dödsorsaksregistret kunde vi studera hur många som insjuknade i förmaksflimmer. Resultaten visade att det fanns en ökad risk att drabbas av förmaksflimmer om man hade förhöjda nivåer av inflammatoriska proteiner i blodet. Särskilt ett protein, kallat ceruloplasmin, verkade vara kopplat till en ökad risk för förmaksflimmer. Flera genetiska varianter på kromosom 3 i anslutning till ceruloplasmin-genen var förenade med förhöjda nivåer av ceruloplasmin. En av dessa genetiska varianter uppvisade även ett samband med ökad risk för förmaksflimmer. Detta skulle möjligen kunna tyda på ett kausalt samband mellan ceruloplasmin och förmaksflimmer, d.v.s. höga nivåer av ceruloplasmin skulle kunna orsaka förmaksflimmer. Den exakta mekanismen bakom detta samband är dock oklar. Vi fann också att en stor variation i storleken hos röda blodkroppar var kopplat till en ökad risk för förmaksflimmer. Likaså fann vi att begynnande åderförkalkningsförändringar i halskärlen var förenat med en förhöjd risk att insjukna i förmaksflimmer. Inget av dessa samband kunde förklaras av tidigare kända riskfaktorer för hjärt-kärlsjukdom. Sambanden kvarstod även efter att patienter med hjärtinfarkt och hjärtsvikt hade censurerats.

Sammanfattningsvis visar denna avhandling att höga nivåer av inflammationsproteinet ceruloplasmin, stor variation i storleken mellan röda blodkroppar, och begynnande åderförkalkning är alla faktorer som är förenade med en förhöjd risk att drabbas av förmaksflimmer. Resultaten tyder också på att ceruloplasmin skulle kunna orsaka förmaksflimmer. Det krävs dock ytterligare studier för att klarlägga de underliggande mekanismerna bakom dessa samband.

Abbreviations

AF	atrial fibrillation
ANOVA	analysis of variance
BMI	body mass index
CABG	coronary artery bypass grafting
CCA	common carotid artery
CHS	Cardiovascular Health Study
CI	confidence interval
CV	coefficient of variation
CRP	C-reactive protein
ECG	electrocardiogram
HDL	high-density lipoprotein
HR	hazard ratio
hs-CRP	high sensitivity C-reactive protein
ICD	the International Classification of Diseases
IL-6	interleukin-6
IMT	intima-media thickness
LDL	low-density lipoprotein
MCV	mean corpuscular volume
MDCS	Malmö Diet and Cancer Study
MDCS-CC	Malmö Diet and Cancer Cardiovascular Cohort
MPP	Malmö Preventive Project
MRI	magnetic resonance imaging

NFκB	nuclear factor kappa B
RAS	renin-angiotensin system
RDW	red blood cell distribution width
TIA	transient ischemic attack
TNF	tumor necrosis factor
SD	standard deviation
SNP	single-nucleotide polymorphism

Chapter 1: Introduction

Atrial fibrillation (AF) is rarely a lone electrical disorder of the heart. Instead, it is often the final common pathway of a variety of cardiac and non-cardiac conditions. The arrhythmia is associated with substantial morbidity, reduced functional status and quality of life, and an increased mortality due to a combination of altered hemodynamics, thromboembolic complications, and impaired mechanical function of the heart muscle. Prognosis and effects of treatment are better the earlier the AF is diagnosed. Early detection of AF is however complicated by the often silent and asymptomatic nature of the arrhythmia. Recognition of AF at an earlier stage might allow introduction of therapies to protect the patient, not only from the complications of the arrhythmia, but also from progression of AF from an easily treated disturbance to a permanent, disabling and life-threatening condition. By the identification of risk factors and potential causes of AF, it would perhaps be possible to prevent the onset of the arrhythmia.

1.1 Epidemiology of atrial fibrillation

AF is the most common sustained cardiac arrhythmia, with a prevalence of 1% to 2% in the general population. Over six million Europeans suffer from this arrhythmia and its prevalence is estimated to at least double in the next 50 years, as the population ages.[1-3] The lifetime risk of developing AF is one in four in individuals aged 40 years or more.[2, 4, 5]

It is estimated that AF is silent in <5% to 35% of the patients, which may lead to an underestimation of the true prevalence.[6-8] The prevalence of AF in the United States is projected to be 5.6 million in 2050, but this estimation is based only on patients with permanent AF. If patients with silent and paroxysmal AF are also included, the prevalence is instead projected to 12.1 to 15.9 million.[2, 9]

In Sweden, 2.9% of the adult population (≥20 years) have been diagnosed with AF according to data from hospital discharge registers, but the true prevalence of the disease is unknown.[10] A study of 68-year old men in Malmö reported a 3.5% prevalence of AF, while a recent screening of 75-year-olds in Halland, Sweden, found a 9.6% prevalence of AF.[11, 12] Previous studies often report a prevalence between 6%

and 8% in patients aged 75 years, although higher prevalence figures have been reported from the Nordic countries and from the UK.[2, 3, 13, 14]

1.2 Clinical characteristics of atrial fibrillation

A normal heartbeat is triggered by an electrical impulse that starts in the sinoatrial (SA) node in the right atrium (**Figure 1.1.**). The electric activity spreads through the walls of the atria and causes them to contract. In AF, the electric activity of the SA-node is no longer working properly. Instead, several electrical impulses with varying origin spread throughout the atria, resulting in uncoordinated atrial contractions; fibrillation.

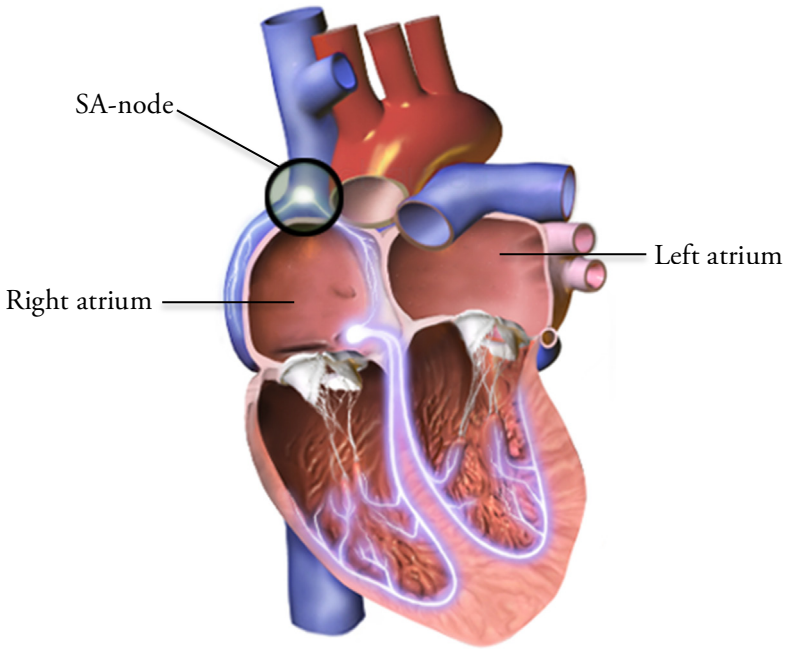

Figure 1.1.
Electrical impulses normally originates from the sinoatrial (SA) node in the right atrium. In atrial fibrillation, this electrical activity is disturbed. Copyright © Blausen Medical Communications, Inc.

AF is characterized by an irregular heart rhythm and an electrocardiogram (ECG) with absent P-waves (**Figure 1.2.**). Symptoms of AF include palpitations, fatigue, dyspnea and chest pain. But the arrhythmia can also be asymptomatic and only discovered with the onset of a stroke or a transient ischemic attack (TIA).

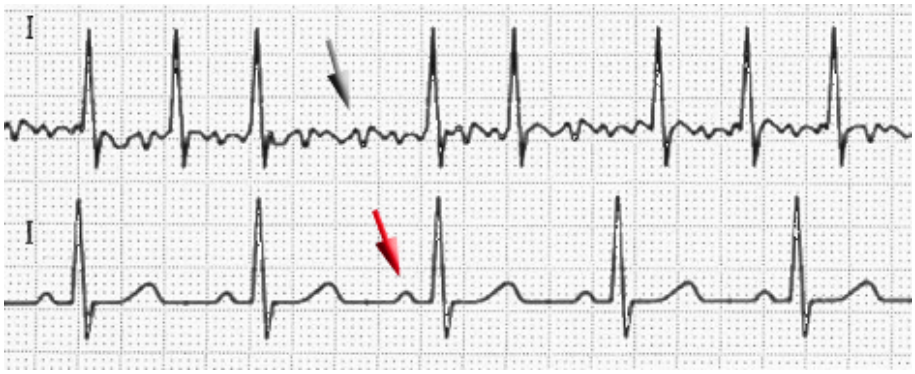

Figure 1.2.
Electrocardiogram (ECG) of atrial fibrillation (top) and normal sinus rhythm (bottom). The red arrow indicates a P-wave, which is absent in atrial fibrillation. Copyright © J. Heuser "Afib ecg"

Clinically, AF is divided into three different categories depending on the presentation and duration of the arrhythmia; paroxysmal, persistent and permanent. Paroxysmal AF is recurrent episodes that self-terminate within 7 days. Persistent AF is when the recurrent episodes are longer than 7 days or requires termination by cardioversion. When cardioversion is no longer an option, the AF is classified as permanent.[15]

1.2.1 Stroke and atrial fibrillation

AF is associated with a five-fold increased risk of stroke, and one in five of all strokes is attributed to this arrhythmia.[11, 16, 17] Ischemic strokes in association with AF are often fatal, and those patients who survive are often left more disabled by their stroke and more likely to suffer a recurrence than patients with other causes of stroke.[18] Paroxysmal AF is associated with the same stroke risk as persistent or permanent AF.[16] As a consequence, AF therapy is mainly focused on thromboembolic prevention. Since the introduction of systematic use of anticoagulants, the absolute number of ischemic strokes has decreased dramatically. In Sweden, the annual rate of ischemic stroke in AF patients between 2005 and 2008 was 25 per 1000 person-years among those treated with anticoagulants and 45 per 1000 person-years in those who were not treated.[19, 20] Furthermore, anticoagulant therapy is the only treatment that has been

shown to reduce AF-related mortality.[21, 22] Studies have also shown that the risk of ischemic stroke without anticoagulant treatment is higher than the risk of intracranial bleeding with anticoagulant treatment, in almost all patients with AF.[16, 20] Various risk stratification schemes comprising the main risk factors for stroke are being used to decide whether a patient should be offered anticoagulant therapy or not. The concomitant diseases that contribute most to all-cause mortality in AF-patients; neoplasm, chronic renal failure and chronic obstructive pulmonary disease, are however not included in these risk scores.[23] The risk of death is doubled by AF, independently of other known predictors of mortality.[24, 25]

In addition to stroke prevention, management of AF also includes ventricular rate control and treatment of concomitant cardiac diseases.

1.3 Risk factors for atrial fibrillation

The strongest predictors of AF are age and sex. The prevalence of AF doubles with each decade of age.[6] In Sweden, less than 0.6% of the AF patients are younger than 60, while as much as 13.4% are in the ages 80-89.[10] Men are more affected than women. After adjusting for age and other predisposing conditions, male sex is associated with a 1.5-fold increased risk of developing AF.[6] Mortality rates are also markedly higher in male AF patients compared to female. The relative risk of death is however higher in women and decreases with age.[23]

Hypertension is a well-documented risk factor for AF.[6, 18, 26] Although hypertension is associated with a modest risk increase (relative risk 1.2-1.5), the high prevalence of hypertension in the general population makes it the most significant population-attributable risk factor beyond both age and sex.[26]

Heart failure and AF are strongly related, as heart failure can be both a cause and consequence of AF.[27] Heart failure is associated with a 4.5 to 5.9-fold increased risk of AF and is found in 30% of all AF patients, while 30-40% of heart failure patients have a concomitant diagnosis of AF.[18, 28] Left ventricular dysfunction is often a consequence of irregular, fast ventricular rate, loss of atrial contractile function, and increased filling pressure. Rate control and maintenance of sinus rhythm have shown to improve left ventricular function in AF patients.[18]

Obesity is associated with both ventricular dysfunction and atrial enlargement, which may precede AF. One in four AF patients is obese.[29] For each unit in body mass index (BMI) the adjusted relative risk of AF increases by 3% to 7%. Obese patients with a BMI above 30 kg/m^2 have twice as high risk of AF compared to people with a normal BMI (<25 kg/m^2).[30, 31] Obesity-promoted natriuretic peptides secreted from cardiomyocytes are linked with increased risk of AF and its predisposing cardiovascular conditions.[32] Natriuretic peptides have also been associated with increased risk of cardioembolic stroke.[33]

The association between obesity and AF is probably partly mediated by diabetes mellitus. Although diabetes shares a risk factor profile with several other conditions predisposing to AF, it has been independently associated with a 1.4 to 1.6-fold increased risk of AF.[34-36] Diabetes mellitus requiring medical treatment is found in 20% of the AF patients.[18]

Valvular heart disease is found in approximately 30% of the AF population.[29, 37] It is associated with a 1.8-fold increased risk for AF in men, and a 3.4-fold increased risk in women.[38]

Heavy alcohol consumption (>36 g/day) is also a well-known risk factor for new-onset AF. Alcohol consumption and subsequent withdrawal from alcohol may result in hyperadrenergic activity, impairment of vagal tone and changes in atrial conduction properties, which are all factors that predispose to AF.[39-41]

Apart from the above-mentioned established risk factors, there are several emerging risk factors for AF including prehypertension[42], hypertrophic cardiomyopathy[43, 44], increased pulse pressure[45], congenital heart disease[46], thyroid dysfunction[47], obstructive sleep apnea[48-50] and excessive physical activity.[51, 52] Potential risk factors are also chronic renal disease[53, 54], tobacco use[55, 56], coronary artery disease[57, 58] and inflammation[59, 60].

1.4 Genetic factors and atrial fibrillation

The human genome constitutes over three billion base pairs organized into 23 chromosome pairs. The number of protein-coding genes in the human genome is estimated to 20 000-25 000.[61] On average, humans are 99.9% genetically similar to any other humans. The 0.1% sequence variation can be due to nucleotide substitutions, deletions and insertions. The most common type of sequence variation is called single nucleotide polymorphism (SNP), and is estimated to account for 90% of all sequence variation.[62] A SNP is a difference in a single nucleotide between individuals that occurs in at least 1% of the population. Large-scale discovery projects have until October 2014 identified over 112 million SNPs in human populations, available in online databases such as dbSNP (www.ncbi.nlm.nih.gov/projects/SNP).
Although the majority of genetic variations are likely to be neutral, a substantial fraction of them might explain the origins of Mendelian and complex diseases. If certain SNPs are known to be associated with a trait, it may be possible to examine stretches of DNA near these SNPs in an attempt to identify the gene or genes responsible for the trait. For common diseases, such as AF, genetic variants are identified through association analysis, which tests for differences in allele frequencies between cases and controls. Association studies have traditionally only been able to evaluate polymorphisms in candidate genes that are known or suggested to be associated with the disease. Recent advances in DNA-sequencing technique have

however made it possible to perform genome-wide association studies (GWAS), where a large number of polymorphisms are tested regardless of previous knowledge of gene function.

Genetics is thought to play an important role in the susceptibility to AF. Having a family member with AF is associated with a 40% increased risk of developing AF.[63] The earliest report of familial AF dates to the early 1940s.[64] But it was not until 2003 that Chen et al identified the first gene for familial AF.[65] They found a gain-of-function mutation in the potassium-channel gene *KCNQ1,* resulting in shortening of the atrial action potential duration and atrial refraction period. Subsequently, mutations have been discovered in several other ion-channel genes.[66]

The first GWAS performed for AF was published in 2007 and identified a region on chromosome 4q25 that showed associations with AF in patients with European and Asian ancestry.[67] In a recent meta-analysis of AF GWAS data, it was shown that carriers of a single-copy of the 4q25 variant had an approximately 65% increased risk of AF.[68] The 4q25 risk region lies upstream of the *PITX2* gene, which encodes a transcription factor that is crucial during embryogenesis and cardiogenesis.[69-72] Subsequent GWAS has identified another eight genetic loci associated with AF.[66] Although the odds ratios for any of these regions are modest, the combined risk of all SNPs in a given individual may be much higher. Still, the causative variants at all of the AF loci remain unknown and the current Heart Rhythm Society/European Heart Rhythm Association guidelines recommend against the testing of individual GWAS-associated SNPs in patients with AF.[73] Furthermore, given that very few causative mutations have been identified in families with AF, the guidelines also state that there is no clinical use for screening known AF-associated genes in patients with AF.[73]

1.5 Inflammation and atrial fibrillation

Multiple prospective studies have reported associations between inflammatory diseases, circulating inflammatory markers and incidence of AF.[60, 74-80] Inflammation may predict AF recurrence after cardioversion[81], and levels of inflammatory markers are directly related to AF severity.[59] These associations could either result from inflammatory processes in the atria in inflammatory conditions or from arrhythmogenic effects of inflammatory mediators, which are increased in blood in inflammatory states. C-reactive protein (CRP), the first inflammatory marker to be associated with AF, was investigated for a causal relationship with AF in a large Mendelian randomization study, i.e., to determine whether genetic variants that confer increased CRP levels also confer increased risk of AF.[60, 82] The results from this study did not support a causal relationship. In addition, inclusion of inflammatory biomarkers (CRP and fibrinogen) has not shown to improve predictive ability beyond

well-established clinical risk factors.[79] Although inflammation is thought to play an important role in the initiation and perpetuation of AF, it is still unclear whether inflammation is a cause or consequence of AF.[80]

1.5.1 Inflammation-sensitive proteins

Inflammation-sensitive proteins (ISPs) or acute-phase proteins are a class of proteins whose plasma concentration increase or decrease in response to inflammation.[83] ISPs are mainly synthesized in the liver and the stimulus for production is likely to be inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α).[84] The magnitude of the increases varies from about 50% in the case of ceruloplasmin and several complement components to as much as 1000-fold in the case of CRP and serum amyloid A.[83] ISPs are therefore useful markers of inflammation. The ISPs have various pathophysiological roles in the immune system and may have both pro-inflammatory and anti-inflammatory effects. Some inhibit growth of infectious agents, while others affect coagulation, vascular permeability or protect against reactive oxygen species. Conditions that commonly lead to substantial changes in ISP concentrations include infection, trauma, surgery, burns, tissue infarction, various immunologically mediated and crystal-induced inflammatory conditions, and advanced cancer. Moderate changes occur after strenuous exercise, heatstroke, and childbirth, while small changes occur after psychological stress and in several psychiatric illnesses.[83] Acute inflammation is a limited beneficial response, particularly during infections, whereas chronic inflammation is a persistent phenomenon that can lead to severe tissue damage.

1.5.2 Ceruloplasmin

Ceruloplasmin (”blue substance from plasma”) is a plasma protein that was first described in 1948 by Holmberg and Laurell.[85, 86] Ceruloplasmin is a member of the so-called multicopper oxidase family of enzymes, which is characterized by presence of three spectroscopically distinct copper sites.[87] Electron transfer between sulfur and copper atoms at the type-I copper sites results in spectroscopic absorption at 600 nm and gives the protein its intense blue color.[88] Ceruloplasmin is thought to function as a copper transporter as it accounts for approximately 95% of the serum copper in healthy adults.[89] Ceruloplasmin is also considered as an acute-phase reactant with serum levels increased by two- to threefold in inflammatory conditions.[89-91] The protein is synthesized in the liver, but expression of the ceruloplasmin gene (*CP*) has been found in several other tissues including kidney, spleen, testis, brain and lung.[92-95]

Ceruloplasmin is thought to play an essential role in iron homeostasis by catalyzing the oxidation of Fe^{2+} to Fe^{3+}, thereby allowing iron to bind to the transport

protein transferrin.[96] Loss-of-function mutations in the *CP* gene (aceruloplasminemia) result in iron accumulation in several organs including the liver and brain, and subsequent neurodegeneration.[97, 98] Ceruloplasmin may also catalyze the oxidation of Cu to Cu^{2+}, which is regarded as the less toxic form of copper.[99] This so-called ferroxidase activity reduces O_2 to H_2O without releasing superoxide or hydrogen peroxide. The reaction is thought to be responsible for ceruloplasmin's ability to block free radical proteolysis and DNA-damage. The ferroxidase activity is increased during inflammation and infection.[100] By contrast, ceruloplasmin has also been reported to induce LDL oxidation.[101] Experiments have shown that ceruloplasmin increases lipid oxidation by a process requiring superoxide released from vascular smooth muscle cells and endothelial cells.[102, 103] In addition, ceruloplasmin is suggested to be involved in angiogenesis and coagulation.[104]

High levels of ceruloplasmin have been associated with several cardiovascular disorders including heart failure, myocardial infarction, stroke, arteriosclerosis, unstable angina, abdominal aortic aneurysm, vasculitis and peripheral arterial disease.[90, 91, 104] In addition it was recently demonstrated that ceruloplasmin is a predictor of all-cause mortality in patients with heart failure.[105] It remains unclear whether ceruloplasmin is also a predictor of AF.

1.6 Red blood cell distribution width

Red blood cell distribution width (RDW) is a measure of the size variation of the erythrocytes, with higher values reflecting higher heterogeneity in cell volumes (i.e. anisocytosis). RDW is clinically used together with mean corpuscular volume (MCV) for classification of anemia. For example, iron deficiency anemia is associated with increased RDW, whereas thalassemia is associated with normal RDW. Recent studies have shown that RDW is associated with a large number of cardiovascular diseases including coronary artery disease, stroke, peripheral artery disease, heart failure, pulmonary embolism, and pulmonary arterial hypertension.[106, 107] RDW is also a predictor of mortality in the general population and in several clinical conditions. By contrast, high RDW was recently found to be a protective factor for new-onset diabetes mellitus.[108] Although low RDW showed associations with high waist circumference, glucose, insulin and triglyceride concentrations, there was also a significant positive relation between RDW and hemoglobin A1c.

Elevated RDW is common in patients with nutritional deficiencies of iron, folate and vitamin B12.[109, 110] High RDW is also found in a number of non-hematological diseases such as chronic hepatobiliary disease[106], hypothyreosis[111], hyperthyreosis[111], systemic lupus erythematosus[112], and inflammatory bowel disease[113]. Furthermore, RDW correlates with several inflammatory markers including high sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate, IL-6, soluble

transferrin receptor, soluble TNF receptor I, and soluble TNF receptor II.[114-118] Increased levels of cytokines in inflammatory states promote anisocytosis by desensitizing bone marrow erythroid progenitor cells and inhibiting red blood cell maturation.[106] Although RDW is emerging as a biomarker of cardiovascular disease, there are few studies focusing on its potential association with incident AF.

1.7 Carotid intima-media thickness

Atherosclerosis is a chronic disease of the arterial wall of medium to large arteries. The term atherosclerosis refers to mushy pathological areas (atheromata), often hardened (sclerosed) by deposition of calcium, that weaken the arterial wall and intrude the lumen of the vessel, thereby progressively restricting the blood flow.[119] The arterial wall consists of three morphologically distinct layers; the tunica intima, tunica media and tunica adventitia (also known as tunica externa) (**Figure 1.3.**).

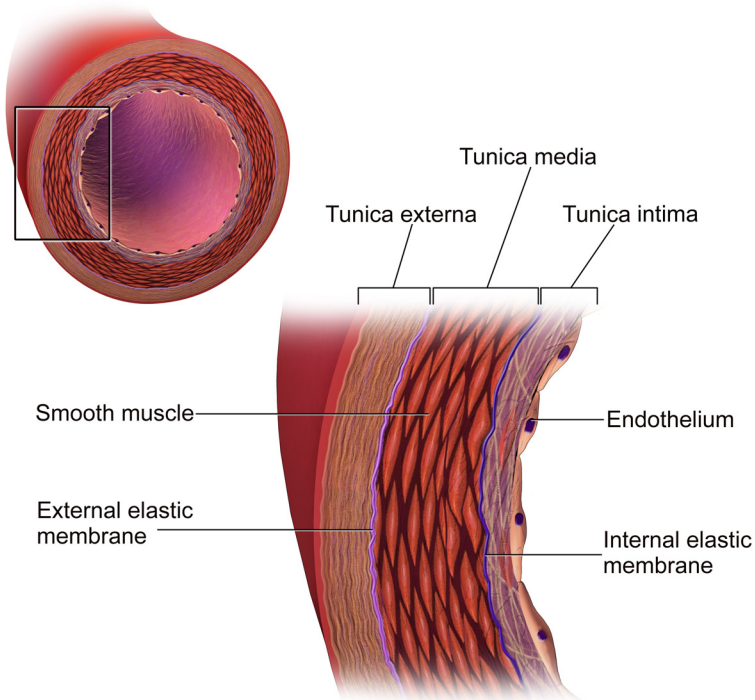

Figure 1.3.
The basic structure of a normal artery. Copyright © Blausen.com staff.

The intima, the innermost layer, consists of a single layer of endothelial cells, subendothelial connective tissue, and a sheet of elastic fibers. This is the layer that is mainly involved in atherosclerosis.[120] Tunica media is thicker than the intima and is mainly composed of longitudinal smooth muscle cells that are surrounded by connective tissue including elastin. The adventitia, the outer layer, is separated from the media by the external elastic layer and consists of connective tissue with interspersed fibroblasts and smooth muscle cells.[121, 122]

The development of atherosclerosis; atherogenesis, is a complex process that is yet to be fully understood. An early hypothesis about the pathophysiology was the so-called "response-to-injury" theory, which initially proposed that a physical injury of the endothelium could initiate atherosclerotic changes of the vessel wall.[123] The hypothesis was later modified so that endothelial dysfunction rather than injury was considered as the key factor of atherosclerosis.[124] The endothelial dysfunction, possibly caused by an irritative stimuli such as dyslipidemia, hypertension or pro-inflammatory mediators, result in an attraction of circulating white blood cells.[125] Parallel changes in the endothelial permeability promote the entry and retention of cholesterol-containing low-density lipoproteins (LDL) into the intima.[126] The white blood cells in the intima differentiate into macrophages that engulf the LDL particles and become so-called foam cells, due to its microscopic appearance.[125] The foam cells aggregate beneath the endothelium and form a visible fatty streak.[127] In humans, fatty streaks can be found in the aorta in the first decade of life, the coronary arteries in the second decade, and the cerebral arteries in the third or fourth decades.[122] Fatty streaks never cause symptoms but may continue to develop to an advanced lesion.[120]

The LDL particles in the intima might undergo oxidization and cause inflammation and further damage to the overlying endothelial cells. The inflammatory response stimulates migration and proliferation of smooth-muscle cells, from the tunica media to the intima. In the intima, the smooth-muscle cells produce extracellular matrix molecules, including interstitial collagen and elastin, and form a fibrous cap that covers a core of lipid and necrotic tissue.[125] The lipid deposits and the cellular proliferation can eventually grow so large that the plaque bulges into the lumen of the artery and greatly reduces the blood flow. The plaque might eventually disrupt and provoke thrombi that can interrupt the blood flow locally or embolize to distal arteries.[128] Atherosclerosis is a systemic, chronic disease and is often present at multiple vascular areas within the same patient. The carotid and coronary artery plaques are closely related.[129] Multiple studies have linked atherosclerotic plaque features between carotid and coronary arteries. Cross-sectional studies have shown associations between carotid intima-media thickness (IMT) and the extent and severity of coronary atherosclerosis based on angiography.[130-133] Additionally, calcification and lipid-rich necrotic core identified in carotid plaque by magnetic resonance imaging (MRI) have been associated with stenosis and calcification on coronary angiography, and carotid intraplaque hemorrhage on MRI has been associated with partially calcified coronary plaques on angiography.[134-136]

The technique of measuring carotid IMT with B-mode ultrasound was first described by in 1998 by Pignoli et al.[137] They showed that the distance between the lumen and intima interface of the common carotid artery from pathologic examinations did not differ from the distance between the echogenic lines seen on the B-mode ultrasound measurement from the same sample. The results suggested that B-mode ultrasound could be used to measure carotid IMT in vivo. Today carotid IMT is an accepted measure of atherosclerosis and is frequently used in both observational studies and randomized controlled studies.[138-146] Clinically, ultrasound is commonly used to identify carotid stenosis in the prevention of ischemic stroke. Measurement of carotid IMT in combination with assessment of plaque is also suggested as a potential tool for risk classification in asymptomatic subjects with intermediate risk for cardiovascular disease.[147-149] The clinical benefit of carotid ultrasound compared to traditional risk factors has however been debated.[150]

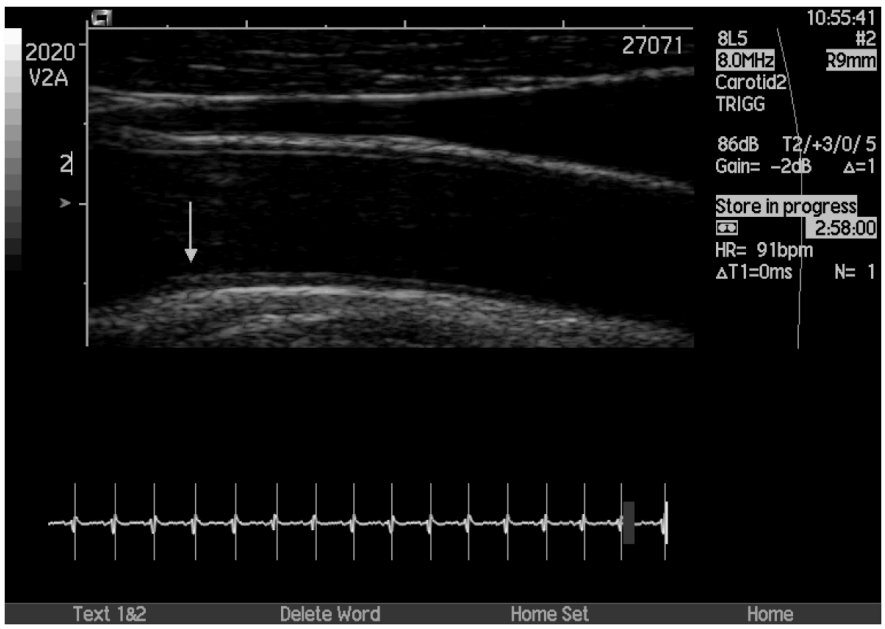

Figure 1.4.
B-mode sonogram of the carotid artery. The arrow indicates the limit between the bifurcation and the common carotid artery. Intima-media thickness of the common carotid artery (CCA-IMT) is measured directly proximally of this point.
Courtesy of Experimental Cardiovascular Unit, Skåne University Hospital, Malmö.

The coexistence of AF and clinical manifestations of atherosclerosis has been shown to increase the risk of future cardiovascular events dramatically.[151] AF has been associated with peripheral artery disease and atherosclerotic plaque in the carotid arteries.[152, 153] Furthermore, it was shown that flow-mediated dilatation, a measure of

endothelial dysfunction, was impaired in patients with AF.[154] Increased carotid IMT is a risk factor for acute myocardial infarction, stroke and heart failure.[138-141] Few studies have however explored whether carotid IMT is a risk factor AF, and the results are inconsistent. The Cardiovascular Health Study (CHS) found no association between carotid IMT or carotid stenosis and incidence of AF.[155, 156] In contrast, the Rotterdam Study showed a significant association between carotid IMT and incidence of AF, especially among women, and a recent case-control study found carotid IMT to be associated with lone AF.[157, 158] Hence, it is still unclear whether IMT predicts future AF.

Chapter 2: Aims of the thesis

2.1 General aim

The general aim of the present thesis was to study conventional and novel cardiovascular risk factors in relation to AF in an urban population.

2.2 Specific aims

- To explore whether ISPs are associated with incidence of AF in middle-aged men without a history of cardiovascular disease (Paper I).
- To investigate whether genetic polymorphisms in the gene encoding ceruloplasmin are associated with elevated ceruloplasmin levels, and whether such genetic polymorphisms are also associated with incidence of AF (Paper II).
- To explore whether RDW levels are associated with incidence of AF in middle-aged subjects without a history of cardiovascular disease (Paper III).
- To examine whether carotid IMT is associated with incidence of AF in middle-aged subjects without a history of cardiovascular disease (Paper IV).

Chapter 3: Methods

3.1 Study populations

The hypotheses of this thesis were tested in subjects from two study populations. **Paper I** and **Paper II** were based on the preventive case-finding program for cardiovascular risk factors and alcohol abuse called the Malmö Preventive Project (MPP). **Paper III** and **Paper IV** were based on the population-based Malmö Diet and Cancer Study (MDCS). In addition, findings from **Paper II** were tested for replication in the Malmö Atrial Fibrillation Cohort, which included subjects from both the MPP and the MDCS.

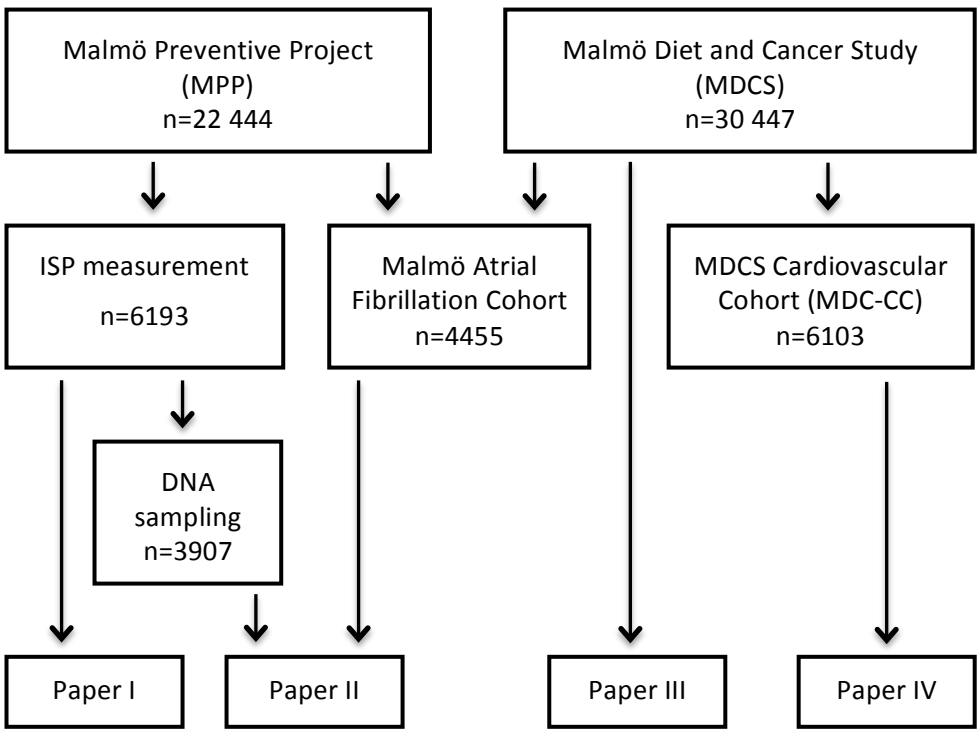

Figure 3.1.
Flow chart of the study populations in Paper I-IV.

3.1.1 Malmö Preventive Project

The Malmö Preventive Project (MPP) was a screening program that started in 1974 with the aim to prevent cardiovascular disease by detecting high-risk individuals and offer preventive interventions.[159] Between 1974 and 1992, a total of 22 444 men (aged 26-61 years) and 10 902 women (aged 28-58 years) attended the screening program with an overall attendance rate of 71%.[160] Males were screened between 1974 and 1984, while females were screened between 1977 and 1992. Baseline examinations included a physical examination and blood sampling. Participants were instructed not to change their normal habits or diet before screening, and to observe an overnight fast preceding the investigation. A short medical history including medication was obtained by a nurse. A self-administered questionnaire was used to obtain information on family disease history, smoking habits, alcohol consumption, physical activity, weight gain, blood pressure medication and history of cardiovascular disease.[159]

The health service authority of Malmö approved and funded the screening program. Participants provided informed consent.

As a part of the program, plasma proteins were determined in a randomly selected 30% of the male cohort. Complete information on all five ISPs was available for 6193 men.[161]

Non-participants were shown to have a higher total and cause-specific mortality and a less favorable socio-economic situation.[162]

3.1.2 Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study (MDCS) was designed in the early 1980s with the main goal to investigate the association between diet and certain forms of cancer.[163]

All men born between 1923 and 1945 and women born between 1923 and 1950 living in Malmö, Sweden, were invited to participate. More young women than men were included in order to be able to study breast cancer in menopausal women. In total, 74 138 subjects were invited by letter and advertisement in local newspapers, in public places and in primary health care centers. A total of 30 447 subjects attended the baseline examinations between March 1991 and September 1996 (41% attendance rate).[164]

At the first visit, the respondents underwent different anthropometric measurements and non-fasting blood samples were drawn. A self-administered questionnaire was handed out and was collected and checked for missing values at a second visit, approximately two weeks later. The questionnaire included information on education, occupation, physical activity, social network, use of tobacco and

alcohol, current health, medical history, current medication and disease in close relatives. Women were also asked about reproductive history. Participants also completed a dietary assessment that combined a 7-day menu-book, a food frequency questionnaire and a 45-minute interview.[165]

After excluding 1998 individuals who failed to complete the questionnaire or did not attend the follow-up diet interview, the cohort consisted of 28 449 subjects (11 246 men and 17 203 women). The cohort has been shown to be representative considering smoking and overweight, but with a higher mortality rate in non-participants.[164]

Malmö Diet and Cancer – Cardiovascular Cohort

A random 50% of participants who entered the MDCS between October 1991 and February 1994 were invited to take part in a study of the epidemiology of carotid artery diseases, the MDCS Cardiovascular Cohort (MDC-CC). During this period, a total of 6103 subjects (2572 men and 3531 women) were examined by B-mode ultrasound of the right carotid artery, and 5540 participants returned to donate blood samples for measurements of blood lipids and glucose.[166]

The study was approved by the regional ethics committee. Participants provided written informed consent.

3.1.3 Malmö Atrial Fibrillation Cohort

The Malmö Atrial Fibrillation (AF) Cohort is a case-control cohort with 4826 unique subjects drawn from the MDCS and re-examinations from the MPP. Cases were subjects with a diagnosis of AF before December 31 2006; controls were matched according to sex, age (±1 year) and follow-up time. A total of 371 subjects (166 cases and 205 controls) from the MPP who were also included in the discovery analyses were excluded from the replication study. After exclusions, there were 4455 subjects (59% male) in the replication analysis in paper II.

3.2 Assessment of main exposures

3.2.1 Paper I-II

Inflammation-sensitive proteins and complement factors

Plasma proteins were determined in a randomly selected 30% of the male cohort in MPP. Plasma levels of five ISPs and two complement factors were assessed by means of an electroimmunoassay. The analysis was performed consecutively at the time of

study entry. The lower detection limits were 20 mg/l for ceruloplasmin, 50 mg/l for α1-antitrypsin and 350 mg/l for orosomucoid, haptoglobin and fibrinogen, respectively. C3 and C4 were originally expressed as the percentages of the mean values from a reference population of blood donors. In order to facilitate the interpretation of the C3 and C4 values, the percentages were converted into grams per liter (C3 100% = 0.98 g/l, C4 100% = 0.20 g/l).[167] The inter-assay coefficient of variation was <5%.[168] Complete information on all five ISPs was available in 6193 men.[161]

It has been shown that all five ISPs are associated with cardiovascular disease with largely the same relative risks for all ISPs.[90] A composite score, i.e., the number of ISPs in the fourth quartile, was therefore constructed from these proteins. The fourth quartiles were as follows: fibrinogen >4.0 g/l, orosomucoid >0.93 g/l, α1-antitrypsin >1.42 g/l, haptoglobin >1.76 g/l, and ceruloplasmin >0.36 g/l. The reliability in terms of internal consistency was fully adequate for this composite score (Cronbach's α = 0.65). The score has been associated with cardiovascular disease and cardiovascular risk factors in many previous studies.[90, 91, 169-171]

Single nucleotide polymorphisms

A subset of the cohort described above later underwent re-examinations, including whole blood sampling for DNA extraction. A total of 3907 individuals had complete information on plasma ceruloplasmin and genotype. We selected 17 tag SNPs across the ceruloplasmin gene (*CP*) and genotyped them using IPLEX on a MassARRAY platform (Sequenom, San Diego, CA, USA) according to the manufacturer's standard protocols. Twenty percent of the samples were run in duplicate without any inconsistencies. All genotypes were called by two investigators.

3.2.2 Paper III

Red blood cell distribution width

Erythrocyte volume was analyzed using a SYSMEX K1000 fully automated assay (Sysmex Europe, Norderstedt, Germany). The analyses were performed consecutively at the time of the screening examination, at the central laboratory of Malmö Hospital, using fresh heparinized blood. RDW was calculated as the width of the erythrocyte distribution curve at a relative height of 20% above the baseline. Reference values were 36.4–46.3 fL in women and 35.1–43.9 fL in men.

3.2.3 Paper IV

Intima-media thickness

IMT of the right carotid artery was assessed by B-mode ultrasound. All ultrasound examinations were performed by trained certified sonographers, using an Acuson 128 (Acuson, Mountain View, California).[172] The bifurcation area of the right common carotid artery was scanned within a predefined "window" comprising 3 cm of the right common carotid artery, the bifurcation, and 1 cm of both the internal and external carotid artery for the presence of plaque, defined as a focal thickening of the IMT >1.2 mm and with an area >10 mm^2. IMT was measured off-line in the far wall of the right distal common carotid artery proximal to the bifurcation.

The leading edges of the inner echo and far wall outer echo were outlined using a specially designed computer-assisted analyzing system along 1 cm of the vessel.[173] Distances between the lines were measured at approximately 30 sites per centimeter by the computer, and the mean value from these measurements was used. The distance between the inner and outer echo of the far wall represents IMT. The maximum IMT in the bifurcation was also measured.

Inter-observer and intra-observer variability with regard to IMT was checked regularly. The mean inter-observer difference was 8.7 ± 6.2% (r = 0.85) and the mean intra-observer difference 9.0 ± 7.2% (r = 0.77).[166]

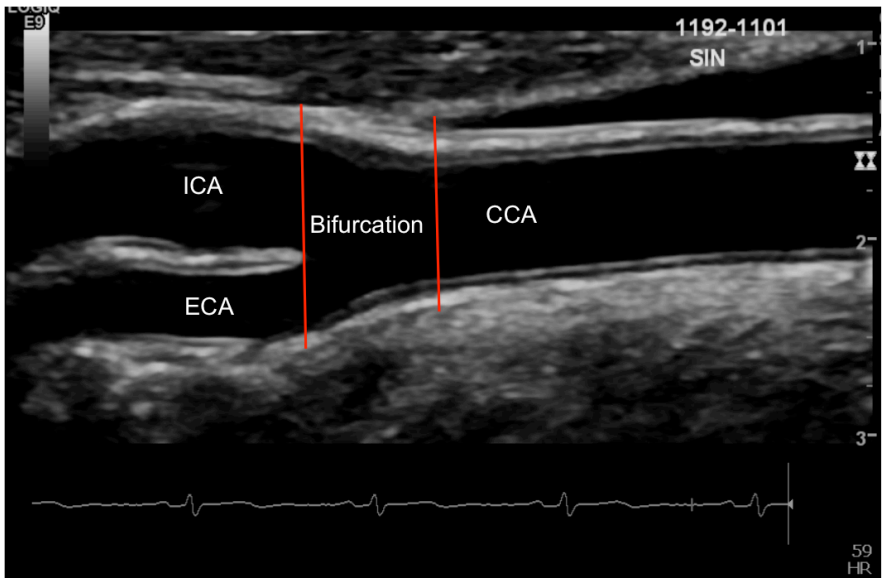

Figure 3.2.
B-mode ultrasonogram of the common carotid artery (CCA), bifurcation, internal carotid artery (ICA) and the external carotid artery (ECA).
Courtesy of Experimental Cardiovascular Research Unit, Skåne University Hospital, Malmö.

3.3 Assessment of covariates

3.3.1 Malmö Preventive Project

Biological factors

All measurements were performed by specially trained nurses. Height (m) and weight (kg) were measured when subjects were wearing light indoor clothing and no shoes. BMI was calculated as weight/height^2 (kg/m^2).

Blood pressure (mm Hg) and heart rate (beats/min) was measured twice in the supine position using a sphygmomanometer after 10 min of rest.

Blood samples were drawn after an overnight fast. Levels of serum total cholesterol, serum triglycerides and blood glucose were determined using standard methods at the laboratory of Malmö University hospital. Diabetes was defined as fasting whole blood glucose ≥ 6.1 mmol/l, 2-h post-glucose load ≥ 10.0 mmol/l, or self-reported diabetes.

Socioeconomic and lifestyle factors

Data on socioeconomic and lifestyle factors was collected from a self-administered questionnaire. Subjects who answered affirmative to the question 'Do you smoke?' or reported a daily smoking of at least ten cigarettes or equivalent amounts of other tobacco goods, were defined as smokers.

Physical inactivity in spare time was assessed using the question 'Are you mostly engaged in sedentary activities in spare time, for example, watching TV, reading, going to the movie?'

Alcohol consumption was assessed using the modified shortened version of the Michigan Alcoholism Screening Test.[174] Subjects with more than two of nine affirmative answers were considered to have high alcohol consumption.

Other variables

Use of anti-hypertensive medication was assessed in the questionnaire. Subjects who reported a physician's diagnosis of angina pectoris or who used nitrates were considered to have angina pectoris. The question 'Do you use any heart drugs?' assessed use of heart medications.

3.3.2 Malmö Diet and Cancer Study

Biological factors

All measurements were performed by specially trained nurses. Height (cm), weight (kg) and waist circumference (cm) were measured when subjects were wearing light indoor clothing and no shoes. BMI was calculated as weight/height^2 (kg/m^2). Blood pressure (mm Hg) was measured once in the supine position after 10 minutes of rest using a mercury-column sphygmomanometer. Blood samples of 45 ml were drawn, immediately separated and stored in a biological bank at -80°C or -140°C according to blood component fraction. Hemoglobin content and leukocyte count were analyzed using a SYSMEX K1000 fully automated assay (Sysmex Europe, Norderstedt, Germany). The analyses were performed consecutively at the time of the screening examination, at the central laboratory of Malmö University Hospital, using fresh heparinized blood.

In the MDC-CC, blood glucose, total cholesterol and HDL-cholesterol, were measured from fasting blood samples, according to standard procedures at the Department of Clinical Chemistry, Malmö University Hospital. The LDL-cholesterol concentration was calculated according to Friedewald's formula.[88] CRP was analyzed in frozen plasma, gathered at the baseline examination, using Tina-quant CRP latex high-sensitivity assay (Roche Diagnostics) on an ADVIA 1650 Chemistry System (Bayer Healthcare).

Presence of diabetes mellitus was defined as a self-reported physician's diagnosis of diabetes or use of anti-diabetic medications. In the MDC-CC, subjects with a fasting whole blood glucose level ≥6.1 mmol/L were also defined as having diabetes.

Socioeconomic and lifestyle factors

Data on socioeconomic and lifestyle factors was collected from a self-administered questionnaire. Smoking was classified into 3 categories: smokers, former smokers and never-smokers. Marital status was classified into 2 groups: unmarried (single, divorced, or widowed) or married (cohabiting). Educational level was classified into low (≤8 years), moderate (9-12 years), and high (college/university) levels. Information on physical activity was retrieved through 18 questions covering a range of activities during all four seasons. An overall leisure time physical activity score was created by multiplying the time spent for each activity (min per week) by an intensity coefficient. The scores were divided into quartiles of physical activity for the analysis. Daily alcohol consumption in men/women was classified as low (<20/15 g/day), intermediate (20–40/15–30 g/day) and high (>40/30 g/day).

Other variables

The dietary intake of folate, B12 and iron was assessed using an interview-based, modified diet history method that combined (i) a 7-day menu book for registration of lunch and dinner, cold beverages including alcohol, drugs and nutrient supplements; (ii) a 168-item questionnaire for assessment of meal patterns, consumption frequencies and portion sizes of regularly eaten foods; and (iii) a 45-min complementary interview. The methods and their relative validity have been described elsewhere.[165, 175, 176] Nutrient intakes were log-transformed and adjusted for total energy intake and method version of the diet assessment for use in the regression models.

Information about previous percutaneous coronary artery intervention was retrieved from the national Swedish Coronary Angiography and Angioplasty Register, and information regarding coronary artery bypass graft surgery came from the Swedish Hospital Discharge Register.

Information on current medications was obtained from the questionnaire.

3.4 Case retrieval and definition of endpoint

Cases of AF were retrieved by linkage of Swedish personal identification numbers to the Swedish Hospital Discharge register and the Swedish Cause of Death register. AF was defined as a primary or contributory diagnosis of atrial fibrillation or atrial flutter using diagnosis codes 427.92 for the International Classification of Diseases 8th edition (ICD-8), 427D (ICD-9), and I48 (ICD-10). As in previous studies, we included both diagnoses of atrial fibrillation and atrial flutter, given the close interrelationship of these diseases.[177] A validation study of 100 cases with a diagnosis of AF in the MDCS showed that the diagnosis was accurate in 95%, likely in 2% and inaccurate in 3%.[26] All subjects were followed from baseline examinations until first hospitalization due to AF, death, emigration or end-of-follow-up-date, whichever came first. Register linkages were updated consecutively during work with the present thesis such that follow-up was extended until December 31, 2006 in Paper I; December 31, 2008 in Paper II and Paper III; and June 30, 2009 in Paper IV.

3.5 Statistical analyses

All analyses were performed in SPSS versions 17, 18 and 20 (SPSS Inc., Chicago, IL, USA) or Stata IC/12.1 (StataCorp, College Station, TX, USA). All tests were two-sided and *P*-values <0.05 were considered statistically significant.

Paper I

Differences in risk factor distributions between patients with or without AF were analyzed using one-way analysis of variance (ANOVA) for continuous variables, and Pearson's chi-squared test for dichotomous variables. Cox proportional hazards regression was used to estimate hazard ratios (HRs) for incidence of AF in relation to number of elevated ISPs, and also in relation to quartiles of individual ISPs. The models were adjusted for cardiovascular risk factors associated with AF at baseline (age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption). Age-adjusted models were also assessed for comparison. The proportional hazards assumption was confirmed by plotting incidence rates over time for different categories of risk factors and by introducing time-dependent variables in the model. Incidence of AF was first analyzed in relation to the number of elevated ISPs and, secondly, in relation to the individual proteins. The Kaplan-Meier estimator was used to study incidence of AF in relation to number of elevated ISPs and across quartiles of individual proteins.

Paper II

One-way ANOVA was used to compare plasma levels of ceruloplasmin across genotypes and also to compare risk factor distributions between different SNPs. Cox proportional hazards regression was used to study the associations between SNPs and incidence of AF. The additive effect per risk allele on AF risk was tested. The models were adjusted for age, smoking, diabetes, BMI, total cholesterol level, systolic blood pressure and ceruloplasmin level. Crude models were assessed for comparison. The proportional hazards assumption was confirmed by plotting incidence rates over time. Logistic regression was used to analyze the association between ceruloplasmin and SNPs in the case-control cohort. The model was adjusted for age, sex, smoking, diabetes, BMI and systolic blood pressure. A crude model was assessed for comparison. Additional sensitivity-analyses were performed after censoring subjects with incident myocardial infarction and/or heart failure. A web-based calculator was used to estimate the Hardy-Weinberg equilibrium.[178]

Paper III

In order to adjust for differences in AF and RDW between men and women, the sample was divided into sex-specific quartiles of RDW, i.e. four groups with equal proportions of men and women in each quartile. Risk factor distributions across quartiles of RDW were analyzed using one-way ANOVA for continuous variables and logistic regression for dichotomous variables. Cox proportional hazards regression with backward stepwise elimination was used to estimate HRs for the incidence of AF in relation to quartiles of RDW. The model was adjusted for cardiovascular risk factors associated with RDW at baseline (age, sex, smoking, diabetes, BMI, waist, diastolic blood pressure, blood pressure medication, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption, hemoglobin concentration and leukocyte count). An age- and sex-adjusted model was also assessed for comparison. The Kaplan-Meier estimator and log-rank test was used to study incidence of AF across quartiles of RDW. Possible interactions between RDW and other risk factors, with respect to incidence of AF, were studied by introducing multiplicative interaction terms in the multivariate model. Independent predictors of RDW were determined by multiple linear regression with backward stepwise elimination.

Paper IV

IMT and hs-CRP were log-transformed due to skewed distributions. The study population was categorized into sex-specific quartiles of IMT in the common carotid artery and in the bifurcation, respectively. One-way ANOVA and logistic regression was used to compare risk factor distributions across quartiles of IMT. Cox proportional hazards regression was used to estimate HRs for the incidence of AF in relation to quartiles of IMT. Three models were used. The first model was adjusted for age and sex. The second model was adjusted for cardiovascular risk factors (age, sex, smoking, diabetes, waist circumference, systolic blood pressure, anti-hypertensive medication, LDL, HDL, education, physical activity and hs-CRP). The third model was in addition adjusted for presence of carotid plaque. Potential interactions between IMT and other risk factors, on the incidence of AF, were estimated by introducing interaction terms in the multivariate model. The Kaplan-Meier estimator and log-rank test was used to study incidence of AF across quartiles of IMT.

Chapter 4: Results

4.1 Paper I

In **Paper I**, the relationship between ISPs and incidence of AF was studied. Complete information on all five ISPs was available in 6193 men.[90] After excluding individuals with a history of AF, heart failure, stroke or cancer, and 17 subjects with missing data on blood pressure or cholesterol, 6031 men remained for analysis. During a mean follow up of 25 years, 667 men (11%) were diagnosed with AF. Baseline plasma levels of ceruloplasmin were significantly higher in men who were hospitalized due to AF during follow-up. Age, BMI, blood pressure, anti-hypertensive medication, angina and alcohol consumption were also significantly higher at baseline in men who developed AF (**Table 4.1.**).

Table 4.1. Baseline characteristics in subjects with and without atrial fibrillation during follow-up

	Atrial fibrillation		***P***
	No (*n*=5364)	**Yes (*n*=667)**	
Age (years)	46.7 ± 3.7	47.8 ± 3.4	<0.001
BMI (kg/m^2)	24.9 ± 3.3	25.7 ± 3.5	<0.001
Systolic blood pressure (mm Hg)	128.7 ± 15.5	132.2 ± 16.4	<0.001
Diastolic blood pressure (mm Hg)	86.9 ± 10.0	89.1 ± 10.7	<0.001
Anti-hypertensive medication (%)	4.2	7.8	<0.001
Cholesterol (mmol/L)	5.69 ± 1.04	5.73 ± 0.98	0.37
Triglycerides (mmol/L)	1.58 ± 1.11	1.58 ± 0.90	0.87
Smokers (%)	48	48	0.33
Diabetes (%)	4.7	4.9	<0.001
Angina (%)	1.2	1.6	<0.001
Heart drug (%)	0.4	0.3	0.72
Physical inactivity (%)	57	54	0.058
High alcohol consumption (%)	13	15	<0.001
Heart rate	68 ± 10	67 ± 10	0.19

Table 4.1. Continued from previous page

	Atrial fibrillation		*P*
	No (*n*=5364)	Yes (*n*=667)	
ISPs (g/L)			
Fibrinogen	3.5 ± 0.80	3.6 ± 0.80	0.12
Haptoglobin	1.38 ± 0.67	1.39 ± 0.73	0.76
Ceroloplasmin	0.316 ± 0.07	0.322 ± 0.07	0.015
α_1-Antitrypsin	1.27 ± 0.27	1.28 ± 0.27	0.50
Orosomucoid	0.82 ± 0.20	0.83 ± 0.21	0.18
Complement factors			
C3	103.0 ± 22.7	103.8 ± 22.6	0.43
C4	120.9 ± 40.2	120.6 ± 40.2	0.89

There was a significant association between the number of elevated ISPs and incidence of AF (**Table 4.2.**). The relationship remained significant after adjustment for potential confounders and HRs were essentially unchanged when patients with incident myocardial infarction (n=139) were censored (data not shown).

Table 4.2. Incidence of atrial fibrillation (AF) in relation to number of elevated inflammation-sensitive proteins (ISPs)

	Number of elevated ISPs				*P*, trend
	None n=2437	One n=1555	Two n =891	Three or more n=1148	
Atrial fibrillation *n* (%)	253 (10.4)	173 (11.1)	96 (10.8)	145 (12.6)	
AF per 1000 person-years	4.00	4.46	4.48	5.54	
Age-adjusted HR	1.0	1.16 (0.96-1.41)	1.19 (0.94-1.51)	1.61 (1.31-1.98)	<0.001
Risk factor adjusted HR[a]	1.0	1.08 (0.88-1.31)	1.07 (0.84-1.36)	1.39 (1.12-1.74)	0.007

[a]Hazard ratios (95% CI) adjusted for age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption

Kaplan-Meier figures showed that the ISPs were elevated several years before the AF hospitalizations (**Figure 4.1.**).

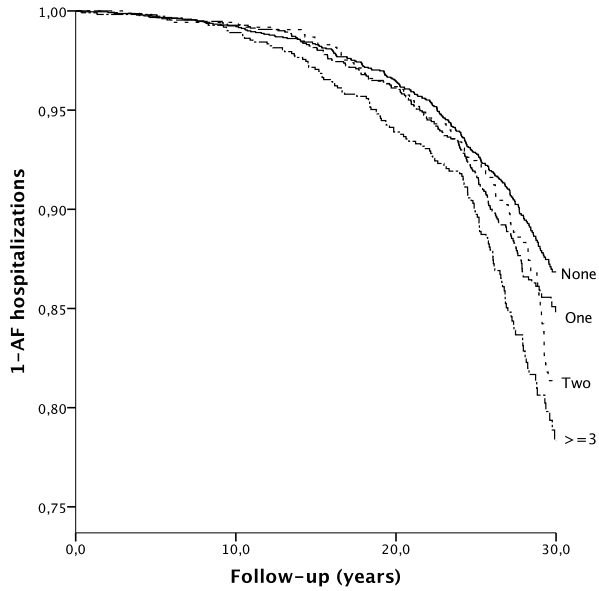

Figure 4.1.
Incidence of hospitalizations due to atrial fibrillation during a mean follow-up time of 25 years, in relation to the number of elevated inflammation-sensitive proteins. Figure reproduced from **Paper I**.

When the ISPs were analyzed individually, ceruloplasmin was the only protein that showed an independent association with incidence of AF after adjustment for potential confounding factors (**Table 4.3.**).

Table 4.3. Incidence of atrial fibrillation in relation to individual inflammation-sensitive proteins and complement factors. Presented as hazards ratios (HR) per standard deviation increase of the plasma protein concentration.

	Age adjusted HR	*P*	+ risk factors[a]	*P*
Fibrinogen	1.13 (1.05-1.22)	0.002	1.07 (0.99-1.16)	0.11
Haptoglobin	1.13 (1.05-1.22)	0.001	1.08 (1.00-1.17)	0.056
Ceruloplasmin	1.17 (1.08-1.26)	<0.001	1.13 (1.04-1.22)	0.003
α1-Antitrypsin	1.07 (0.99-1.16)	0.087	1.04 (0.96-1.12)	0.34
Orosomucoid	1.13 (1.05-1.22)	0.001	1.06 (0.98-1.15)	0.14
C3	1.10 (1.02-1.19)	0.020	1.01 (0.93-1.09)	0.90
C4	1.01 (0.93-1.09)	0.80	0.96 (0.89-1.05)	0.37

Standard deviation values for the plasma proteins were 0.80 g/L for fibrinogen, 0.68 g/L for haptoglobin, 0.067 g/L for ceruloplasmin, 0.27 g/L for α_1-antitrypsin, 0.20 g/L for orosomucoid 0.22 g/L for C3, 0.080 g/L for C4

[a]Adjusted for age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption

Subjects in the highest quartile of ceruloplasmin had a significantly higher risk of being hospitalized with a diagnosis of AF compared to subjects in the lowest quartile (**Figure 4.2.**) Censoring patients with myocardial infarction did not alter this association.

Complement factors C3 and C4 did not show any significant association with incidence of AF. Neither did they seem to modify the relationship between the ISPs and incidence of AF.

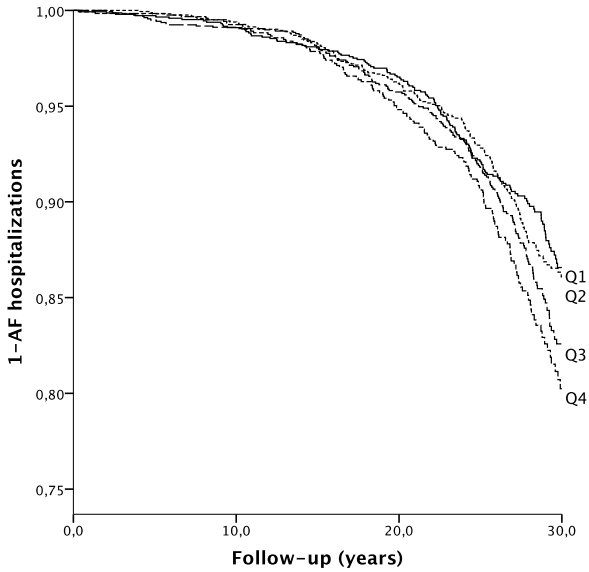

Figure 4.2.
Incidence of hospitalizations due to atrial fibrillation during a mean follow-up time of 25 years, in relation to quartiles of ceruloplasmin. Figure reproduced from **Paper I**.

4.2 Paper II

In **Paper II**, we investigated whether genetic polymorphisms in the gene encoding ceruloplasmin are associated with elevated levels of ceruloplasmin, and whether such genetic polymorphisms are also associated with incidence of AF. Clinical data and DNA was available in 3900 individuals from the MPP, and in 4455 individuals from the Malmö AF Cohort. Baseline characteristics for the two cohorts are presented in **Table 4.4.**

Table 4.4. Baseline characteristics of the study cohorts

	Malmö Preventive Project		Malmö AF Cohort	
	Incident AF			
	Yes	No	AF cases	Controls
n	520	3380	2247	2208
Age (years)	47±3	46±4	65±8	65±8
Male sex (%)	100	100	59.6	58.8
Current smoker (%)	42.3	41.8	42.4	42.6
Diabetes (%)	2.9	3.3	7.7	5.0
Systolic blood pressure (mm Hg)	131±16	127±15	147±20	148±21
BMI (kg/m^2)	25±3	25±3	26±4	27±4

AF, atrial fibrillation; BMI, body mass index
Values are presented as means ± standard deviation unless otherwise stated.

During a mean follow-up of 28.8 years, 520 men from the MPP were hospitalized with a diagnosis of AF.

Nine of 17 tested SNPs were associated with elevated levels of ceruloplasmin. Six of these SNPs remained significantly associated with ceruloplasmin when they were fitted in an additive model adjusted for potential confounding factors (**Table 4.5**). One of these SNPs, rs11708215, also showed a significant association with incidence of AF, with a HR of 1.24 (95% confidence interval (CI): 1.06–1.44) per copy of the minor C allele (P = 0.006)(**Table 4.6**). The association was independent of potential confounding factors (age, smoking, total cholesterol level, systolic blood pressure, diabetes and BMI) and was even stronger if subjects with incident heart failure or myocardial infarction prior to the diagnosis of AF were censored (HR: 1.38 per risk allele, 95% CI: 1.18–1.63, $P = 9.7 \times 10^{-5}$).

Table 4.5. Associations between single nucleotide polymorphisms (SNPs) and plasma levels of ceruloplasmin

SNP	Number of subjects	Minor allele frequency	*P*-value for relationship with ceruloplasmin (two degrees of freedom)[a]	*P*-value for relationship with ceruloplasmin (one degree of freedom)[a]	r^2
rs11708215	3831	0.12	9×10^{-10}	0.001	0.051
rs11709714	3830	0.49	8×10^{-5}	1×10^{-5}	0.046
rs11714000	3764	0.07	2×10^{-9}	0.12	0.051
rs13075891	3794	0.10	2×10^{-10}	0.002	0.052
rs13095764	3816	0.05	0.015	0.86	0.043
rs16861579	3833	0.20	0.017	0.95	0.043
rs16861582	3784	0.30	0.007	0.016	0.043
rs17195505	3806	0.04	0.005	0.026	0.043
rs17838831	3834	0.15	3×10^{-6}	0.006	0.047

[a]Adjusted for age, smoking, cholesterol level, systolic blood pressure, diabetes and BMI.

Table 4.6. Associations between single nucleotide polymorphisms (SNPs) and incidence of atrial fibrillation (AF) in three models

SNP	HR for AF[a]	HR for AF[b]	HR for AF[c]
rs11708215	1.24 (1.06–1.44) *P* = 0.006	1.23 (1.06–1.43) *P* = 0.007	1.38 (1.18–1.63) *P* = 9.7x10-5
rs11709714	1.07 (0.95–1.21)		
rs13075891	1.21 (1.00–1.46) *P* = 0.05	1.18 (0.98–1.43) *P* = 0.09	
rs16861582	1.12 (0.98–1.28)		
rs17195505	0.86 (0.61–1.20)		
rs17838831	1.15 (0.98–1.36)		

HR, hazard ratio; CI, confidence interval

[a]HR (95% CI) and *P*-value per allele, crude model.

[b]HR (95% CI) and *P*-value per allele, adjusted for age, smoking, diabetes, body mass index, cholesterol level, systolic blood pressure and ceruloplasmin level.

[c]HR (95% CI) and *P*-value per allele, after adjustments for above risk factors; subjects with a hospital diagnosis of heart failure or myocardial infarction before incidence of AF were censored (*n* = 126).

The relationship between the SNP rs11708215 and AF was tested for replication in an independent case-control study, the Malmö AF Cohort. The results from this study are presented in **Table 4.8.** We found that rs11708215 remained significantly associated with AF in this cohort, with an odds ratio of 1.13 (95% CI: 1.02-1.26) per minor allele (P = 0.02). After excluding 219 individuals with prior myocardial infarction or heart failure and adjustment for potential risk factors, the odds ratio was 1.16 (95% CI 1.04–1.29, P = 0.008).

Table 4.7. Distribution of different genotypes for the single nucleotide polymorphism (SNP) rs11708215 in the Malmö Preventive Project and the Malmö Atrial Fibrillation Cohort

SNP	TT	CT	CC
Malmö Preventive Project *n*	3080	581	170
Malmö Atrial Fibrillation Cohort *n*	2480	1265	197

Table 4.8. Association between single nucleotide polymorphism (SNP) rs11708215 and atrial fibrillation (AF) in the Malmö Atrial Fibrillation Cohort

SNP	OR (95% CI) of AF per allele	OR (95% CI) of AF per allele[a]	OR (95% CI) of AF per allele[a,b]
rs11708215	1.13 (1.02–1.26) P = 0.02	1.14 (1.03–1.27) P = 0.02	1.16 (1.04–1.29) P = 0.008

OR, odds ratio; CI, confidence interval
P-values per allele.
[a]Adjusted for age, sex, smoking, diabetes, body mass index and systolic blood pressure.
[b]Subjects with a history of myocardial infarction and/or heart failure before AF diagnosis were excluded (n = 219).

4.3 Paper III

In **Paper III**, we studied the association between RDW and incidence of AF. Subjects with a history of AF, heart failure, myocardial infarction or stroke were excluded. Subjects with missing data on any variables or with a leukocyte count above 20×10^9 per liter were also excluded. After exclusions, 27 124 individuals remained for analysis.

In order to adjust for differences in AF and RDW between men and women, the study population was divided into sex-specific quartiles of RDW, i.e. quartiles with equal fractions of men and women in each quartile.

The baseline characteristics of the study population in relation to sex-specific quartiles of RDW are presented in **Table 4.9**. High RDW was positively associated with age, smoking, use of nitrates, history of revascularization and alcohol consumption, and inversely associated with diabetes, BMI, waist circumference, diastolic blood pressure, anti-hypertensive medication, being married and a high level of education. With regard to hematological variables, increased RDW was associated with increased leukocyte count and MCV but a small decrease in hemoglobin and mean corpuscular hemoglobin concentration. The strongest predictor for RDW was smoking, followed by age and BMI (see **Paper III** for further details).

Table 4.9. Baseline characteristics of the study population in relation to sex-specific quartiles of red blood cell distribution width

MDCS (***n***=27 124)	Quartiles of red blood cell distribution width				
	Q1	**Q2**	**Q3**	**Q4**	***P*, trend**
Red blood cell distribution width, fL (men/women)	<38.2/<38.6	38.2-40.1/38.6-40.5	40.2-42.5/40.6-42.7	>42.5/>42.7	
***n* (men/women)**	2298/4189	2651/4155	2636/4216	2508/4171	
Age (years)	56.7 ± 6.9	57.7 ± 7.4	58.4 ± 7.8	59.0 ± 7.9	<0.001
Smokers (%)	14.3	21.4	29.8	47.0	<0.001
Diabetes (%)	4.4	2.9	2.0	1.9	<0.001
BMI (kg/m^2)	26.2 ± 4.0	25.9 ± 3.9	25.6 ± 3.9	25.0 ± 4.0	<0.001
Waist (cm)	84.8 ± 12.7	84.4 ± 12.9	83.7 ± 12.7	82.5 ± 13.1	<0.001
Systolic blood pressure (mm Hg)	141 ± 20	141 ± 20	141 ± 20	141 ± 20	0.25
Diastolic blood pressure (mm Hg)	86 ± 10	86 ± 10	85 ± 10	85 ± 10	<0.001

Table 4.9. Continued from previous page

	Quartiles of red blood cell distribution width				
	Q1	**Q2**	**Q3**	**Q4**	***P*, trend**
Anti-hypertensive medication (%)	16.8	16.3	15.6	15.3	0.006
Lipid-lowering medication (%)	2.6	2.1	2.4	2.1	0.15
Nitroglycerine treatment (%)	0.9	0.9	1.0	1.3	0.05
History of coronary revascularization (%)	0.3	0.5	0.7	0.6	0.006
Physical activity, (% in top quartile)	23.5	24.7	25.4	24.8	0.05
Married (%)	68.2	65.5	63.7	60.5	<0.001
High alcohol consumption (%)	3.1	3.5	4.3	6.4	<0.001
High education (%)	31.9	33.0	31.7	30.0	0.005
Leukocytes (millions/mL)	6.1 ± 1.5	6.3 ± 1.6	6.4 ± 1.7	6.7 ± 1.8	<0.001
Mean Corpuscular Volume (fL)	84.5 ± 16.8	87.9 ± 8.2	89.9 ± 7.6	93.1 ± 4.7	<0.001
Mean Corpuscular Hemoglobin Concentration (g/L)	340± 38	340 ± 46	339 ± 44	338 ± 26	0.03
Hemoglobin (g/L)	142 ± 12	142 ± 12	142 ± 12	141 ± 12	0.001
Iron intake[a] (mg/day)	15 (5-58)	15 (4-65)	15 (4-53)	15 (3-62)	<0.001[b]
B12 intake[a] (µg/day)	5.5 (0-99)	5.7 (0-103)	5.6 (0-117)	5.8 (0-121)	<0.001[b]
Folate intake[a] (µg/day)	243 (56-861)	242 (33-1086)	241 (46-879)	232 (51-855)	<0.001[b]

[a]Intake of iron, B12 and folate are presented as median (interquartile range) due to skewed distributions. All other values are means ± SD, unless otherwise stated.
[b]*P*-values for log-transformed value for intake of iron, B12 and folate.

During a mean follow-up of time of 13.6 years, 1894 subjects (1011 men and 883 women) were hospitalized with a diagnosis of AF. The associations between incidence of AF and different quartiles of RDW are presented in **Table 4.10.** and **Figure 4.3**. There was a significant association between RDW and incidence of AF (age- and sex-adjusted HR: 1.26, 95% CI: 1.11–1.44 for fourth versus first quartile of RDW). The association remained significant and was even slightly stronger after adjustments for potential confounding factors (HR: 1.33, 95% CI: 1.16–1.53 for fourth versus first quartile of RDW). The final multivariable model can be seen in **Paper III**.

Table 4.10. Incidence of atrial fibrillation in relation to sex-specific quartiles of red blood cell distribution width

	Quartiles of red blood cell distribution width					
	Q1	**Q2**	**Q3**	**Q4**	***P*, trend**	**HR per 1 SD[b]**
Red blood cell distribution width, fL (men/women)	<38.2/<38.6	38.2-40.1/38.6-40.5	40.2-42.5/40.6-42.7	>42.5/>42.7		
n	6787	6806	6852	6679		
Atrial fibrillation, *n* (%)	376 (5.5)	482 (7.1)	493 (7.2)	543 (8.1)		
Age- and sex-adjusted HR	1.00	1.17 (1.02-1.34)	1.12 (0.98-1.28)	1.26 (1.11-1.44)	0.003	1.07 (1.02-1.11)
+ risk factors[a]	1.00	1.20 (1.05-1.38)	1.18 (1.03-1.35)	1.33 (1.16-1.53)	<0.001	1.08 (1.04-1.12)

[a]Risk factors: Age, sex, smoking, diabetes, body mass index (BMI), waist circumference, diastolic blood pressure, blood pressure medication, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption, hemoglobin and leukocyte count were entered; but BMI, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption and leukocyte count were eliminated from the stepwise regression model.
[b]Hazard ratio per 1 standard deviation increase (3.43 fL)

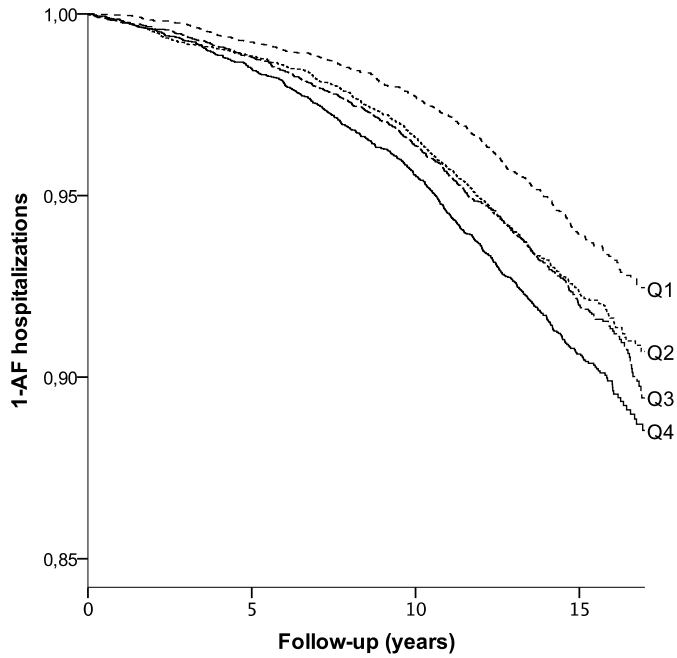

Figure 4.3.
Incidence of hospitalizations due to atrial fibrillation over a mean follow-up of 13.6 years, in relation to quartiles of red blood cell distribution width. *P* for trend across quartiles <0.001 (log rank test). Figure reproduced from **Paper III**.

Censoring individuals with incident heart failure or myocardial infarction did not alter the associations between RDW and AF. Stratified analysis showed that there was little difference between men and women, and little difference between subjects above and below median age.

High RDW could be the result of a high proportion of large cells, a high proportion of small dense cells or a combination of both. The analysis was therefore stratified by quartiles of MCV. The association between RDW and AF was more significant in the lower quartiles of MCV, with no association in subjects in the highest MCV quartile (**Table 4.11.**).

Table 4.11. Incidence of atrial fibrillation (AF) in relation to quartiles of RDW stratified according to quartile of mean corpuscular volume (MCV)

MCV (*n*=27 124)	AF (*n*)	Hazard Ratio Q1 vs. Q4 of RDW[a]	Hazard Ratio Q1 vs. Q4 of RDW, adjusted[b]	Hazard Ratio per 1 SD of RDW, adjusted[b]
Q1 (*n*=6799)	464	1.67 (1.08-2.59)	1.67 (1.07-2.60)	1.25 (1.09-1.43)
Q2 (*n*=6841)	454	1.46 (1.03-2.08)	1.47 (1.03-2.10)	1.18 (1.02-1.37)
Q3 (*n*=6737)	480	1.36 (0.97-1.91)	1.48 (1.06-2.08)	1.15 (1.01-1.31)
Q4 (*n*=6747)	496	1.43 (0.53-3.83)	1.26 (0.47-3.39)	1.01 (0.93-1.11)

[a]Adjusted for age and sex

[b]Risk factors: age, sex, smoking, diabetes, body mass index, waist circumference, diastolic blood pressure, blood pressure medication, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption, hemoglobin concentration and leukocyte count were entered.

4.4. Paper IV

In **Paper IV,** the relationship between carotid IMT and incidence of AF was explored. Subjects with a history of AF, heart failure or myocardial infarction were excluded. Clinical data was available for 4846 individuals (1944 men and 2902 women). The baseline distribution of cardiovascular risk factors in relation to sex-specific quartiles of IMT in the common carotid artery (CCA-IMT) is presented in **Table 4.12.** CCA-IMT was positively associated with age, diabetes, carotid plaque, waist circumference, systolic- and diastolic blood pressure, antihypertensive medication, LDL and hs-CRP, and inversely associated with HDL and education level.

Table 4.12. Baseline characteristics in relation to sex-specific quartiles of intima-media thickness in the common carotid artery (CCA-IMT)

MDCS (*n*=4846)	Sex-specific quartiles of CCA-IMT				
	Q1	Q2	Q3	Q4	*P*, trend
IMT range, men (mm)	0.36-0.67	0.68-0.76	0.77-0.87	0.88-2.06	
IMT range, women (mm)	0.36-0.65	0.66-0.73	0.74-0.82	0.83-1.85	
***n* (men/women)**	497/689	466/812	498/731	483/670	
Carotid plaque (%)	20.8	28.3	33.6	48.7	<0.001
Age (years)	54.8 ± 5.7	56.6 ± 5.8	58.6 ± 5.6	60.1 ± 5.4	<0.001
Smokers (%)	22.2	21.9	20.7	22.9	0.89
Diabetes (%)	5.2	6.2	7.5	10.7	<0.001
Waist circumference (cm)	82.3 ± 12.0	82.2 ± 12.4	83.5 ± 13.0	85.5 ± 13.1	<0.001
Systolic blood pressure (mm Hg)	134 ± 17	139 ± 18	143 ± 19	149 ± 20	<0.001
Diastolic blood pressure (mm Hg)	85 ± 9	86 ± 9	87 ± 9	89 ± 9	<0.001
Anti-hypertensive medication (%)	11.1	12.6	15.6	21.0	0.001
Physical activity (% in top quartile)	24.6	24.8	25.7	25.0	0.71
Married (%)	68.0	67.8	66.9	68.7	0.83
High alcohol consumption (%)	3.4	4.5	3.1	2.9	0.22
High education (%)	32.3	28.0	27.6	24.5	<0.001
Low-density lipoproteins (mmol/L)	4.0 ± 1.0	4.1 ± 1.0	4.2 ± 1.0	4.4 ± 1.0	<0.001
High-density lipoproteins (mmol/L)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	<0.001

Table 4.12. Continued from previous page.

	Sex-specific quartiles of CCA-IMT				
	Q1	**Q2**	**Q3**	**Q4**	***P*, trend**
Total leukocytes (millions/mL)	6.1 ± 2.7	5.9 ± 1.5	6.0 ± 1.6	6.2 ± 1.7	0.17
hs-CRP[a] (mg/L)	1.1 (0.6-2.3)	1.3 (0.6-2.6)	1.4 (0.7-2.9)	1.5 (0.8-3.0)	<0001[b]

[a]hs-CRP levels are presented as medians (interquartile range in brackets) due to skewed distributions.
[b]*P*-value for log-transformed hs-CRP. All other values are means ± standard deviation unless otherwise stated.

During a mean follow-up time of 15.3 years, 353 individuals (181 men and 172 women) were hospitalized with a diagnosis of AF. There was a significant relationship between incidence of AF and quartiles of CCA-IMT, (age- and sex- adjusted HR: 1.82, 95% CI: 1.30–2.55 for fourth versus first quartile of CCA-IMT)(**Table 4.13, Figure 4.4**). The associations remained significant after adjustments for cardiovascular risk factors and carotid plaque. Similar relationships were also found in the carotid bifurcation.

Table 4.13. Incidence of first atrial fibrillation hospitalization in relation to sex-specific quartiles of intima-media-thickness in the common carotid artery (CCA-IMT)

MDCS (***n*=4846**)	Sex-specific quartiles of IMT				
	Q1	**Q2**	**Q3**	**Q4**	**HR per SD[a]**
***n* (men/women)**	497/689	466/812	498/731	483/670	
Atrial fibrillation, *n* (%)	50 (4.2)	89 (7.0)	86 (7.0)	128 (11.1)	
Per 1000 person-years	2.7	4.5	4.6	7.6	
Age and sex adjusted HR	1.00	1.46 (1.03-2.07)	1.26 (0.88-1.79)	1.82 (1.30-2.55)	1.21 (1.09-1.35)
+Risk factors[b]	1.00	1.39 (0.98-1.97)	1.17 (0.82-1.66)	1.61 (1.14-2.27)	1.15 (1.03-1.29)
+Carotid plaque[c]	1.00	1.36 (0.96-1.93)	1.12 (0.79-1.61)	1.52 (1.08-2.16)	1.12 (1.00-1.25)

[a]Hazard ratios per 1 standard deviation increase of log-IMT.
[b]Risk factors: age, sex, smoking, diabetes, waist circumference, systolic blood pressure, anti-hypertensive medication, LDL, HDL, education, physical activity and hs-CRP (log-transformed).
[c]Model adjusted for all risk factors including carotid plaque.

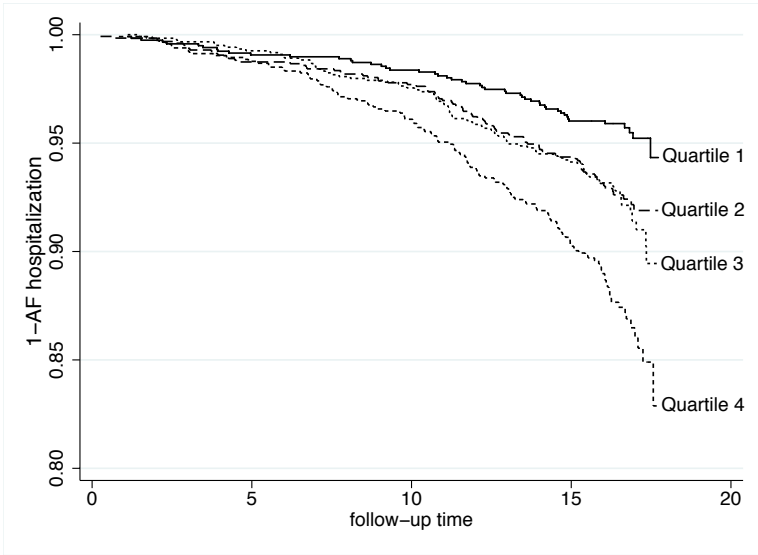

Figure 4.4.
Incidence of first atrial fibrillation hospitalization during a mean follow-up of 15.3 years, in relation to quartiles of intima-media thickness in the common carotid artery (CCA-IMT). Figure reproduced from **Paper IV**.

Sex-stratified analysis showed that the association between CCA-IMT and AF was significant among men (HR: 2.00, 95% CI: 1.24-3.23 for fourth versus first quartile) but not in women (HR: 1.08, 95% CI: 0.65-1.81 for fourth versus first quartile). A similar relationship was found for IMT in the carotid bifurcation. We did not observe any significant interaction between IMT and sex with respect to incidence of AF. The relationship between IMT and AF was essentially unchanged after censoring individuals with incident heart failure and myocardial infarction.

Chapter 5: Discussion

Many risk factors for AF have already been established. Still, the exact pathophysiological mechanisms that underlie AF remain unrevealed, and studies proposing causal factors are rare. Ceruloplasmin, RDW and carotid IMT have recently been associated with incidence of cardiovascular disease. However, all of them are sparsely studied in relation to AF.

Ceruloplasmin has been related to a number of cardiovascular diseases including myocardial infarction, heart failure and stroke.[90, 91, 104, 105] Although there are several studies showing associations between a wide range of inflammatory markers and AF, there are few prospective studies in patients without a history of AF and none of these studies have, to our knowledge, included ceruloplasmin.[60, 77-80]

RDW has emerged as a prognostic marker for a variety of cardiovascular disorders.[106, 107] Findings from a study of patients undergoing coronary angiography indicated a possible association with AF.[179] It is however unclear whether RDW is also a risk factor for AF in patients without a history of cardiovascular disease.

Increased carotid IMT has been shown to predict future cardiovascular events and has also been associated with incidence of heart failure.[138-141] Few studies have focused on the potential association between carotid IMT and incidence of AF, and the results are inconsistent.[155-157]

We studied these topics in large population-based cohorts with middle-aged subjects who were followed for up to 32 years.

5.1 Methodological considerations

5.1.1 Study design and representativity

This thesis is based on data from the two prospective urban population-based cohorts MPP and MDCS. Large sample sizes and long follow-up time enabled identification of a large number of disease events. Another advantage of the prospective study design is that information on the exposure variable was collected before the onset of disease, which lowers the risk of reverse causation and potential recall bias.[180] Apart from information on incident cardiovascular disease during follow-up, information on

exposure variables and covariates were limited to that collected during baseline examinations. It is likely that levels of exposure variables and covariates have changed before disease manifestation. Ideally, repeated measurements of these factors would be beneficial. Changed exposure levels over time would however probably bias the results towards null, suggesting that the HRs presented in these studies are underestimations of the actual risks.

It should be noted that there was a significant time-lapse between baseline examinations and blood sampling for genotyping in **Paper II**, which may have led to a bias since the patients may have died before genotyping. This would however also result in an underestimation of the actual risk associated with the identified risk gene variant.

Representativity of the MPP and MDCS cohorts has been described previously.[162, 164] The participation rate in the MPP was 71%, while the corresponding figure in the MDCS was 40.8%. The low participation rate in the MDCS makes it important to emphasize the main differences between participants and non-participants in order to assess the external validity of the findings. Overall, non-participants had in comparison with participants a higher mortality before and during the recruitment period, as well as during follow-up. Participants reported a better subjective health compared to non-participants, but were found to be representative considering smoking, overweight and socio-demographic structure.

In the MPP, about 25% of the screened subjects took part in various interventions to prevent cardiovascular disease and alcohol abuse. Population level comparisons with non-participants in similar age groups have shown that these interventions did not reduce cardiovascular mortality or morbidity. Non-participants had however a higher total and cause-specific mortality than participants of the MPP, and were also characterized by a less favorable socio-economic situation. The lower mortality rates among participants of both the MPP and the MDCS suggest a selection of more healthy subjects. Such selection bias indicates that the risk estimates found in the study populations are most likely underestimations of the true values in the general population.

5.1.2 Validity of endpoints

In all studies, information on morbidity and mortality was obtained by record linkage with the Swedish Cause of Death Register and the Swedish Hospital Discharge Register, which includes data from Malmö municipality from 1970 and forward and nationwide from 1987 and forward. A validation study has showed that a diagnosis of AF was accurate in 95%, likely in 2% and inaccurate in 3%.[26] The case validity is identical with corresponding figures from a validation study of the Danish National Patient Registry.[39] Additional validation studies of the Swedish Hospital Discharge

Register have also confirmed a high validity of myocardial infarction and heart failure as primary diagnoses.[181-183]

Some cases of AF might be treated only in primary care and are therefore not included in this study. Information on the sensitivity of AF diagnoses in the Swedish Hospital Discharge Register is not available since national databases on diagnoses from primary care still do not exist. Furthermore, electrocardiographic information was not available at baseline, suggesting that some cases might have had AF already when entering the study. However, the estimates of prevalence (1%) and incidence (4.3 per 1000 person-years) in the MDCS were largely comparable with estimates from other epidemiological studies of AF.[26] Mean age at baseline in the MPP was 47 years, and considering the fact that AF is strongly related to age, the number of cases with AF at baseline is considered to be small.

We did not discriminate between atrial fibrillation and atrial flutter, given the close relationship between these diagnoses. Neither do we know if the incident cases were classified as paroxysmal, persistent or permanent AF. The findings in this thesis can therefore only be generalized to the same broad definition of AF.

5.1.3 Validity of risk factors

The assessment of plasma protein levels with electroimmunoassay is an established and reliable method.[168] However, the estimations of plasma protein levels were based on a single blood test at baseline, and the intra-individual variation is a possible source of misclassification. A random intra-individual variation would, if anything, bias the results towards a negative finding.

Genotyping was successful in 14 of the 17 tested SNPs. The call rate was higher than 96% for all 14 SNPs. Twenty percent of the samples were run in duplicates without any inconsistences.

RDW was measured only once at baseline, and it is unclear to what extent RDW might have changed during follow-up. A study of healthy subjects demonstrated however that yearly variations in RDW are comparable to those of red blood cells and other hematological measures and that the intra-individual variability is small.[184]

IMT was measured only in the right carotid artery, whereas many other studies calculated the mean value from both sides.[148, 185] The reproducibility of the IMT measurements and prediction of cardiovascular events in the present study is however comparable with the results from other large population-based cohort studies, which scanned both sides.[138, 148, 185] It is still possible that measuring IMT on both sides could further improve the prognostic value of IMT.[186]

5.1.4 Confounding

There may be other factors, apart from the studied exposures, that are associated with both the exposure and the disease. If the prevalence between these factors differs between the groups being compared, they may confound the observed association between the disease and the studied exposure. In observational studies, confounding is always a concern due to lack of randomization. Adjustment for factors that are mediators in the causal pathway of the disease could potentially underestimate the association between the studied exposure and outcome, while leaving out genuine confounders could cause spurious relationships. Adequate adjustment for confounding is dependent on information on confounders being available and accurately measured.

The main confounders, age and sex, are considered to be measured with fairly high precision. Weight and waist circumference were measured by trained nurses, but the information was only collected at baseline. It is possible that some participants developed obesity during follow-up.

Blood pressure was only measured on a single occasion. Blood pressure is a powerful risk predictor for incident cardiovascular disease (e.g., coronary events, stroke, heart failure, etc.) in these cohorts, which should strengthen its internal validity.[187-189] But we cannot rule out that multiple measurements at baseline or updated information on blood pressure could have weakened the observed relationship between the studied risk factors and incident AF.

Baseline exposures in terms of lifestyle, medical treatment and socio-economic circumstances, were obtained from a self-administered questionnaire. The reliability and validity on such data may be questioned, as unhealthy subjects tend to underreport their unhealthy habits, and it is possible that such measurement error may have affected the observed estimates.

CRP was not used in clinical practice at the time when the MPP study started and was therefore not available in **Paper I** and **II**. It is possible that adjustment for CRP might have altered our findings.

Although we adjusted our analysis for several biological, life-style, and socio-demographic factors, we cannot rule out the possibility of residual confounding.

Mendelian randomization

The golden standard to avoid confounding is a randomized controlled study where potential confounders are equally distributed between the study groups before the individuals are randomly allocated one or other of the studied exposures. Mendelian randomization studies can under the right circumstances provide a study design comparable with randomized controlled trials.[190] Whereas in a randomized controlled trial the randomization occurs at entry into the trial, in Mendelian randomization the randomization occurs during gamete formation and conception. Mendelian randomization is based on the general assumption that the association between a

disease and a genetic polymorphism that mimics the link between a proposed exposure and disease is not susceptible to reverse causation or confounding. The assumption is based on Mendel's law of independent assortment, which states that alleles assort independently of one another during gamete formation. The net result is a natural randomization of confounding factors. Thus, if a genetic polymorphism is associated with the levels of a plasma protein, and the same polymorphism is also associated with the disease, it may be inferred that the plasma levels of the protein is causally influencing the disease. Because the quantity of the protein cannot cause the genotype but the genotype can affect the quantity of the protein. There are however a number of certain criteria that must be fulfilled when using the Mendelian randomization approach, which are further discussed in the following section.

5.2 Main findings and interpretation

5.2.1 Ceruloplasmin and atrial fibrillation

In **Paper I**, we found that a composite score of five acute phase proteins was significantly associated with incidence of AF. When the proteins were considered individually, ceruloplasmin was the only one that showed a significant association with incidence of AF. A subsequent Mendelian randomization study in **Paper II** showed that genetic polymorphisms in the promoter region of the gene encoding ceruloplasmin are associated with elevated plasma levels of ceruloplasmin. One of these polymorphisms was also reproducibly associated with incidence of AF, suggesting a causal relationship between ceruloplasmin and AF.

Several previous studies have suggested that inflammation plays a role in initiating and perpetuating AF, and that inflammation correlates with duration of AF and cardioversion success rate.[60, 77-80] There are however limited data from prospective population-based studies with patients without a history of AF.

Bruins et al. were the first to propose a direct link between inflammation and AF after observing that the increased incidence of AF after coronary bypass surgery coincided with the peak elevation of CRP.[191] Furthermore, patients with persistent AF were shown to have higher CRP levels than patients with paroxysmal AF, and both groups had higher CRP levels than controls.[59] A study of elderly Americans, followed during a mean time of 6.9 years, reported increased incidence of AF in subjects with high CRP.[77] While a study of middle-aged healthy subjects, followed over four years, reported that CRP was associated with AF only in the presence of raised levels of complement C3 and C4.[192] Reports from the Framingham Heart study and from the MDCS found an independent association between CRP and AF, but no improvement in disease discrimination.[78, 79] A Danish Mendelian

randomization study including 47 000 individuals showed that elevated plasma CRP was significantly associated with increased risk of AF, but that genetically elevated CRP was not.[60] This suggests that plasma CRP per se does not increase AF risk. However, inflammation could still play an important role in the pathophysiology of AF.

Another study from the Framingham cohort reported that a panel of 12 inflammatory markers predicted incidence of AF.[193, 194] Osteoprotegerin was the only marker that was significantly associated with AF, but these associations were attenuated after adjustments for myocardial infarction and heart failure.

To our knowledge there are no previous studies on ceruloplasmin and incidence of AF.

In order to be able to evaluate a potential causal relationship between ceruloplasmin and AF using a Mendelian randomization approach, there are three different criteria that must be fulfilled.[190] First, the instrumental variable; the ceruloplasmin genotype, must be associated with the exposure variable; plasma ceruloplasmin. In our study, baseline levels of ceruloplasmin differed significantly between genotypes for the SNP rs11708215 (P=9×10^{-10}) with two degrees of freedom, and also when fitted in an additive model (P=0.001) with one degree of freedom. This SNP was estimated to contribute 5.1% to the total variation in plasma ceruloplasmin, why the first criteria is considered to be satisfied.

Second, the ceruloplasmin genotype must be unrelated to factors that are likely to confound the association between ceruloplasmin levels and AF. Although the independent assortment of alleles at conception provides the theoretical justification for this, our models were still adjusted for age, smoking, total cholesterol, systolic blood pressure, diabetes and BMI.

Finally, the ceruloplasmin genotype must not be associated with AF in any other pathway than via plasma ceruloplasmin, if potential confounding factors are also taken into account. This third criterion is hard to prove. However, the SNPs in our study were selected upon their previously known associations with the Ceruloplasmin *(CP)* gene. Furthermore, the SNP rs11708215 is located in the promoter region of the *CP* gene at chromosome 3q23-24, and is therefore assumed to be functional.[195] In addition, we were able to replicate our findings in an independent cohort, which reduces the possibility that the associations are due to population stratification. The significant association between the SNP and AF remained after taking plasma level of ceruloplasmin into account, suggesting that an additional mechanistic pathway might be present. Another explanation, which has also been put forward in other Mendelian randomization experiments with larger effect estimates than expected, might be that the association between rs11708215 and AF represents a lifelong genetic risk, whereas the association between ceruloplasmin and AF represents a risk that is present over a more limited time frame.[196, 197] There is also the possibility that canalization (also known as developmental compensation) may have occurred to some extent, which means that the effect of life-long elevation of ceruloplasmin may be buffered by

compensatory responses that have evolved somewhere between conception and study entry.[190, 198]

The promoter region where rs11708215 is located is also strongly associated with several transcription factors including nuclear factor kappa B (NFκB). NFκB is thought to be involved in altering ion channel transcription, thereby causing electrical remodeling of the heart.[199] It has also been shown that common genetic variants of the gene encoding the receptor for IL-6 are reproducibly associated with AF risk.[200] Interestingly, NFκB is activated by cytokines, including by binding of IL-6 to its receptor.[201, 202]

The underlying mechanism for ceruloplasmin causing AF is however uncertain. Ceruloplasmin has been shown to have multiple roles in copper transportation, iron homeostasis, coagulation, angiogenesis and defense against oxidative stress.[104] The copper-dependent ferroxidase activity, by which Fe^{2+} (ferrous iron) is oxidized to Fe^{3+} (ferric iron), is thought to be responsible for the ability of ceruloplasmin to protect from free-radical proteolysis, inhibit lipid oxidation, and block protein- and DNA-damage.[96, 100, 203-205] By contrast, ceruloplasmin has been reported to induce LDL oxidation in smooth muscle cells and endothelial cells by a superoxide-dependent mechanism.[101] Removal of one of ceruloplasmin's copper atoms completely blocked oxidant activity. In addition, removal of loosely bound copper from ceruloplasmin may be induced by reactive oxidative species. The free copper may catalyze more reactive oxidative species and may also give direct effects on signal transduction and transcription.[206] Reactive oxygen species may cause ectopic firing as well as atrial electrical and structural remodeling by altering ion channels, disturbing Ca^{2+} homeostasis and remodeling gap junctions.[207, 208] If ion channels are altered and action potential is decreased by reactive oxygen species, this could cause inexcitable areas, which could promote re-entry arrhythmias.[207]

Whether ceruloplasmin is a protective or a pathological protein seem to be dependent of the oxidative status. However, it remains unclear whether an elevated level of ceruloplasmin is a cause of oxidative stress or a protective reaction against oxidative stress.

Further mechanistic studies are warranted to elucidate the causal pathway between ceruloplasmin and AF. Future studies will also need to address whether these associations are to be found in populations with other ancestries than European.

5.2.2 Red blood cell distribution width and atrial fibrillation

In **Paper III**, we found that increased RDW was significantly associated with incidence of AF in middle-aged subjects without a history of cardiovascular disease. The risk estimates remained significant and even showed a slight increase after adjusting for potential cardiovascular confounders. The strongest predictors of RDW were smoking, age and BMI.

Increased RDW has been found to be associated with a large number of cardiovascular diseases such as coronary artery disease, stroke, peripheral artery disease, heart failure, pulmonary embolism, and pulmonary arterial hypertension.[106] Studies have also shown that RDW is associated with a large number biomarkers and life style factors associated with cardiovascular disease.[107] The potential associations with AF are however sparsely explored. The findings of a study of patients undergoing coronary artery bypass grafting (CABG) indicated a possible association between RDW and AF.[179] Another recent study of CABG patients reported that preoperative RDW levels were found to be significantly higher in patients who developed AF than in those who did not.[209] RDW was also associated with paroxysmal AF and non-valvular AF in two separate case-control studies.[210, 211] To our knowledge, our study is the first large prospective study to show that RDW is a risk factor for AF in subjects without a history of cardiovascular disease.

Comparisons between different studies of RDW are complicated by the fact that there are different ways to express RDW indices. Automated blood cell counters calculate RDW from the red blood cell volume histogram.[109] RDW is often expressed as a percentage coefficient of variation (CV), and is calculated by dividing the standard deviation of the red blood cell volume with the mean corpuscular volume (MCV). The result is multiplied by 100 in order to be expressed as a percentage. RDW is expressed as CV percentage for several manufacturers (Abott, ABX, Beckman Coulter, and Siemens).[109] However, RDW may also be expressed as a direct measurement of the width of the distribution, which gives a measure in fL that is independent of MCV.[109, 212] This is an advantage when the relationship between RDW and outcome is studied at different levels of MCV. This index variant is obtained by the Sysmex automated blood cell counter, which is the model being used in **Paper III**. The reference intervals are different between different manufacturers and may vary even between different models from the same manufacturer.[109, 110, 212] The International Council for Standardization in Haematology has suggested a standardized statistical method for the analysis of RDW.[213] However, at present any clinical use of RDW must be evaluated by the comparison with reference values established for each model of analyzer.

Our findings suggest that RDW is a novel risk factor for incidence of AF. The mechanism behind this relationship is unclear but could result from a direct effect of changes in erythrocyte volume and function on the heart, or may reflect other pathophysiological processes acting independently on both erythrocytes and the heart. A direct effect of altered erythrocyte function on the heart seems reasonable, as erythrocytes both carry oxygen to tissues and have an important role in cardiovascular regulation through release of extracellular nucleotides and other mediators.

Inflammation and oxidative stress have been suggested to be associated with increased RDW as these states may reduce red blood cell survival.[214] Inflammation may also increase RDW by altering iron metabolism or by inhibiting the production of, or response to erythropoietin.[215] It was found that higher levels of

proinflammatory cytokines were associated with higher erythropoietin levels among non-anemic older adults, but lower erythropoietin levels in anemic persons.[216] This suggests that in a proinflammatory state the increase in erythropoietin is a compensatory mechanism for maintaining normal hemoglobin levels and that anemia occurs when the compensatory increment in erythropoietin production is unsustainable. Proinflammatory cytokines are also thought to disrupt erythropoiesis by inhibiting the proliferation of erythroid progenitor cells and down-regulating erythropoietin receptor expression.[215]

We found RDW to be associated with high leukocyte count. However, adjustments for leukocytes in our study did not alter the association between RDW and AF, suggesting that inflammation might play a minor role in the mechanistic pathway between RDW and AF.

Erythropoiesis has been shown to be related to activation of the renin-angiotensin system (RAS) and the RAS has been suggested to play a significant role in the pathogenesis of AF in experimental studies.[217-219] Hence, the RAS may be a possible link between RDW and AF and pharmacological inhibition of the RAS has shown some promise to reduce new onset AF in patients at increased risk.[18, 220, 221] Indeed, studies have reported associations between high RDW and impaired renal function.[215, 222] It was suggested that these associations were at least partly mediated through underlying chronic inflammation, which is common in patients with chronic kidney disease and typically leads to abnormal erythrocyte maturation. We did however not have information on glomerular filtration rate in our study, which is a limitation.

Malnutrition and deficiency of iron, B12 or folate are other factors associated with high RDW via effects on erythropoiesis.[115] Although intake of iron, B12 and folate all differed significantly across quartiles of RDW in our study, and B12 and folate remained independent predictors of RDW in the multivariable linear regression model, adjustment for these variables had marginal effect on the association between RDW and AF.

High RDW could be due to either a high proportion of large cells, indicating high erythrocyte turnover, or high proportion of small dense cells, or both of these possibilities. In our study, the association between RDW and incidence of AF was mainly observed in subjects with a low MCV, and RDW was not related to incidence of AF in subjects with MCV in the top quartile. This suggests that factors associated with high MCV, such as alcohol intake and deficiency of folate or B12, are unlikely to be the cause of the observed association between RDW and AF.

Since RDW is also a risk factor for heart failure and myocardial infarction, the association between RDW and AF could possibly be mediated through these diseases. However, censoring incident cases of heart failure and myocardial infarction during follow-up did not alter the risk estimates, indicating that the observed associations are independent of heart failure and myocardial infarction. Subclinical forms of these diseases could however still play a role.

Although we conclude that RDW is a risk factor for incident AF, we do not know the underlying causal relationship. RDW could be a marker of poor function and adverse properties of the red cells per se, or associated with other disease promoting factors. More studies are needed to determine why raised RDW is associated with AF.

5.2.3 Carotid intima-media thickness and atrial fibrillation

In **Paper IV**, we found that carotid IMT was associated with incidence of AF in middle-aged subjects without a history of cardiovascular disease. The risk estimates remained significant after adjustment for several cardiovascular risk factors. Multivariate analysis showed that both carotid IMT and carotid plaque were significantly associated with incident AF. Sex-stratified analysis indicated that the associations were significant in men but not in women.

Several large population-based studies have shown that carotid IMT is strongly associated with incident myocardial infarction, coronary heart disease and stroke.[139, 140, 185, 223-225] Few studies have however explored whether carotid IMT is a risk factor for AF, and the results are inconsistent. The Cardiovascular Health Study (CHS) found no association between carotid IMT or carotid stenosis and incidence of AF.[155, 156] In contrast, the Rotterdam Study showed a significant association between carotid IMT and incidence of AF, especially among women, and another recent population-based cohort study found carotid atherosclerosis to be a strong predictor of incident AF in both men and women.[152, 157] In addition a recent case-control study found carotid IMT to be associated with lone AF.[158]

It should be noted that the CHS included self-reported AF, while the other two studies, as well as our own study, only included patients with a physician's diagnosis of AF. Mean age also differed significantly between the studies, being lowest in our study and highest in the CHS. As AF is strongly age-related, it is possible that death is a competing risk that might have reduced associations between carotid IMT and AF in older age groups. This might also explain why the Rotterdam Study found a stronger relationship in women, while we observed a stronger relationship in men. However, there were no evidences of interaction between sex and IMT in our study or in the Rotterdam Study, why there is still the possibility that the observed sex-differences are simply due to chance.

There are several potential mechanisms that might explain the association between carotid IMT and AF. IMT likely reflects hypertensive medial hypertrophy and it is possible that IMT could be seen as a marker of the cumulative effect of hypertension or a physiological adaptation to changes in blood flow and wall tension.[148] Arterial stiffness and elevated pulse-pressure have been demonstrated to predispose to the development of AF.[45, 226] Increased systolic cardiac afterload may give rise to cardiac remodeling involving ventricular hypertrophy, impaired

ventricular relaxation, increased intra-atrial pressure, and left-atrial enlargement and dysfunction.[227-229] It is however notable that carotid IMT remained significantly associated with AF in our study after adjustment for blood pressure and anti-hypertensive medication.

Atherosclerosis in the coronary arteries might also cause ischemia and transitory hypoperfusion in the atrium, resulting in fibrosis and subsequent development of AF.[230] It has however been debated whether IMT could be used as a marker for atherosclerosis or not. Plaque area or plaque volume has been suggested as more accurate measures of atherosclerosis than IMT.[231-233] In our study, carotid IMT and carotid plaque were significantly associated with AF independently from one another, suggesting that carotid IMT and plaque might affect AF through partly different mechanisms. These findings were in concordance with previous studies from the MDCS cohort showing that carotid IMT was significantly associated with incident stroke even in the absence of carotid plaque.[140] Hence, it is possible that IMT and plaque might reflect different biological aspects of atherogenesis.

Previous studies from the MDCS cohort have also shown that carotid IMT is a risk factor for incident myocardial infarction and heart failure.[139, 141] However, risk estimates remained essentially unchanged in our study after censoring incident cases of myocardial infarction and heart failure during follow-up. This supports the conclusion that carotid IMT is a risk factor for incidence of AF.

Chapter 6: Conclusions

In this thesis, inflammation-sensitive proteins, red blood distribution width and carotid intima-media thickness were studied in relation to incidence of atrial fibrillation in the general population. From a series of analyses in the population-based cohorts Malmö Preventive Project and Malmö Cancer and Diet Study the following conclusions were drawn:

- A score of five inflammation sensitive proteins (ceruloplasmin, fibrinogen, haptoglobin, orosomucoid and α_1-antitrypsin) were significantly associated with incidence of atrial fibrillation in middle-aged men.
- Plasma levels of ceruloplasmin were associated with incidence of atrial fibrillation.
- Genetic polymorphisms in the promoter of the ceruloplasmin gene were associated with elevated plasma levels of ceruloplasmin.
- One of these polymorphisms was also associated with incidence of atrial fibrillation, suggesting a causal relationship between ceruloplasmin and atrial fibrillation.
- Red blood cell distribution width was a risk factor for incidence of atrial fibrillation in middle-aged men and women from the general population.
- Carotid intima-media thickness was associated with incidence of atrial fibrillation in middle-aged subjects from the general population, after adjustment for several potential confounding factors.

Taken together the results from this project indicate that low-grade inflammation, irregular red blood cell volume, and subclinical atherosclerosis are all potential indicators of an increased risk of future hospitalization due to atrial fibrillation. Further studies are needed to investigate the underlying mechanisms and in particular elucidate the causal pathway between ceruloplasmin and atrial fibrillation.

Chapter 7: Perspectives

Ceruloplasmin, RDW and carotid IMT are all factors that could potentially help to identify individuals at risk for AF. The indication of a causal link between ceruloplasmin and AF gives hope of discovering new drug targets and adds further possibilities of early risk stratification. Risk scores for prediction of AF including conventional clinical risk factors have so far shown limited predictive accuracy.[194, 234, 235] It should therefore be of great interest to add other risk factors to these scores in attempts to improve the predictive capacity. Of note, ceruloplasmin, RDW and IMT are not only risk factors for AF but have also shown associations with other cardiovascular diseases. This lack of specificity could cause problems if the risk factors are to be used as predictive tools in clinic. The results for RDW suggest that the properties and function of the red cells could be of great importance, which so far has received limited scientific attention. RDW is already included as a component of the Intermountain Risk Score IMRS, a predictor of mortality that has shown associations with incidence of a number of cardiovascular diseases including AF.[179] Furthermore, current evidence suggests that IMT may add useful information on vascular risk for people at intermediate risk according to the Framingham risk score, but findings are inconsistent between studies and the improvement in risk classification is in some cases modest.[143] In addition, current guidelines advice against screening of known AF genes.[73] However, rather than using clinical risk factors, biomarkers or genetic variants alone, one should seek to combine each of these risk factors to improve the detection of AF. The quest for further refinements in clinical risk stratification schemes to improve the prediction of high risk AF subjects who develop complications is perhaps an old concept that is now overtaken by new developments in thromboprophylaxis and the continuing need for everyday practicality and simplicity. Current guidelines advocate that the focus in clinical practice should rather be on identifying truly low risk patients who do not need any antithrombotic therapy.[236] It remains to be evaluated whether the risk factors studied in this thesis could be used in the identification of such low risk patients. Nonetheless, our findings provide new insights in the complex pathophysiology of AF, and an awareness of the burden of AF in the urban population.

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Paper I

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CARDIOVASCULAR DISEASE

Inflammation-sensitive proteins and risk of atrial fibrillation: a population-based cohort study

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Abstract Low-grade inflammation has been repeatedly associated with cardiovascular diseases but the relationship with incidence of atrial fibrillation (AF) remains unclear. We explored the association between elevated plasma levels of inflammation-sensitive proteins (ISPs) and incidence of AF in a population-based cohort. Plasma levels of five ISPs (fibrinogen, haptoglobin, ceruloplasmin, α_1-antitrypsin and orosomucoid) and two complement factors (C3 and C4) were measured in 6,031 men (mean age 46.8 years) without history of myocardial infarction, heart failure, stroke or cancer. Incidence of hospitalizations due to AF during a mean follow-up of 25 years was studied both in relation to individual inflammatory proteins and the number of elevated ISPs. During follow-up, 667 patients were hospitalized with a diagnosis of AF. After adjustment for potential confounding factors, the hazard ratios (HR) for AF were 1.00 (reference), 1.08 (95% CI: 0.88–1.31), 1.07 (CI: 0.84–1.36), and 1.40 (CI: 1.12–1.74), respectively, in men with none, one, two and three or more ISPs in the 4th quartile (P for trend = 0.007). Ceruloplasmin was the only individual ISP significantly associated with incidence of AF after adjustment for confounding factors (HR 1.17 per standard deviation, 95% CI: 1.08–1.26). In conclusion, a score of five ISPs was associated with long-term incidence of hospitalizations due to AF in middle-aged men. Of the individual ISPs, a significant association was observed for ceruloplasmin, a protein previously associated with copper metabolism and oxidative stress.

Keywords Atrial fibrillation · Inflammation · Epidemiology

Abbreviations

AF	Atrial fibrillation
BMI	Body mass index
CRP	C-reactive protein
CVD	Cardiovascular disease
ISPs	Inflammatory sensitive proteins
LDL	Low-density lipoprotein

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in the general population, and a major cause of morbidity and mortality [1–4]. Lifetime risk for development of AF has been estimated to 1 in 4 in individuals aged 40 years or more [5]. High age, hypertension, obesity, myocardial infarction and heart failure are major risk factors for AF in the general population [6–8].

It is now widely accepted that systemic low-grade inflammation is a risk factor for cardiovascular disease (CVD). Many studies have reported relationships between raised plasma levels of inflammatory markers and incidence of myocardial infarction and stroke [9–13]. In studies of patients with AF, inflammation has been associated with perpetuation of AF and poor conversion rates [14]. However, whether inflammation is associated with incidence of AF remains unclear. C-reactive protein (CRP) was associated

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with increased incidence of AF in a study of elderly Americans [15] and in the Framingham study [16]. A study of healthy subjects reported that CRP was associated with incidence of AF, but only in the presence of high C3 and C4 levels [17]. A recent study reported that a panel of 12 inflammatory markers predicted incidence of AF. However, no individual marker predicted AF when incidence of myocardial infarction was taken into account [18].

Studies from the Malmö Preventive Study, Sweden, have shown that elevated plasma levels of five inflammation-sensitive proteins (ISPs; fibrinogen, haptoglobin, ceruloplasmin, α_1-antitrypsin and orosomucoid) are risk factors for myocardial infarction, heart failure and stroke. These proteins have been associated with incidence of cardiovascular disease, both when studied individually, and as a composite score of inflammation [9, 19]. The purpose of this population-based cohort study was to explore whether these ISPs are associated with incident AF during a long-term follow-up among middle-aged men without history of CVD.

Methods

Between 1974 and 1984, 22444 men, aged 26–61 years, participated in a screening program for the detection of individuals at high risk for CVD. The participation rate was 71%. As a part of the program, plasma levels of five ISPs were determined for a random sample of 6,193 men. After exclusion of men with a history of cancer, atrial fibrillation, heart failure, myocardial infarction or stroke, and 17 subjects with missing data on blood pressure and cholesterol, 6,031 men remained. Mean age was 46.8 ± 3.7 years.

The health service authority of Malmö approved and funded the screening program. The regional ethics committee approved the data linkage with the Swedish population- and hospital registers. Participants provided informed consent.

Baseline examination

Blood samples were drawn after an overnight fast. Levels of serum cholesterol were determined using standard methods at the laboratory of Malmö University hospital. Diabetes was defined as fasting whole blood glucose ≥6.1 mmol/l, 2-h post-glucose load ≥ 10.0 mmol/l, or self-reported diabetes [20]. Body mass index (BMI) was calculated as weight/height^2. Blood pressure (mm Hg) was measured twice in the right arm using a sphygmomanometer after 10 min of rest. Data on smoking habits and antihypertensive treatment was ascertained from a questionnaire. Physical inactivity in spare time was assessed using the question 'Are you mostly engaged in sedentary activities in spare time, for example, watching TV, reading, going to the movie?'

Subjects who reported a physician's diagnosis of angina pectoris or who used nitrates were considered to have angina pectoris. The question 'Do you use any heart drugs?' assessed use of heart medications.

Alcohol consumption was assessed using the modified shortened version of the Michigan Alcoholism Screening Test [21]. Men with more than two of nine affirmative answers were considered to have high alcohol consumption.

ISPs, complement factors

Plasma levels of five ISPs and two complement factors were assessed by means of an electroimmunometric assay [22]. The analysis was performed consecutively at the time of study entry. The lower detection limits were 20 mg/l for ceruloplasmin, 50 mg/l for α_1-antitrypsin and 350 mg/l for orosomucoid, haptoglobin and fibrinogen, respectively. C3 and C4 were originally expressed as the percentages of the mean values from a reference population of blood donors. In order to facilitate the interpretation of the C3 and C4 values, the percentages have been converted into grams/litre (C3 100% = 0.98 g/l, C4 100% = 0.20 g/l) [23]. The inter-assay coefficient of variation was <5% [22].

In accordance with previous studies from this cohort, relations with incidence of AF are presented in relation to the number of raised ISPs (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, and ceruloplasmin) in the top quartile [9, 19, 23]. We have previously shown that all ISPs are associated with incidence of CVD, with largely the same relative risks for all individual ISPs [9]. The reliability in terms of internal consistency was fully adequate for this composite score (Cronbach's a = 0.65). Median values for the ISPs were 3.46 g/l (interquartile range, 3.0–4.0 g/l) for fibrinogen, 0.80 g/l (0.67–0.93 g/l) for orosomucoid, 1.28 g/l (1.09–1.42 g/l) for a_1-antitrypsin, 1.30 g/l (0.89–1.75 g/l) for haptoglobin, and 0.30 g/l (0.26–0.35 g/l) for ceruloplasmin.

Of the complement factors, median (interquartile range) was 0.98 g/l (0.86–1.15) for C3 and 0.23 g/l (0.18–0.28) for C4.

Follow-up

All men were followed from the baseline examination until first hospitalization due to AF, death, emigration from Sweden, or December 31, 2006. AF was defined as a diagnosis of either atrial fibrillation or atrial flutter as in previous studies [6–8, 24], given the close relationship between these diseases [25]. Subjects were considered to have AF if diagnosed with a primary or contributory hospital discharge diagnosis code 427.92 (ICD-8), 427D

(ICD-9) or I48 (ICD-10). Since inflammation is associated with increased incidence of myocardial infarction, which could cause AF, we performed secondary analyses, in which cases with incident myocardial infarction (ICD codes I21 or 410) during follow-up were followed until the day of the infarction and censored after that. The Swedish Hospital Discharge register was used for case-retrieval. A validation study has shown that case misclassification of AF in national registers is small [8].

Statistics

One-way ANOVA and Pearson χ^2 were used to compare men with and without AF during follow-up. Cox proportional hazards regression was used to compare incidence of AF between men in different categories of ISPs and to estimate hazard ratios (HR) adjusted for potential confounding factors. In accordance with previous studies [9, 19], incidence of AF was first analysed in relation to the number of elevated ISPs, and secondly, in relation to the individual proteins. The fit of the proportional hazards model was confirmed by plotting the AF incidence rates over time for different categories of risk factors, and by introducing time-dependent variables in the model. The model was adjusted for cardiovascular risk factors associated with AF. All analyses were performed in SPSS version 17 or in PASW Statistics 18 (SPSS Inc, Chicago, Illinois, USA).

Results

During a mean follow-up of 25 years, 667 men (11%, 4.4 per 1,000 person years) were diagnosed with AF. Baseline levels of ceruloplasmin were significantly higher in men who were hospitalized due to AF during follow-up ($P < 0.05$). Age, BMI, blood pressure, anti-hypertensive medication, diabetes, angina, and high alcohol consumption were also significantly higher in men who developed AF (Table 1).

Incidence of atrial fibrillation in relation to ISPs

Incidence of AF was significantly associated with the number of elevated ISPs (Table 2). The ISPs were elevated several years before the incident events of AF (Figs. 1, 2). The relationship remained significant after adjustments for possible confounding factors. Other independent predictors

Table 1 Baseline characteristics in subjects with and without atrial fibrillation during follow-up

	Atrial fibrillation		*P*
	No ($n = 5364$)	Yes ($n = 667$)	
Age (years)	46.7 ± 3.7	47.8 ± 3.4	<0.001
BMI (kg/m^2)	24.9 ± 3.3	25.7 ± 3.5	<0.001
Systolic BP (mm Hg)	128.7 ± 15.5	132.2 ± 16.4	<0.001
Diastolic BP (mm Hg)	86.9 ± 10.0	89.1 ± 10.7	<0.001
Anti-hypertensive medication (%)	4.2	7.8	<0.001
Cholesterol (mmol/l)	5.69 ± 1.04	5.73 ± 0.98	0.37
Triglycerides (mmol/l)[#]	1.58 ± 1.11	1.58 ± 0.90	0.87
Smokers (%)	48	48	0.33
Diabetes (%)	4.7	4.9	<0.001
Angina (%)	1.2	1.6	<0.001
Heart drug (%)	0.4	0.3	0.72
Physical inactivity (%)	57	54	0.058
High alcohol consumption (%)	13	15	<0.001
Heart rate[#]	68 ± 10	67 ± 10	0.19
ISPs (g/l)			
Fibrinogen	3.5 ± 0.80	3.6 ± 0.80	0.12
Haptoglobin	1.38 ± 0.67	1.39 ± 0.73	0.76
Ceruloplasmin	0.316 ± 0.07	0.322 ± 0.07	0.015
α_1-Antitrypsin	1.27 ± 0.27	1.28 ± 0.27	0.50
Orosomucoid	0.82 ± 0.20	0.83 ± 0.21	0.18
Complement factors			
C3[#]	103.0 ± 22.7	103.8 ± 22.6	0.43
C4[#]	120.9 ± 40.2	120.6 ± 40.2	0.89

[#] Results on C3, C4, triglycerides and heart rate was based on 5,817, 5,813, 6,019 and 5,984 subjects, respectively

Table 2 Incidence of atrial fibrillation in relation to number of elevated ISPs

	Number of elevated ISPs				*P* for trend
	None $n = 2437$	One $n = 1555$	Two $n = 891$	Three or more $n = 1148$	
Atrial fibrillation *n* (%)	253 (10.4)	173 (11.1)	96 (10.8)	145 (12.6)	
AF per 1,000 person-years	4.00	4.46	4.48	5.54	
Age adjusted HR	1.0	1.16 (0.96–1.41)	1.19 (0.94–1.51)	1.61 (1.31–1.98)	<0.001
+ Risk factors[a]	1.0	1.08 (0.88–1.31)	1.07 (0.84–1.36)	1.39 (1.12–1.74)	0.007

[a] Hazard ratios (95% CI) adjusted for age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption

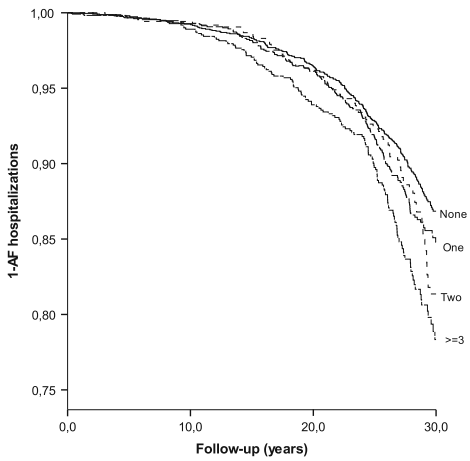

Fig. 1 Incidence of hospitalizations due to AF over a mean follow-up of 25 years, in relation to the number of elevated ISPs

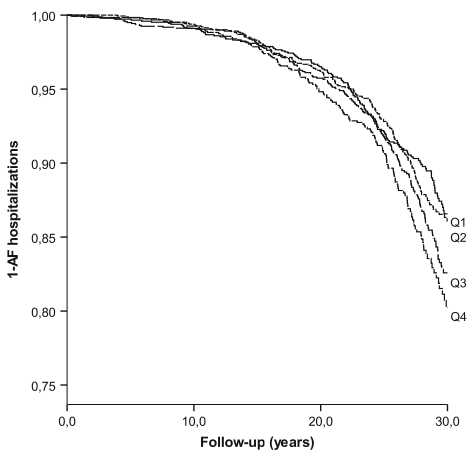

Fig. 2 Incidence of hospitalizations due to AF over a mean follow-up of 25 years, in relation to quartiles of ceruloplasmin

for AF in the risk factor adjusted analysis were age (HR per year: 1.09, 95% confidence interval (CI): 1.07–1.12), BMI (HR per kg/m^2: 1.06, 95% CI: 1.04–1.09), systolic blood pressure (HR per mm Hg: 1.008, 95% CI: 1.003–1.013), anti-hypertensive medication (HR: 1.38, 95% CI 1.02–1.86), smoking (HR: 1.23, 95% CI: 1.04–1.46) and high alcohol consumption (HR: 1.44, 95% CI: 1.16–1.79). No significant relationship was observed for cholesterol, diabetes or physical activity.

In secondary analyses, censoring was performed at incident myocardial infarction. A total of 528 men had incident AF without previous or concomitant myocardial infarction. The association between ISPs and AF was essentially unchanged. After adjustment for risk factors, the HRs were 1.00 (reference), 1.13 (95% CI: 0.91–1.41), 1.16 (95% CI: 0.89–1.53) and 1.51 (95% CI: 1.18–1.93), respectively, for men with none, 1, 2, and 3 or more ISPs in the top quartile (P for trend = 0.002).

Incidence of atrial fibrillation in relation to individual ISPs

When expressed as age-adjusted HR per standard deviation increase of plasma concentration, all ISPs except α_1-antitrypsin showed significant associations with incidence of AF. After adjustments for confounding factors, ceruloplasmin was the only protein which remained significantly associated with incidence of AF (Table 3). This association was unchanged when subjects with myocardial infarction were censored from the analysis.

Complement factors and incidence of AF

There was no significant association between complement C3 or C4 and incidence of AF (Table 3). Incidence of AF was explored for different combinations of high complement C3 or C4 and high ISP levels. There was no

Table 3 Incidence of atrial fibrillation in relation to individual inflammation-sensitive proteins and complement factors

	Age adjusted HR	*P*	+ Risk factors[a]	*P*
Fibrinogen	1.13 (1.05–1.22)	0.002	1.07 (0.99–1.16)	0.11
Haptoglobin	1.13 (1.05–1.22)	0.001	1.08 (1.00–1.17)	0.056
Ceruloplasmin	1.17 (1.08–1.26)	<0.001	1.13 (1.04–1.22)	0.003
α_1-Antitrypsin	1.07 (0.99–1.16)	0.087	1.04 (0.96–1.12)	0.34
Orosomucoid	1.13 (1.05–1.22)	0.001	1.06 (0.98–1.15)	0.14
C3	1.10 (1.02–1.19)	0.020	1.01 (0.93–1.09)	0.90
C4	1.01 (0.93–1.09)	0.80	0.96 (0.89–1.05)	0.37

Presented as hazards ratios (HR) per standard deviation increase of the plasma protein concentration

Standard deviation values for the plasmaproteins were 0.80 g/l for fibrinogen, 0.68 g/l for haptoglobin, 0.067 g/l for ceruloplasmin, 0.27 g/l for α_1-antitrypsin, 0.20 g/l for orosomucoid 0.22 g/l for C3, 0.080 g/l for C4

[a] Adjusted for age, BMI, systolic blood pressure, anti-hypertensive medication, angina, total cholesterol, smoking, diabetes, physical activity and alcohol consumption

indication that C3 or C4 modified the relationship between the ISPs and incidence of AF.

Discussion

It is now widely accepted that systemic low-grade inflammation is a risk factor for cardiovascular disease and many studies have reported relationships between inflammatory markers and incidence of myocardial infarction and stroke. However, whether inflammation is associated with incidence of AF remains unclear. The present study showed that a composite score of five acute phase proteins was significantly associated with incidence of AF. When considered individually, only ceruloplasmin showed a significant association with AF. These relationships remained after adjustment for several potential confounding factors.

Recent studies of different groups of patients suggest that inflammation has a role in initiating and perpetuating AF, and that inflammation correlates with duration of AF and cardioversion success rate [14]. However, there is limited data from prospective population-based studies. In general, the relationship between inflammation and AF does not seem to be as strong as the associations with myocardial infarction, heart failure and stroke [9, 19].

Schnabel et al. recently reported that a panel of 12 inflammatory markers predicted incidence of AF. They found only osteoprotegerin to be significantly associated with AF, and no individual marker predicted AF when incidence of myocardial infarction was taken into account. Fibrinogen, which was the only biomarker also examined in the present study, did not show any significant relationship with AF in that study [18]. A study of 5,806 men and women (mean age 73 ± 5 years) who were followed during a mean time of 6.9 years reported increased incidence of AF in subjects with high C-reactive protein (CRP) [15]. A study of 1,032 middle-aged healthy subjects, followed over 4 years, reported that CRP was associated with AF only in the presence of raised levels of complement C3 [17]. Recent reports from the Framingham Heart study and from the Malmö Diet and Cancer study found an independent association between CRP and AF, but no improvement in disease discrimination [16, 24]. Mean age of these studies were approximately 58 years, as compared to the mean age of 46.8 years in the present study. A recent 'Mendelian randomization study' including 47,000 individuals showed that elevated plasma CRP was associated with increased risk of AF. Importantly, genetically elevated CRP levels were not associated with AF in that study. This suggests that plasma CRP per se does not increase AF risk [26]. However, there could still be an important role for other markers of the underlying systemic inflammation. To our knowledge, there is no previous study on ceruloplasmin in relation to incidence of atrial fibrillation.

The results in the present study indicate that elevated levels of acute-phase proteins, in particular ceruloplasmin, may precede AF. The association between AF and ceruloplasmin is markedly stronger than the relationship with the other ISPs in our study. Ceruloplasmin has strong oxidant effects and has been shown to induce LDL oxidation [27]. The protein contains seven copper atoms per molecule, accounting for approximately 95% of the total circulating copper in healthy adults [28]. Removal of one of the copper atoms completely blocks the oxidative activity of ceruloplasmin [27]. Turnlund et al. [29] showed that long-term high copper intake resulted in significantly elevated ceruloplasmin enzymatic activity. Emerging evidence implicates a role of oxidative stress in the initiation and maintenance of AF, in which oxidative stress may cause remodelling of the atrium [30–32]. This could be a possible link between ceruloplasmin and incidence of AF.

It has been suggested that raised levels of inflammatory markers may not be a reflection of the arrhythmia itself but a result of confounding by the underlying cardiac pathology [33]. However, mean age was rather low in this study, individuals with known cardiovascular diseases were excluded and incidence of AF occurred many years after the baseline examination. It therefore seems unlikely that the present results can be explained by reverse causation. Several pieces of evidence also indicate that inflammation could have a causal role in the initiation and perpetuation of AF, possibly by inducing structural remodeling of the atrium. This hypothesis is partly based on histological studies which demonstrated inflammatory infiltrates in AF patients and in animal models of AF [34–36].

Hypertension and obesity are the two major risk factors for AF, and inflammation has been suggested as a mediator of these associations [37]. Although the model was adjusted for baseline levels of these factors, it is still possible that some men developed hypertension and obesity during the follow-up period. Longitudinal studies have shown that low-grade systemic inflammation is a risk factor for development of hypertension and obesity [38, 39].

Some methodological issues need to be considered. The endpoints of this study were retrieved using the Swedish hospital discharge register. Some cases of atrial fibrillation might only be treated in primary care, and are therefore not included in this study. However, the estimates of prevalence and incidence in a recent validation study from this population were largely comparable with other epidemiological studies of AF [8]. Since all diagnoses were settled during hospital stay, most cases are assumed to be valid. A validation study of cases retrieved from the Swedish national registers showed that the validity of the diagnosis is very high [8].

Another limitation is that electrocardiographic information at baseline was not available, suggesting that some cases may have had AF already when entering the study. However, since mean age at baseline was only 47 years, and considering the fact that AF is strongly related to age, the number of cases with AF at baseline is assumed to be small. CRP was not used in clinical practice at the time when this study started and was therefore not available in our study.

Mean age in this study was rather low, and very few participants used any heart drug at the time when the study started. We do not know how many received anti-arrhythmic drugs during the follow-up period. If anything, however, anti-arrhythmic drugs would weaken the association between ISPs and atrial fibrillation, thereby increasing the risk of a false negative result.

We do not know if the incident cases were classified as paroxysmal, persistent or permanent AF. However, the subcategories of AF overlap to a great extent. Most cases of paroxysmal AF seem to evolve into persistent and permanent AF [40]. The arrhythmia is discovered in different phases in different patients depending on what symptoms the patient experience.

As plasma levels were only measured at baseline, the levels of ISPs may have changed before disease manifestation. This is a limitation. However, this "regression dilution" might result in an underestimation of the actual risk [41].

Previous studies have shown that the ISPs studied here are risk factors for developing myocardial infarction [9]. One question is whether the increased risk of AF could be explained by the increased incidence of myocardial infarction in men with low-grade inflammation. However, when excluding cases with incident myocardial infarctions during follow-up, risk estimates remained significant and even increased slightly, which indicates that the relationship between ISPs and AF is independent of myocardial infarction. However, we were unable to account for patients with silent myocardial infarction, i.e. who were not hospitalized.

It should be mentioned that the primary analysis using a score of all five ISPs was decided a priori based on the methods used in previous studies [9, 19]. The analyses of individual ISPs could therefore be considered as secondary analyses. However, the *P*-value for the relationship between ceruloplasmin and incidence of atrial fibrillation remains significant even after a Bonferroni-correction.

In conclusion, our study shows that a score of five inflammation sensitive proteins is associated with long-term incidence of hospitalizations due to atrial fibrillation in middle-aged men. When inflammatory markers were considered individually, a significant association was observed with ceruloplasmin.

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Conflicts of interest Gunnar Engström is employed as senior epidemiologist by AstraZeneca R&D, Lund, Sweden. Samuel Adamsson Eryd, J. Gustav Smith, Olle Melander and Bo Hedblad have no potential conflicts of interest.

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Paper II

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Ceruloplasmin and atrial fibrillation: evidence of causality from a population-based Mendelian randomization study

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Abstract. Adamsson Eryd S, Sjögren M, Smith JG, Nilsson PM, Melander O, Hedblad B, Engström G (Department of Clinical Sciences, Lund University, Malmö; and Department of Cardiology, Lund University, Lund, Sweden). Ceruloplasmin and atrial fibrillation: evidence of causality from a population-based Mendelian randomization study. *J Intern Med* 2013; doi: 10.1111/joim.12144.

Objectives. Inflammatory diseases and inflammatory markers secreted by the liver, including C-reactive protein (CRP) and ceruloplasmin, have been associated with incident atrial fibrillation (AF). Genetic studies have not supported a causal relationship between CRP and AF, but the relationship between ceruloplasmin and AF has not been studied. The purpose of this Mendelian randomization study was to explore whether genetic polymorphisms in the gene encoding ceruloplasmin are associated with elevated ceruloplasmin levels, and whether such genetic polymorphisms are also associated with the incidence of AF.

Design. Genetic polymorphisms in the ceruloplasmin gene (*CP*) were genotyped in a population-based cohort study of men from southern Sweden (Malmö Preventive Project; $n = 3900$). Genetic polymorphisms associated with plasma ceruloplasmin concentration were also investigated for association with incident AF ($n = 520$) during a mean follow-up of 29 years in the same cohort. Findings were replicated in an independent case–control sample (The Malmö AF cohort; $n = 2247$ cases, 2208 controls).

Results. A single nucleotide polymorphism (rs1170 8215, minor allele frequency 0.12) located in the *CP* gene promoter was strongly associated with increased levels of plasma ceruloplasmin ($P = 9 \times 10^{-10}$) and with AF in both the discovery cohort [hazard ratio 1.24 per risk allele, 95% confidence interval (CI) 1.06–1.44, $P = 0.006$] and the replication cohort (odds ratio 1.13, 95% CI 1.02–1.26, $P = 0.02$).

Conclusions. Our findings indicate a causal role of ceruloplasmin in AF pathophysiology and suggest that ceruloplasmin might be a mediator in a specific inflammatory pathway that causally links inflammatory diseases and incidence of AF.

Keywords: atrial fibrillation, biomarker, cohort study, epidemiology, gene polymorphism, protein.

Abbreviations: AF, atrial fibrillation; CI, confidence interval; CRP, C-reactive protein; BMI, body mass index; HR, hazard ratio; IL-6, interleukin-6; LDL, low-density lipoprotein; NFκB, nuclear factor kappa B; OR, odds ratio; SNP, single nucleotide polymorphism.

Introduction

Relationships between inflammatory diseases, raised plasma concentrations of inflammatory markers and incidence of atrial fibrillation (AF) have been documented in several prospective studies [1–8]. These associations could potentially result either from inflammatory processes in the atria in inflammatory conditions or from arrhythmogenic effects of inflammatory mediators, which are increased in blood in inflammatory states.

The first inflammatory marker to be associated with AF, C-reactive protein (CRP) [4], was investigated for a causal relation with AF in a large Mendelian randomization study, that is, to determine whether genetic variants that confer increased CRP levels also confer increased risk of AF; the results from this study did not support a causal relationship [5]. We recently found in the Malmö Preventive Project that amongst a panel of seven inflammatory markers, only ceruloplasmin was independently associated with increased AF

risk [9]. Ceruloplasmin is an acute-phase reactant that is involved in the metabolism of iron and is an important carrier of circulating copper. Both oxidative and anti-oxidative functions have been reported for ceruloplasmin, and it may induce LDL oxidation as well as protect against free radical proteolysis [10, 11]. The findings of several studies indicate that oxidative stress might play a central role in the initiation and maintenance of AF mediated by electrophysiological remodelling of the atrium [12–14]. It remains unclear whether ceruloplasmin acts as a passive marker of inflammation or as a causal mediator in the development of AF.

The purpose of this study was to further explore the relationship between ceruloplasmin and AF risk using Mendelian randomization methods. We investigated whether genetic polymorphisms in the gene encoding ceruloplasmin are associated with elevated ceruloplasmin levels, and whether such genetic polymorphisms are also associated with incidence of AF.

Methods

Discovery cohort

Between 1974 and 1984, a total of 22 444 men aged 26–61 years participated in a screening programme for the detection of individuals at high risk of cardiovascular diseases in Malmö, Sweden (The Malmö Preventive Project). The participation rate was 71%. As a part of the programme, plasma levels of ceruloplasmin were determined for a random sample of 6455 men [9, 15, 16]. Of these men, 3907 were later re-examined in follow-up studies including DNA sampling [17]; the mean age at baseline was 46.3 ± 3.6 years. After excluding seven subjects with a previous hospital diagnosis of AF, 3900 men remained in the cohort.

The health service authority of Malmö approved and funded the screening programme. The regional ethics committee approved the data linkage with the Swedish population and hospital registers (No. 85/2004, LU 244-02). All participants provided written informed consent.

Baseline examination

Blood samples were drawn after an overnight fast. Levels of serum cholesterol were determined using standard methods at the laboratory of Malmö University Hospital. Diabetes was defined as fasting whole-blood glucose concentration ≥6.1 mmol L^{-1}, 2-h postglucose load ≥10.0 mmol L^{-1} or self-reported diabetes. Body mass index (BMI) was calculated as body weight divided by height squared. Blood pressure (mm Hg) was measured twice in the right arm using a sphygmomanometer after resting for 10 min. Data on smoking habits were collected from a questionnaire.

Plasma levels of ceruloplasmin were assessed by means of an electroimmunoassay [18]. The analysis was performed consecutively at the time of study entry. The lower detection limit for ceruloplasmin was 20 mg L^{-1}, and the coefficient of variation was <5% [18].

Follow-up

All men were followed from the baseline examination until first hospitalization due to AF, death, emigration from Sweden or 31 December 2008. AF was defined as a diagnosis of either atrial fibrillation or atrial flutter, given the close inter-relationship between these disorders, as in previous studies [6, 19]. Subjects were considered to have AF if diagnosed with a primary or contributory hospital discharge code according to the International Classification of Diseases 8th revision (ICD-8) 427.92, ICD-9 427D or ICD-10 I48. Because a relationship between ceruloplasmin and AF could in theory be mediated via an increased risk of other types of heart disease, we also performed an analysis in which subjects with a hospital diagnosis of myocardial infarction or heart failure prior to or at the time of the diagnosis of AF were censored. In this analysis, the follow-up time was considered only up to the time of the myocardial infarction or heart failure event. The Swedish Hospital Discharge Register was used for case retrieval [20].

Selection, genotyping and quality control of single nucleotide polymorphisms

We selected 17 tag single nucleotide polymorphisms (SNPs) across the ceruloplasmin gene (*CP*) and genotyped them using IPLEX on a MassARRAY platform (Sequenom, San Diego, CA, USA) according to the manufacturer's standard protocols. Twenty per cent of the samples were run in duplicate without any inconsistencies. All genotypes were called by two investigators.

Replication in independent samples

SNPs significantly associated with AF in the discovery analyses were tested for replication in a

case–control study, the Malmö AF cohort. This cohort consisted of 4826 unique subjects (62% male) drawn from the Malmö Diet and Cancer Study [19] and re-examinations from the Malmö Preventive Project as described previously [21]. Cases were subjects with a diagnosis of AF before 31 December 2006; controls were matched according to sex, age (±1 year), time of baseline examination (±1 year) and follow-up time. A total of 371 subjects (166 cases and 205 controls) from the Malmö Preventive Project who were also included in the discovery analyses were excluded from the replication study. After exclusions, there were 4455 subjects (59% male) in the replication analysis.

We also attempted to perform *in silico* replication of our findings using data from the AFGen study, a meta-analysis of genomewide association studies of individuals of European ancestry [21]. However, neither the SNP of interest nor any appropriate proxy was available in this data set.

Statistical analysis

Univariate analysis of variance (ANOVA) was used to compare plasma levels of ceruloplasmin across genotypes and also to compare risk factor distributions for the different SNPs. Associations between SNPs and AF were examined using Cox proportional hazards regression, in which the additive effect per copy of the minor allele on AF risk was tested. The analysis was adjusted for age, sex, smoking, diabetes, BMI and systolic blood pressure. The Cox proportional hazard assumption was confirmed by plotting incidence rates over time.

Logistic regression analysis was used to examine the association between ceruloplasmin and SNPs in the case–control cohort. An additional sensitivity analysis was also performed after censoring individuals with incident myocardial infarction and/or heart failure. The Hardy–Weinberg equilibrium was calculated using a Web-based calculator [22]. All analyses were performed using PASW STATISTICS 18 (SPSS Inc, Chicago, IL, USA) or STATA/IC 12.1 (StataCorp, College Station, TX, USA).

Results

Genetic determinants of plasma levels of ceruloplasmin

The baseline characteristics of the discovery study cohort are presented in Table 1. Baseline levels of ceruloplasmin differed significantly ($P < 0.05$) between genotypes in nine of 17 SNPs (two degrees of freedom). The strongest association between levels of ceruloplasmin and genotype was observed for rs13075891 ($P = 2 \times 10^{-10}$) and rs11708215 ($P = 9 \times 10^{-10}$). These two SNPs also showed the strongest pairwise correlation ($r^2 = 0.56$; Table S1). When the SNPs were fitted in an additive model (one degree of freedom), six SNPs remained significantly associated with ceruloplasmin levels (Table 2). The models were adjusted for age, smoking, cholesterol, systolic blood pressure, diabetes and BMI. Age, cholesterol and smoking were also significantly and positively associated with plasma levels of ceruloplasmin.

Genetic variants and AF

During a mean follow-up of 28.8 years, 520 men were hospitalized with AF. One SNP, rs11708215,

Table 1 *Baseline characteristics of the study cohorts*

	Malmö Preventive Project		Malmö AF cohort	
	Incident AF			
	Yes	No	AF cases	Controls
n	520	3380	2247	2208
Age (years)	47 ± 3	46 ± 4	65 ± 8	65 ± 8
Male sex (%)	100	100	59.6	58.8
Current smoker (%)	42.3	41.8	42.4	42.6
Diabetes (%)	2.9	3.3	7.7	5.0
Systolic blood pressure (mmHg)	131 ± 16	127 ± 15	147 ± 20	148 ± 21
BMI (kg m^{-2})	25 ± 3	25 ± 3	26 ± 4	27 ± 4

AF, atrial fibrillation; BMI, body mass index.
Values are presented as means ± standard deviation unless otherwise stated.

Table 2 *Associations between single nucleotide polymorphisms (SNPs) and plasma levels of ceruloplasmin*

SNP	Number of subjects	Minor allele frequency	*P*-value for relationship with ceruloplasmin (two degrees of freedom)[a]	*P*-value for relationship with ceruloplasmin (one degree of freedom)[a]	r^2
rs11708215	3831	0.12	9×10^{-10}	0.001	0.051
rs11709714	3830	0.49	8×10^{-5}	1×10^{-5}	0.046
rs11714000	3764	0.07	2×10^{-9}	0.12	0.051
rs13075891	3794	0.10	2×10^{-10}	0.002	0.052
rs13095764	3816	0.05	0.015	0.86	0.043
rs16861579	3833	0.20	0.017	0.95	0.043
rs16861582	3784	0.30	0.007	0.016	0.043
rs17195505	3806	0.04	0.005	0.026	0.043
rs17838831	3834	0.15	3×10^{-6}	0.006	0.047

[a]Adjusted for age, smoking, cholesterol level, systolic blood pressure, diabetes and body mass index.

showed a significant association with incidence of AF, with a hazard ratio (HR) of 1.24 [95% confidence interval (CI) 1.06–1.44] per copy of the minor C allele ($P = 0.006$; Table 3). The relationship remained significant after adjustment for potential covariates (age, smoking, cholesterol level, systolic blood pressure, diabetes and BMI) and was even stronger if individuals with incident heart failure or myocardial infarction prior to the diagnosis of AF were censored in the analysis (HR 1.38 per risk allele, 95% CI 1.18–1.63, $P = 9.7 \times 10^{-5}$). Allele characteristics for rs11708215 are shown in Table 4.

Replication in an independent sample

The relationship between rs11708215 and ceruloplasmin remained significantly associated with AF in the replication study (the Malmö AF cohort; Table 5) with an odds ratio (OR) of 1.13 (95% CI 1.02–1.26) per minor allele ($P = 0.017$). After excluding 219 individuals with prior myocardial infarction or heart failure and adjustment for potential risk factors, the OR was 1.16 (95% CI 1.04–1.29, $P = 0.008$).

Discussion

In this Mendelian randomization study, we showed that an SNP (rs11708215) in the promoter region of *CP* is associated with increased levels of the gene product in blood and with increased risk of AF. The results were replicated and confirmed in an independent cohort in which the relationships remained significant after adjusting for potential covariates and also after exclusion of individuals with a history of heart failure or myocardial infarction.

Table 3 *Associations between single nucleotide polymorphisms (SNPs) and incidence of atrial fibrillation (AF) in three models*

SNP	HR for AF[a]	HR for AF[b]	HR for AF[c]
rs11708215	1.24 (1.06–1.44); $P = 0.006$	1.23 (1.06–1.43); $P = 0.007$	1.38 (1.18–1.63); $P = 9.7 \times 10^{-5}$
rs11709714	1.07 (0.95–1.21)		
rs13075891	1.21 (1.00–1.46); $P = 0.05$	1.18 (0.98–1.43); $P = 0.09$	
rs16861582	1.12 (0.98–1.28)		
rs17195505	0.86 (0.61–1.20)		
rs17838831	1.15 (0.98–1.36)		

HR, hazard ratio; CI, confidence interval.
[a]HR (95% CI) and *P*-value per allele, crude model.
[b]HR (95% CI) and *P*-value per allele, adjusted for age, smoking, diabetes, body mass index, cholesterol level, systolic blood pressure and ceruloplasmin level.
[c]HR (95% CI) and *P*-value per allele, after adjustments for above risk factors; subjects with a hospital diagnosis of heart failure or myocardial infarction before incidence of AF were censored ($n = 126$).

Table 4 *Allele characteristics for the single nucleotide polymorphism rs11708215*

	Genotype			
	TT	CT	CC	*P* trend
n	3080	581	170	
Plasma level of ceruloplasmin, μmol L^{-1}	307	324	325	0.001
AF, *n* (%)	389 (12.6)	93 (16.0)	30 (17.7)	
Incidence (95% CI) of AF, hazard ratio	1.0	1.27 (1.02–1.60)	1.47 (1.02–2.14)	0.006
Incidence (95% CI) of AF, adjusted hazard ratio[a]	1.0	1.25 (0.99–1.57)	1.50 (1.03–2.17)	0.007

AF, atrial fibrillation; CI, confidence interval.
[a]Adjusted for age, smoking, body mass index, diabetes, cholesterol level, systolic blood pressure and plasma level of ceruloplasmin.

Table 5 *Association between single nucleotide polymorphism (SNP) rs11708215 and atrial fibrillation (AF) in the Malmö AF cohort*

SNP	OR (95% CI) of AF per allele	OR (95% CI) of AF per allele[a]	OR (95% CI) of AF per allele[a,b]
rs11708215	1.13 (1.02–1.26); *P* = 0.02	1.14 (1.03–1.27); *P* = 0.02	1.16 (1.04–1.29); *P* = 0.008

OR, odds ratio; CI, confidence interval.
P-values per allele.
[a]Adjusted for age, sex, smoking, diabetes, body mass index and systolic blood pressure.
[b]Subjects with a history of myocardial infarction and/or heart failure before AF diagnosis were excluded (*n* = 219).

The results indicate that the SNP rs11708215 in the *CP* gene might affect the risk of AF by modifying plasma levels of ceruloplasmin. However, adjustments for plasma levels of ceruloplasmin did not eliminate the significant association between the SNP and AF, suggesting that an additional mechanistic pathway might be present. Another explanation, which has also been put forward in other Mendelian randomization experiments with larger effect estimates than expected [23], might be that the association between rs11708215 and AF represents a lifelong genetic risk, whereas the association between ceruloplasmin and AF represents a risk that is present over a more limited time frame.

It is also possible that the relationships between genetic variations in the *CP* gene, ceruloplasmin and incidence of AF could be explained by other genes in the same chromosomal region. However, this seems less likely as the SNP associated with ceruloplasmin, and AF is located in the promoter region upstream of the *CP* gene at chromosome 3q23-24. This region is strongly associated with binding of several transcription factors including nuclear factor kappa B (NFκB). NFκB is thought to be involved in altering ion channel transcription, thereby causing electrical remodelling of the heart [24]. A recent study has also shown that common genetic variants of the gene encoding the receptor for interleukin-6 (IL-6) are reproducibly associated with AF risk [25]. Of note, NFκB is activated by cytokines, including by binding of IL-6 to its receptor [26, 27].

Ceruloplasmin is a plasma protein that is considered to be an acute-phase reactant with levels increased by two to threefold in inflammatory conditions [15, 16, 28]. A high level of ceruloplasmin is a risk factor for several cardiovascular disorders including myocardial infarction, arteriosclerosis, unstable angina, abdominal aortic aneurysm, vasculitis and peripheral arterial disease [29]. The protein is thought to function as a copper transporter as it accounts for approximately 95% of the serum copper in healthy adults [30]. It has also been proposed that ceruloplasmin is involved in iron homeostasis, coagulation, angiogenesis and immune function [29].

The findings of several studies also suggest that ceruloplasmin may have effects on heart muscle, which might be mediated through copper. It was shown that left ventricular hypertrophy, commonly observed in combination with AF, was related to

accumulation of loosely bound copper in patients with type-2 diabetes [31]. Patients with genetically elevated copper levels (Mb Wilson) also show signs of cardiomyopathy, possibly due to deposition of copper in the heart [32–34], and a relatively high frequency of atrial arrhythmias has been described [35]. By contrast, loss-of-function mutations in the *CP* gene (aceruloplasminaemia) result in neurodegeneration and accumulation of iron in several organs including the liver and brain, but no reported effects on copper metabolism or cardiac disease [36, 37].

Ceruloplasmin has been reported to posses both oxidative and anti-oxidative functions. It was shown that ceruloplasmin catalyses the oxidation of Fe^{2+} to Fe^{3+} [38, 39], as well as the oxidation of Cu^{+} to Cu^{2+} [40]. The reaction reduces O_2 to H_2O without releasing superoxide or hydrogen peroxide. This so-called ferroxidase activity is essential for iron homeostasis and is thought to be responsible for the ability of ceruloplasmin to block free radical-induced proteolysis and DNA damage. The ferroxidase activity is increased, for example, during inflammation and infection [11]. By contrast, ceruloplasmin has been reported to possess oxidative effects and may induce LDL oxidation [10]. Experiments have shown that ceruloplasmin increases lipid oxidation by a process requiring superoxide released from vascular smooth muscle cells and endothelial cells [41, 42]. Removal of one of the seven copper atoms completely blocked oxidant activity [10]. In addition, removal of loosely bound copper from ceruloplasmin may be induced by reactive oxygen species. The free copper may then catalyse the formation of more reactive oxygen species and may also lead to direct effects on signal transduction and transcription [43]. Thus, the role of ceruloplasmin as a protective or pathological protein might be dependent on the oxidative status. However, it remains unclear whether an elevated level of ceruloplasmin is a cause of oxidative stress or instead a compensatory reaction against oxidative stress.

Oxidative stress is thought to play a central role in the initiation and continuation of AF, and several mechanisms might be involved in this process. Reactive oxygen species may cause ectopic firing as well as atrial electrical and structural remodelling by altering ion channels [44, 45]. Reactive oxygen species have been linked to abnormal Ca^{2+} homeostasis and gap junctions [44, 45]. If ion channels are altered and action potential is decreased by reactive oxygen species, this could cause inexcitable areas which could promote re-entry arrhythmias [44].

We were able to replicate our findings for the rs11708215 SNP in an independent case–control cohort, which further strengthens our hypothesis that ceruloplasmin may cause AF.

There are some potential limitations of our study that should be considered. First, all diagnoses in this study were made during hospitalization, and the cases should therefore be valid. Furthermore, a validation study of cases retrieved from the Swedish national registers showed that the validity of the diagnosis was very high [19]. However, some cases of AF might only be treated in primary care and are therefore not included in this study. Nevertheless, the estimates of prevalence and incidence in a recent validation study of this population were largely comparable with estimates in other epidemiological studies of AF [19]. Secondly, electrocardiographic information was not available at baseline, suggesting that some cases may have had AF already when entering the study. However, because the mean age was only 47 years, and AF is strongly related to age, the number of cases with AF at baseline is assumed to be small. Finally, there was a significant time-lapse between baseline examinations and blood collection for genotyping, which may have lead to a bias as patients with AF may have died before genotyping. However, this would result in an underestimation of the actual risk associated with the minor allele of rs11708215.

In conclusion, our results show that genetic polymorphisms in the promoter of the *CP* gene are associated with elevated plasma levels of ceruloplasmin. One of these polymorphisms is also reproducibly associated with increased risk of AF. Our findings indicate a causal relationship between ceruloplasmin and AF, but additional work is necessary to elucidate the causal pathway.

Conflict of interest statement

No conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pairwise correlations (r^2) between single-nucleotide polymorphisms (SNPs) in the ceruloplasmin gene.■

Paper III

Original Article | Journal of INTERNAL MEDICINE

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Red blood cell distribution width is associated with incidence of atrial fibrillation

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Abstract. Adamsson Eryd S, Borné Y, Melander O, Persson M, Smith JG, Hedblad B, Engström G (Department of Clinical Sciences, Lund University, Malmö; and Department of Cardiology, Lund University, Lund, Sweden). Red blood cell distribution width is associated with incidence of atrial fibrillation. *J Intern Med* 2014; **275**: 84–92.

Objectives. Red blood cell distribution width (RDW), a measure of variation in erythrocyte volume, has been associated with several cardiovascular disorders, but the relationship with atrial fibrillation (AF) remains unclear. We investigated the association between RDW and incidence of first hospitalization due to AF in a population-based cohort.

Design. Red blood cell distribution width was measured in 27 124 subjects from the general population (age 45–73 years, 62% women) with no history of AF, heart failure, myocardial infarction or stroke. The association between baseline RDW and incidence of AF identified from the Swedish Hospital Discharge Register was evaluated.

Results. During a mean follow-up of 13.6 years, 1894 subjects (53% men) were hospitalized with a diagnosis of AF. After adjustment for potential confounding factors, including cardiovascular disease risk factors, nutrient intake (iron, vitamin B12 and folate) and several haematological parameters (haemoglobin concentration, mean corpuscular volume and corpuscular haemoglobin content), the hazard ratio (HR) for incidence of AF was 1.33 [95% confidence interval (CI) 1.16–1.53] for the fourth versus first quartile of RDW (P for trend <0.001). The results were essentially unchanged when subjects with incident myocardial infarction or hospitalizations because of heart failure were censored from the analysis (HR 1.30, 95% CI 1.13–1.51; P for trend = 0.001).

Conclusion. Red blood cell distribution width was associated with incidence of AF independently of several cardiovascular, nutritional and haematological factors in this study of middle-aged subjects from the general population.

Keywords: atrial fibrillation, cohort study, epidemiology, red blood cell distribution width, risk factors.

Introduction

The red blood cell distribution width (RDW) is a measure of variation in erythrocyte volume, with higher values reflecting greater heterogeneity in cell volumes (anisocytosis). RDW is typically used together with mean corpuscular volume (MCV) to determine the cause of an underlying anaemia. For example, iron deficiency anaemia is associated with increased RDW, whereas thalassaemia is associated with normal RDW. In the past few years, RDW has also emerged as a prognostic marker in a variety of cardiovascular disorders other than anaemia. Associations have been reported between elevated RDW, within the normal range, and poor prognosis in patients with heart failure and acute myocardial infarction [1–9]. Furthermore, we recently reported a significantly increased incidence of hospitalizations due to heart failure in initially healthy subjects with raised RDW [3]. The findings of a study in patients undergoing coronary angiography indicated a possible association between RDW and atrial fibrillation [10]. The mechanisms underlying these relationships remain unclear, although several explanations have been proposed. In a recent study of patients with systolic left heart failure, high RDW was found to be associated with significantly decreased heart rate variability [11]. This suggests that the cardiovascular autonomic function could be impaired in subjects with high RDW. It has also been suggested that RDW could be a marker of oxidative stress [12], which has been associated with atrial fibrillation [13]. However, it remains

unclear whether RDW is a risk factor for atrial fibrillation in patients without a history of cardiovascular disease.

The aim of this population-based cohort study was to explore the major determinants of raised RDW levels in the general population and assess whether RDW is independently associated with the long-term incidence of atrial fibrillation in subjects without a history of cardiovascular disease.

Methods

Study population

All men born between 1923 and 1945 and women born between 1923 and 1950 living in Malmö, Sweden, were invited to participate in the Malmö Diet and Cancer Study (MDCS). Between March 1991 and September 1996, the respondents underwent clinical examinations at the screening centre and completed a self-administered questionnaire including a dietary assessment. Of an eligible population of 74 000, a total of 30 447 individuals attended the baseline examinations. After excluding 1998 individuals who did not undergo the full clinical examinations or failed to complete the questionnaire or the dietary assessment, the cohort consisted of 28 449 subjects (11 246 men and 17 203 women) [14]. Subjects with a history of atrial fibrillation, heart failure, myocardial infarction or stroke ($n = 1120$) were excluded. Another 184 subjects were excluded because of missing data on RDW, body mass index (BMI), waist circumference, systolic blood pressure, diastolic blood pressure, diabetes mellitus, leucocyte count or haemoglobin concentration. Subjects with a leucocyte count of more than 20×10^9 L^{-1} ($n = 21$) were also excluded; such high values were considered to be due to illness or measurement error. After exclusions, 27 124 subjects with a mean age of 58 ± 7.6 years remained for analyses. The cohort has been shown to be representative of the general population with regard to the proportions of smokers and overweight individuals, but the mortality rate was higher than in nonparticipants [14]. The regional ethics committee approved the MDCS. All participants provided written informed consent.

Baseline examinations

A self-administered questionnaire was used to obtain information on smoking habits, alcohol use, the levels of education and physical activity, marital status, medical history and current medications. For the purpose of these analyses, smoking was classified into three categories: smokers, former smokers and nonsmokers. Marital status was classified into two groups: unmarried (single, divorced or widowed) or married (cohabiting). Level of education was classified into low (≤8 years), moderate (9–12 years) and high (college/university). Information on physical activity was retrieved through 18 questions covering a range of activities during all four seasons. An overall leisure time physical activity score was created by multiplying the time spent for each activity (min per week) by an intensity coefficient. The scores were divided into quartiles of physical activity for the analysis. Daily alcohol consumption in men/women was classified as low (<20/15 g day^{-1}), intermediate (20–40/15–30 g day^{-1}) and high (>40/30 g day^{-1}).

Blood pressure was measured using a mercury column sphygmomanometer after resting for 10 min in the supine position. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive medication. Body weight, height and waist circumference were measured. Baseline diabetes mellitus was defined as a self-reported physician's diagnosis of diabetes or use of antidiabetic medications.

Erythrocyte volume, haemoglobin content and leucocyte count were analysed using a SYSMEX K1000 fully automated assay (Sysmex Europe, Norderstedt, Germany). The analyses were performed consecutively at the time of the screening examination, at the central laboratory of Malmö Hospital, using fresh heparinised blood. RDW was calculated as the width of the erythrocyte distribution curve at a relative height of 20% above the baseline. Reference values were 36.4–46.3 fL in women and 35.1–43.9 fL in men.

Information about previous percutaneous coronary artery intervention was retrieved from the national Swedish Coronary Angiography and Angioplasty Register, and information regarding coronary artery bypass graft surgery came from the Swedish Hospital Discharge Register.

The dietary intake of folate, B12 and iron was assessed using an interview-based, modified diet history method that combined (i) a 7-day menu book for registration of lunch and dinner, cold

beverages including alcohol, drugs and nutrient supplements; (ii) a 168-item questionnaire for assessment of meal patterns, consumption frequencies and portion sizes of regularly eaten foods; and (iii) a 45-min complementary interview. The methods and their relative validity have been described elsewhere [15–17]. Nutrient intakes were log-transformed and adjusted for total energy intake [15] and method version of the diet assessment [16] for use in the regression models.

Follow-up and definitions of end-points

All subjects were followed from baseline until a first hospitalization due to atrial fibrillation, death, emigration from Sweden or end of follow-up (31 December 2008). As in previous studies [18–23], the term 'AF' was defined as a primary or contributory diagnosis of either atrial fibrillation or atrial flutter, because of the close relationship between these diagnoses. Cases were retrieved by linkage of Swedish personal identification numbers to the Swedish Hospital Discharge Register and Cause of Death Register using International Classification of Diseases (ICD) diagnosis codes: 8th edition (ICD-8), 427.92; ICD-9, 427D; and ICD-10, I48. A validation study of 100 cases with a diagnosis of AF in the present cohort showed that the diagnosis was accurate in 95%, likely in 2% and inaccurate in 3% [24].

Statistical analysis

The sample was categorized into sex-specific quartiles of RDW (i.e. four groups with equal proportions of men and women in each quartile). One-way analysis of variance (ANOVA) and logistic regression were used to compare risk factor distributions across quartiles of RDW. Cox proportional hazards regression with backward stepwise elimination (P for removal >0.10) was used to estimate hazard ratios (HRs) adjusted for potential confounding factors, in relation to quartile of RDW and per one standard deviation increase in RDW. The proportional hazards assumption was confirmed by plotting the incidence rates over time. The model was adjusted for cardiovascular disease risk factors associated with RDW at baseline. Age, BMI, waist circumference, diastolic blood pressure and leucocyte count were fitted as continuous variables, whereas sex, smoking, diabetes, antihypertensive medication, lipid-lowering medication, physical activity, marital status and education level were fitted as dichotomous variables. Possible interactions between RDW and the other risk factors on the incidence of AF were investigated by introducing interaction terms in the multivariate model.

The Kaplan–Meier estimator and log-rank test were used to investigate the incidence of AF across quartiles of RDW. Multiple linear regression with backward stepwise elimination (P for removal >0.10) was used to determine independent predictors of RDW.

A total of 521 individuals (1.9%) had missing data for any of the following variables: smoking, physical activity, marital status, level of education and alcohol consumption. Two different strategies were used to handle missing values: in the main analysis, missing data were coded as dummy variables in separate categories and, secondly, a multiple imputation method was evaluated [25]. The results were essentially the same for both methods. All analyses were performed using IBM SPSS STATISTICS version 20 (IBM Corporation, Armonk, NY, USA).

Results

Baseline characteristics and RDW

The mean values of RDW at the baseline examination were 40.5 ± 3.8 fL (interquartile range 38.1–42.6) in men and 40.8 ± 3.4 (interquartile range 38.5–42.8) in women.

The baseline characteristics of the study population in relation to sex-specific quartiles of RDW are presented in Table 1. High RDW was positively associated with age, smoking, use of nitrates, history of revascularization and alcohol consumption, and inversely associated with diabetes, BMI, waist circumference, diastolic blood pressure, antihypertensive medication, being married and a high level of education. With regard to haematological variables, increased RDW was associated with increased leucocyte count and MCV but a small decrease in haemoglobin and mean corpuscular haemoglobin concentration.

Independent predictors of RDW at baseline

Table 2 shows the independent predictors of RDW in a multivariable linear regression model. Smoking was the strongest predictor of RDW followed by age and BMI. Waist circumference, use of blood pressure medication and iron intake did not show any significant association with RDW and were

Table 1 *Baseline characteristics of the study population in sex-specific quartiles of red blood cell distribution width*

	Quartiles of red blood cell distribution width				
MDC (n = 27124)	Q1	Q2	Q3	Q4	P for trend
Red blood cell distribution width, fL (men/women)	<38.2/<38.6	38.2–40.1/ 38.6–40.5	40.2–42.5/ 40.6–42.7	>42.5/>42.7	
n (men/women)	2298/4189	2651/4155	2636/4216	2508/4171	
Age (years)	56.7 ± 6.9	57.7 ± 7.4	58.4 ± 7.8	59.0 ± 7.9	<0.001
Smokers (%)	14.3	21.4	29.8	47.0	<0.001
Diabetes (%)	4.4	2.9	2.0	1.9	<0.001
BMI (kg m^{-2})	26.2 ± 4.0	25.9 ± 3.9	25.6 ± 3.9	25.0 ± 4.0	<0.001
Waist (cm)	84.8 ± 12.7	84.4 ± 12.9	83.7 ± 12.7	82.5 ± 13.1	<0.001
Systolic blood pressure (mmHg)	141 ± 20	141 ± 20	141 ± 20	141 ± 20	0.25
Diastolic blood pressure (mmHg)	86 ± 10	86 ± 10	85 ± 10	85 ± 10	<0.001
Antihypertensive medication (%)	16.8	16.3	15.6	15.3	0.006
Lipid-lowering medication (%)	2.6	2.1	2.4	2.1	0.15
Nitroglycerine treatment (%)	0.9	0.9	1.0	1.3	0.05
History of coronary revascularization (%)	0.3	0.5	0.7	0.6	0.006
Physical activity (% in top quartile)	23.5	24.7	25.4	24.8	0.05
Married (%)	68.2	65.5	63.7	60.5	<0.001
High alcohol consumption (%)	3.1	3.5	4.3	6.4	<0.001
High education level (%)	31.9	33.0	31.7	30.0	0.005
Leucocytes (10^6 mL^{-1})	6.1 ± 1.5	6.3 ± 1.6	6.4 ± 1.7	6.7 ± 1.8	<0.001
Mean corpuscular volume (fL)	84.5 ± 16.8	87.9 ± 8.2	89.9 ± 7.6	93.1 ± 4.7	<0.001
Mean corpuscular haemoglobin concentration (g L^{-1})	340 ± 38	340 ± 46	339 ± 44	338 ± 26	0.03
Haemoglobin (g L^{-1})	142 ± 12	142 ± 12	142 ± 12	141 ± 12	0.001
Iron intake[a] (mg day^{-1})	15 (5–58)	15 (4–65)	15 (4–53)	15 (3–62)	<0.001*
B12 intake[a] (μg day^{-1})	5.5 (0–99)	5.7 (0–103)	5.6 (0–117)	5.8 (0–121)	<0.001*
Folate intake[a] (μg day^{-1})	243 (56–861)	242 (33–1086)	241 (46–879)	232 (51–855)	<0.001*

[a]Presented as median (interquartile range), due to skewed distribution. All other values are means ± SD, unless otherwise stated.
**P*-values for log-transformed data (i.e. intake of iron, B12 and folate).

hence removed from the stepwise regression model.

Incidence of AF and RDW

During a mean follow-up time of 13.6 years, 1894 subjects (1011 men and 883 women) were hospitalized with a diagnosis of AF (5.1 hospitalizations per 1000 person-years). The incidence of AF was significantly associated with RDW (age- and sex-adjusted HR 1.26, 95% CI 1.11–1.44 for fourth versus first quartile of RDW; HR per 1 SD 1.07, 95% CI 1.02–1.11). The association remained significant and was even slightly stronger after adjustment for potential confounding factors (HR 1.33, 95% CI 1.16–1.53 for fourth versus first quartile of RDW; HR per 1 SD 1.08, 95% CI 1.04–1.12; Tables 3 and 4 and Fig. 1). The adjusted HR (per 1 SD) was 1.08 (95% CI 1.02–1.14) for men and 1.12 (95% CI 1.05–1.21) for women, and the adjusted HR (per 1 SD) was 1.14 (95% CI 1.02–1.27) for subjects below the median age (57.5 years) and 1.08 (95% CI 1.03–1.13) for subjects above 57.5 years at baseline.

The increased risk of AF was essentially unchanged when dietary intake of folate, iron and B12 was added to the model: HR 1.32, 95% CI

Table 2 *Independent predictors of red blood cell distribution width*

	B (95% CI)	*P*-value
Age (per 1 year)	0.08 (0.08–0.09)	<0.001
Sex (women vs. men)	0.21 (0.11–0.31)	<0.001
Diabetes (yes vs. no)	−1.16 (−1.39 to −0.93)	<0.001
BMI (per 1 kg m^{-2})	−0.07 (−0.08 to −0.06)	<0.001
Diastolic blood pressure (per mmHg)	−0.006 (−0.01 to −0.001)	0.009
Lipid-lowering medication (yes vs. no)	−0.39 (−0.65 to −0.13)	0.003
Nitroglycerine treatment or history of revascularization (yes vs. no)	0.44 (0.12 to 0.77)	0.008
Current smoker (vs. never)	2.18 (2.07 to 2.28)	<0.001
Former smoker (vs. never)	0.34 (0.24 to 0.43)	<0.001
Single (vs. married)	0.33 (0.20 to 0.47)	<0.001
Divorced (vs. married)	0.18 (0.07 to 0.28)	0.001
Widow/widower (vs. married)	0.17 (0.02 to 0.33)	0.03
High education level (vs. low)	0.19 (0.11 to 0.28)	<0.001
Physical activity (highest quartile vs. lowest quartile)	0.09 (0.003 to 0.18)	0.04
Leucocytes (per 10^9 L^{-1})	0.14 (0.11 to 0.16)	<0.001
Haemoglobin (per 1 g L^{-1})	−0.02 (−0.03 to −0.018)	<0.001
B12 intake (per 1 μg day^{-1})	0.19 (0.15 to 0.23)	<0.001
Folate intake (per 1 μg day^{-1})	−0.19 (−0.23 to −0.14)	<0.001

Coefficient of determination of the entire model $r^2 = 0.135$.

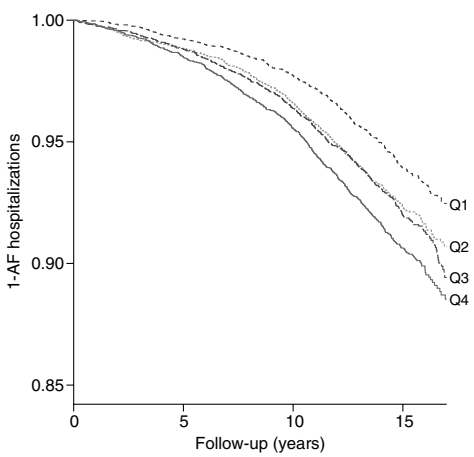

Fig. 1 *Incidence of hospitalizations due to AF over a mean follow-up of 13.6 years, in relation to quartiles of red blood cell distribution width. P for trend across quartiles < 0.001 (log-rank test).*

1.15–1.52 for the fourth versus first quartile of RDW; this information was available for 26 796 subjects. RDW showed no significant interactions with any of the other risk factors on incidence of AF.

In secondary analyses, censoring was performed at incident heart failure or myocardial infarction. A total of 1691 individuals had incident AF without previous or concomitant heart failure or myocardial infarction. The association between RDW and AF was essentially unchanged in these analyses (Table 4). After adjustment for risk factors, the HRs were 1.00 (reference), 1.18 (95% CI 1.02–1.36), 1.15 (95% CI 1.00–1.33) and 1.30 (95% CI 1.13–1.51), respectively, for the four quartiles of RDW (*P* for trend = 0.001).

High RDW could be the result of a high proportion of large cells, a high proportion of small dense cells or a combination of both. The analysis was therefore stratified by quartiles of MCV (Table 5). The association between RDW and AF was more significant in the lower quartiles of MCV, with no association in subjects in the highest MCV quartile

Table 3 *Incidence of atrial fibrillation with regard to sex-specific quartiles of red blood cell distribution width*

	Quartiles of red blood cell distribution width				*P* for trend	HR per 1 SD[b]
	Q1	Q2	Q3	Q4		
Red blood cell distribution width, fL (men/women)	<38.2/ <38.6	38.2–40.1/ 38.6–40.5	40.2–42.5/ 40.6–42.7	>42.5/>42.7		
n	6787	6806	6852	6679		
Atrial fibrillation, *n* (%)	376 (5.5)	482 (7.1)	493 (7.2)	543 (8.1)		
Age- and sex-adjusted HR	1.00	1.17 (1.02–1.34)	1.12 (0.98–1.28)	1.26 (1.11–1.44)	0.003	1.07 (1.02–1.11)
Additional risk factors[a]	1.00	1.20 (1.05–1.38)	1.18 (1.03–1.35)	1.33 (1.16–1.53)	<0.001	1.08 (1.04–1.12)

[a]Additional risk factors were entered into the model: age, sex, smoking, diabetes, body mass index (BMI), waist, diastolic blood pressure, blood pressure medication, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption, haemoglobin concentration and leucocyte count; but BMI, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption and leucocyte count were eliminated from the stepwise regression model.
[b]Hazard ratio per 1 standard deviation increase (3.43 fL).

Table 4 *Final multivariable model for incidence of atrial fibrillation (AF)*

	All incident AF		Incident AF without prior HF or MI	
	HR[a] (95% CI)[b]	*P*-value	HR[a] (95% CI)[c]	*P*-value
RDW (Q4 vs. Q1)	1.33 (1.16–1.53)	<0.001	1.30 (1.13–1.51)	<0.001
Age (per 1 year)	1.11 (1.10–1.12)	<0.001	1.11 (1.10–1.11)	<0.001
Male sex	1.35 (1.20–1.53)	<0.001	1.37 (1.21–1.56)	<0.001
Smoking (yes vs. no)	1.21 (1.07–1.37)	0.003	1.16 (1.02–1.33)	0.03
Diabetes (yes vs. no)	1.28 (1.04–1.59)	0.02	1.27 (1.00–1.61)	0.05
Waist circumference (per cm)	1.02 (1.02–1.03)	<0.001	1.03 (1.02–1.03)	<0.001
Diastolic blood pressure (per 10 mmHg)	1.07 (1.02–1.12)	0.007	1.08 (1.03–1.13)	0.003
Blood pressure medication (yes vs. no)	1.65 (1.49–1.83)	<0.001	1.62 (1.45–1.82)	<0.001
Haemoglobin (per 1 g L^{-1})	0.99 (0.99–1.00)	0.005	0.99 (0.99–1.00)	0.02

HF, heart failure; MI, myocardial infarction; RDW, red blood cell distribution width; Q, quartile; HR, hazard ratio; CI, confidence interval.
[a]HRs in the final model; body mass index, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption and leucocyte count were eliminated from the stepwise regression model.
[b]Model based on 1894 AF cases.
[c]Model based on 1691 AF cases.

(Table 5). MCV was not associated with incidence of AF and did not show any significant interaction with RDW.

Discussion

Felker *et al.* [26] first reported that elevated RDW is a novel predictor of morbidity and mortality amongst patients with chronic heart failure. In subsequent studies, these findings have been validated and it has also been demonstrated that RDW is associated with several other types of cardiovascular disorders [1–9]. To our knowledge, no study has focused on the relationship between RDW and incidence of AF.

In the present prospective cohort study, we found that increased RDW is significantly associated with incidence of first hospitalization due to AF amongst middle-aged subjects. The risk estimates remained significant and even showed a slight increase after adjustment for cardiovascular disease risk factors.

Table 5 *Incidence of atrial fibrillation (AF) in quartiles of red blood cell distribution width (RDW) stratified according to quartile (Q) of mean corpuscular volume (MCV)*

MCV (n = 27 124)	AF (n)	Hazard ratio Q1 vs. Q4 of RDW[a]	Hazard ratio Q1 vs. Q4 of RDW, adjusted[b]	Hazard ratio per 1 SD of RDW, adjusted[b]
Q1 (n = 6799)	464	1.67 (1.08–2.59)	1.67 (1.07–2.60)	1.25 (1.09–1.43)
Q2 (n = 6841)	454	1.46 (1.03–2.08)	1.47 (1.03–2.10)	1.18 (1.02–1.37)
Q3 (n = 6737)	480	1.36 (0.97–1.91)	1.48 (1.06–2.08)	1.15 (1.01–1.31)
Q4 (n = 6747)	496	1.43 (0.53–3.83)	1.26 (0.47–3.39)	1.01 (0.93–1.11)

[a]Adjusted for age and sex.
[b]Risk factors: age, sex, smoking, diabetes, body mass index, waist, diastolic blood pressure, blood pressure medication, lipid-lowering medication, nitroglycerine treatment, history of revascularization, physical activity, marital status, education level, alcohol consumption, haemoglobin concentration and leucocyte count were entered.

This result suggests that RDW is a novel risk factor for incidence of AF. The mechanism behind this relationship is unclear but could result from a direct effect of changes in erythrocyte volume and function on the heart, or may reflect other pathophysiological processes acting independently on both erythrocytes and the heart. A direct effect of changes in erythrocyte function on the heart seems plausible, as erythrocytes both carry oxygen to tissues and have an important role in cardiovascular regulation through release of extracellular nucleotides and other mediators [27]. Erythrolysis is associated with increased release of free radicals [28], which are thought to be detrimental to the heart. Pathophysiological processes, which have been suggested to be associated with increased RDW, include oxidative stress and inflammation as these states may reduce red blood cell survival and lead to a more mixed population of erythrocytes in the circulation [29]. Inflammation may also increase RDW by altering iron metabolism or by inhibiting the production of or response to erythropoietin [30].

In the present study, we found that RDW was associated with a high leucocyte count. Adjustment for leucocyte count did not, however, alter substantially the association between RDW and AF, suggesting that inflammation might play a minor role in the mechanistic pathway between RDW and AF. This observation is consistent with the findings of other studies of inflammatory markers and AF, in which AF has shown a weaker association with systemic inflammation than other types of heart disease, including heart failure, myocardial infarction and stroke [18, 31–33].

Malnutrition and deficiency of iron, B12 or folate are other factors associated with high RDW through effects on erythropoiesis [34]. Indeed intake of iron, B12 and folate all differed significantly across quartiles of RDW in the present study, and B12 and folate remained independent predictors of RDW in the multivariable linear regression model (Table 2). However, adjustment for these variables had essentially no effect on the association between RDW and AF.

High RDW could be due to either a high proportion of large cells, indicating high erythrocyte turnover, or high proportion of small dense cells, or both of these possibilities. The association between RDW and incidence of AF was mainly observed in subjects with a low MCV, and RDW was not related to incidence of AF in subjects with MCV in the top quartile. Therefore, we speculate that factors associated with high MCV, such as alcohol intake and deficiency of folate or B12, are unlikely to be the cause of the present results. As RDW has been shown to be a risk factor for heart failure and myocardial infarction, the increased risk of AF could potentially be explained by the association between AF and these diseases. However, censoring incident cases of heart failure and myocardial infarction during follow-up did not change the risk estimates significantly, indicating that the observed relation between RDW and AF is independent of heart failure and myocardial infarction. Of note, it should be acknowledged that subclinical forms of heart failure and myocardial infarction may still be important.

Some limitations of this study should be considered. First, although the analysis was adjusted for diabetes and use of lipid-lowering drugs, we did not have information, for example, on HbA1c and lipid levels for the entire cohort. Secondly, the endpoints of this study were retrieved using the

Swedish Hospital Discharge Register. Some cases of AF might be treated only in primary care and therefore would not be included in this study. However, the estimates of prevalence and incidence in a validation study from this population were largely comparable with those from other epidemiological studies of AF. We have previously shown very high validity of AF diagnosis in the MDCS [24]. Additional validation studies of these registers have also confirmed a high validity of heart failure and myocardial infarction as the primary diagnosis [35–37]. Thirdly, we do not have detailed information about the AF cases at the time of diagnosis. All patients in this study were without a history of AF at baseline examination, according to hospital registers. However, electrocardiographic information was not available at baseline, suggesting that some subjects may have already had AF at study entry. Nevertheless, the mean age of participants in this study was 57 years, and the prevalence of AF is low in this age group. Finally, RDW was only measured once at baseline examination, raising the question of whether the RDW may have changed before disease manifestation. However, such changes would be likely to reduce the HRs and bias the results towards null.

In conclusion, in this study of middle-aged subjects from the general population, we found that RDW was associated with incidence of AF independently of potential confounding factors.

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Conflict of interest statement

The authors have no potential conflict of interests to declare.

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Carotid intima-media thickness is associated with incidence of hospitalized atrial fibrillation

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ABSTRACT

Objective: Carotid intima-media thickness (IMT) is a measure of arterial thickening and a risk predictor for myocardial infarction and stroke. It is unclear whether IMT also predicts atrial fibrillation (AF). We explored the association between IMT and incidence of first AF hospitalization in a population-based cohort.
Methods: IMT was measured in 4846 subjects from the general population (aged 46–68 years, 60% women) without a history of AF, heart failure or myocardial infarction. The Swedish in-patient register was used for retrieval of AF cases. IMT was studied in relation to incidence of AF.
Results: During a mean follow-up of 15.3 years, 353 subjects (181 men, 172 women, 4.8 per 1000 person-years) were hospitalized with a diagnosis of AF. After adjustment for cardiovascular risk factors, the hazard ratio (HR) for incidence of AF was 1.61 (95% confidence interval (CI): 1.14–2.27) for 4th vs. 1st quartile of IMT in the common carotid artery. This relationship was also independent of occurrence of carotid plaque. The results were similar for IMT in the bifurcation.
Conclusion: Carotid IMT was independently associated with incidence of hospitalized AF in this study of middle-aged subjects from the general population. The results suggest that arterial thickening can predict future AF.

1. Introduction

Atrial fibrillation (AF) is a highly prevalent condition in the elderly. AF is associated with substantial morbidity and mortality, with a five-fold increased risk of stroke and a three-fold increased incidence of congestive heart failure (HF) [1]. AF is a heterogeneous disorder and different disease processes, such as ischemia, fibrosis or myocardial strain, could ultimately result in the substrates necessary to cause and sustain AF. Many risk factors for atherosclerosis are associated with increased incidence of AF, but there are also interesting exceptions. For example, higher levels of low-density lipoprotein (LDL) cholesterol and physical inactivity have shown to be protective of AF in some studies [2].

Atherosclerosis develops slowly and a long subclinical period precedes the clinical manifestations. It has been reported that the coexistence of AF and clinical manifestations of atherosclerosis increases the risk of future cardiovascular events dramatically [3]. AF has been associated with peripheral artery disease and atherosclerotic plaque in the carotid arteries [4,5]. It was also reported that flow-mediated dilatation, a measure of endothelial dysfunction, was impaired in patients with AF [6].

Carotid intima-media thickness (IMT) is widely accepted as a measure of arterial thickening. Increased carotid IMT is a risk predictor for acute myocardial infarction (MI) and stroke and has also been associated with incidence of HF [7–10]. Few studies have explored whether carotid IMT is a risk predictor of AF, and the results are inconsistent [11–13]. The Cardiovascular Health Study (CHS) found no association between carotid IMT or carotid stenosis and incidence of AF [11,12]. In contrast the Rotterdam Study showed a significant association between carotid IMT and incidence of AF, especially among women, and a recent case–control study found carotid IMT to be associated with lone AF [13,14]. Hence, it is still unclear whether IMT predicts future AF. This population-based

Abbreviations: AF, atrial fibrillation; CCA, common carotid artery; CI, confidence interval; HF, heart failure; MI, myocardial infarction; hs-CRP, high-sensitive C-reactive protein; HDL, high-density lipoprotein; HR, hazard ratio; IMT, intima-media thickness; LDL, low-density lipoprotein; MDC, Malmö Diet and Cancer cohort; SD, standard deviation.

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cohort study sought to investigate whether carotid IMT is associated with incidence of first AF hospitalization.

2. Materials and methods

2.1. Study population

All men born between 1923 and 1945 and women born between 1923 and 1950 living in Malmö, Sweden, were invited to participate in the Malmö Diet and Cancer (MDC) study. Details of the study have been described previously [15]. Between March 1991 and September 1996, the respondents participated in clinical examinations at the screening centre and a self-administered questionnaire including a dietary assessment. A total of 30 447 individuals from the eligible population of 74 000 individuals attended the baseline examinations. After excluding 1998 individuals who failed to complete either the questionnaire, the clinical examinations or the dietary assessment, the cohort consisted of 28 449 subjects (11 246 men and 17 03 women). A random 50% of participants who entered the MDC study between October 1991 and February 1994 were also invited to take part in a study of the epidemiology of carotid artery diseases [16]. During this period, a total of 6103 subjects (2572 men and 3531 women) were examined by B-mode ultrasound of the right carotid artery, and 5540 participants returned to donate fasting blood samples for measurements of blood lipids and glucose.

Subjects with a history of hospitalization due to AF, HF or MI (in total 159 subjects) were excluded from analysis. Furthermore, 536 subjects with missing information on carotid IMT, high-sensitive C-reactive protein (hs-CRP), waist circumference, lipoproteins and education level were also excluded (Fig. 2).

Mean age was 57.4 ± 5.9 in excluded subjects ($n = 1257$) and 57.5 ± 6.0 in those who were included in the study ($n = 4846$). The proportion of men was 50% and 40% respectively. Incidence of first AF hospitalization was higher in excluded subjects (7.7 per 1000 person years vs. 4.8 per 1000 person years).

The study was approved by the ethics committee at Lund University, Lund, Sweden (LU 51/90). All participants provided written informed consent.

2.2. Baseline examinations

A self-administered questionnaire was used to obtain information on smoking habits, alcohol use, education, physical activity, marital status, medical history and current medications. Smoking was classified into 3 categories: smokers, former smokers and never-smokers. Marital status was classified into 2 groups: unmarried (single, divorced, or widowed) or married (cohabiting). Educational level was classified into low (8 years), moderate (9–12 years), and high (college/university) levels. Information on physical activity was explored through 18 questions covering a range of activities in the 4 seasons. An overall leisure time physical activity score was created by multiplying the number of minutes per week for each activity by an intensity coefficient. The scores were divided into quartiles of physical activity when used in the analysis. Information on daily alcohol intake was assessed through a validated diet history method where food and beverages was registered in a "menu book" on 7 consecutive days [17]. Daily alcohol consumption in men/women was classified as low (<20/15 g), medium (20–40/15–30 g), and high (>40/30 g). Blood pressure was measured once in the supine position after 10 min rest using a mercury-column sphygmomanometer. Hypertension was defined as systolic blood pressure ≥140/90 mm Hg or current use of blood pressure lowering medication. Body weight, height and waist circumference was measured. Presence of diabetes mellitus was defined as a self-reported physician's diagnosis of diabetes, use of anti-diabetic medications or a fasting whole blood glucose level ≥6.1 mmol/L. Blood glucose, total and high-density lipoprotein (HDL)-cholesterol, were measured from fasting blood samples, according to standard procedures at the Department of Clinical Chemistry, Malmö University Hospital. The LDL-cholesterol concentration was calculated according to Friedewald's formula [18]. Hs-CRP was analyzed in frozen plasma, gathered at the baseline examination, using Tina-quant CRP latex high-sensitivity assay (Roche Diagnostics) on an ADVIA 1650 Chemistry System (Bayer Healthcare). Total leukocyte count was analyzed using an SYSMEX K1000 fully automated assay (Sysmex Europe, Norderstedt, Germany). The analyses were performed consecutively at the time of the screening examination, at the central laboratory of the Malmö University Hospital, using fresh heparinized blood.

2.3. Carotid artery measurement

Participants underwent B-mode ultrasonography of the right carotid artery by trained certified sonographers, using an Acuson 128 (Acuson, Mountain View, California). Presence of carotid plaque, defined as a focal thickening of the IMT >1.2 mm, was assessed. In short, the bifurcation area of the right common carotid artery was scanned within a predefined "window" comprising 3 cm of the right common carotid artery (CCA), the bifurcation, and 1 cm of both the internal and external carotid artery for the presence of plaque. IMT was measured off-line in the far wall of the right distal CCA as the mean thickness over a 10-mm segment proximal to the bifurcation according to the leading edge principle, using a specially designed computer-assisted analyzing system [19]. The maximum IMT in the bifurcation was also measured. [20–23]

Intra-observer and inter-observer variability with regard to IMT was checked regularly. The mean intra-observer difference was 8.7 ± 6.2% ($r = 0.85$) and the mean inter-observer difference 9.0 ± 7.2% ($r = 0.77$). [16]

2.4. Follow-up and definitions of end-points

AF was defined as a primary or contributory diagnosis of AF or atrial flutter as in previous studies [24,25]. All subjects were followed from baseline until the first hospitalization with a diagnosis of AF, death, emigration from Sweden or end of follow-up (June 30, 2009). In secondary analyses, subjects who experienced a nonfatal MI or HF during the follow-up period were followed until the day of hospitalization and censored thereafter. Subjects who were diagnosed with AF concomitantly with the MI or HF diagnosis during follow-up were also censored from this secondary analysis, given the close relationship between these diagnoses. Cases were retrieved by linkage of Swedish personal identification numbers to the Swedish Hospital Discharge Register and the Swedish Cause of Death Register using diagnosis codes 427.92 for the International Classification of Diseases 8th edition (ICD-8), 427D (ICD-9), and I48 (ICD-10). A validation study of 100 cases with AF diagnosis in the present cohort showed that AF was definite in 95%, probable in 2% and incorrect in 3% [24].

2.5. Statistics

IMT and hs-CRP were log-transformed due to skewed distributions. The sample was categorized into sex-specific quartiles of IMT in the CCA and in the bifurcation, respectively, i.e. four groups with equal proportions of men and women in each quartile. One-way ANOVA and logistic regression was used to compare risk factor distributions across the quartiles of IMT. Cox proportional hazards regression was used to estimate hazard ratios (HR) adjusted for

Table 1
Baseline characteristics in relation to sex-specific quartiles of intima-media thickness in the common carotid artery (CCA-IMT).

MDC ($n = 4846$)	Sex-specific quartiles of CCA-IMT				*P*, trend
	Q1	Q2	Q3	Q4	
IMT range, men (mm)	0.36–0.67	0.68–0.76	0.77–0.87	0.88–2.06	
IMT range, women (mm)	0.36–0.65	0.66–0.73	0.74–0.82	0.83–1.85	
N (men/women)	497/689	466/812	498/731	483/670	
Carotid plaque (%)	20.8	28.3	33.6	48.7	<0.001
Age (years)	54.8 ± 5.7	56.6 ± 5.8	58.6 ± 5.6	60.1 ± 5.4	<0.001
Smokers (%)	22.2	21.9	20.7	22.9	0.89
Diabetes (%)	5.2	6.2	7.5	10.7	<0.001
Waist circumference (cm)	82.3 ± 12.0	82.2 ± 12.4	83.5 ± 13.0	85.5 ± 13.1	<0.001
Systolic blood pressure (mm Hg)	134 ± 17	139 ± 18	143 ± 19	149 ± 20	<0.001
Diastolic blood pressure (mm Hg)	85 ± 9	86 ± 9	87 ± 9	89 ± 9	<0.001
Anti-hypertensive medication (%)	11.1	12.6	15.6	21.0	0.001
Physical activity (% in top quartile)	24.6	24.8	25.7	25.0	0.71
Married (%)	68.0	67.8	66.9	68.7	0.83
High alcohol consumption (%)	3.4	4.5	3.1	2.9	0.22
High education (%)	32.3	28.0	27.6	24.5	<0.001
Low-density lipoproteins (mmol/L)	4.0 ± 1.0	4.1 ± 1.0	4.2 ± 1.0	4.4 ± 1.0	<0.001
High-density lipoproteins (mmol/L)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	<0.001
Total leukocytes (millions/mL)	6.1 ± 2.7	5.9 ± 1.5	6.0 ± 1.6	6.2 ± 1.7	0.17
Hs-CRP[a] (mg/L)	1.1 (0.6–2.3)	1.3 (0.6–2.6)	1.4 (0.7–2.9)	1.5 (0.8–3.0)	<0001[b]

[a] hs-CRP levels are presented as medians (interquartile range in brackets) due to skewed distributions.
[b] *P*-value for log-transformed CRP. All other values are means ± standard deviation unless otherwise stated.

potential confounding factors, in relation to quartiles of IMT and per 1 standard deviation (SD) increase of log-IMT. The proportional hazards assumption was confirmed by plotting the AF incidence rates over time. Three different models were tested. The first model was adjusted for age and sex. The second model was adjusted for cardiovascular risk factors. The third model was in addition adjusted for presence of carotid plaque. Age, waist circumference, systolic blood pressure, LDL, HDL and hs-CRP were fitted as continuous variables. Sex, smoking, carotid plaque, diabetes, education level and physical activity were fitted as dichotomous variables. Possible interactions between IMT and the other risk factors, with respect to incidence of hospitalized AF, were studied by introducing interaction terms in the multivariate model.

The Kaplan–Meier estimator was used to study incidence of first AF hospitalization across quartiles of IMT.

All analyses were performed in Stata/IC 12.1 (StataCorp, College Station, Texas, USA).

3. Results

3.1. Baseline characteristics

Median CCA-IMT (interquartile range) was 0.77 mm in men (0.68–0.87 mm) and 0.73 mm (0.66–0.82 mm) in women. The relationships between sex-specific quartiles of CCA-IMT and cardiovascular risk factors are presented in Table 1. CCA-IMT was positively associated with age, diabetes, carotid plaque, waist circumference, systolic- and diastolic blood pressure, anti-hypertensive medication, LDL and hs-CRP, and inversely associated with HDL and education level.

IMT in the bifurcation was associated with the same risk factors as CCA-IMT and in addition positively associated with smoking, civil status and total leukocyte count (data not shown).

3.2. Risk factors for incidence of hospitalized atrial fibrillation

During a mean follow-up time of 15.3 years, 353 subjects (181 men, 172 women, 4.8 per 1000 person-years) were hospitalized with a diagnosis of AF. The relationships between different cardiovascular risk factors and incidence of AF are presented in Table 2. Age, presence of carotid plaque, use of anti-hypertensive medication, waist circumference and log CCA-IMT were positively associated with incidence of AF, while LDL was inversely associated.

Incidence of AF was significantly associated with quartiles of CCA-IMT (age and sex adjusted hazard ratio (HR): 1.82, 95% confidence interval (CI): 1.30–2.55 for 4th vs. 1st quartile of CCA-IMT). The relationship remained significant after adjustment for potential confounding factors and presence of carotid plaque (HR: 1.52, CI: 1.08–2.16 for 4th vs. 1st quartile of CCA-IMT) (Table 3, Fig. 1).

Incidence of AF also showed a significant relationship with IMT in the bifurcation (risk factor adjusted HR: 1.66, CI: 1.06–2.61, for 4th vs. 1st quartile of IMT) (Table 4).

Sex-specific multivariate analysis showed that the association between CCA-IMT and incident AF was significant among men (HR: 2.00, CI: 1.24–3.23 for 4th vs. 1st quartile), but not in women (HR: 1.08, 0.65–1.81 for 4th vs. 1st quartile) (Supplemental Table 1). A similar relationship was found for IMT in the bifurcation. No

Table 2
Results from Cox proportional hazards regression of incidence of first atrial fibrillation hospitalization.

	Age and sex adjusted	+Risk factors
Age, per year	1.13 (1.11–1.15)	1.11 (1.09–1.14)
Male (vs. female)	1.69 (1.37–2.08)	1.24 (0.94–1.65)
Carotid plaque (yes vs. no)	1.46 (1.17–1.81)	1.37 (1.09–1.71)
Systolic blood pressure, per 10 mm Hg	1.11 (1.06–1.18)	1.04 (0.98–1.11)
Anti-hypertensive medication (yes vs. no)	1.74 (1.37–2.22)	1.40 (1.08–1.81)
Waist, per 1 cm	1.03 (1.02–1.04)	1.02 (1.01–1.03)
Diabetes (yes vs. no)	1.66 (1.21–2.27)	1.19 (0.85–1.65)
Smoking (current vs. never)	1.18 (0.88–1.58)	1.11 (0.82–1.50)
Education (high vs. low)	0.90 (0.70–1.15)	0.95 (0.74–1.22)
Physical activity (highest quartile vs. lowest quartile)	0.78 (0.58–1.06)	0.84 (0.62–1.14)
LDL, per 1 mmol/L	0.88 (0.78–0.98)	0.85 (0.76–0.95)
HDL, per 1 mmol/L	0.85 (0.62–1.17)	1.18 (0.85–1.65)
Log hs-CRP, per 1 SD	1.21 (1.09–1.35)	1.10 (0.98–1.23)
Log CCA-IMT, per 1 SD	1.21 (1.09–1.35)	1.12 (1.00–1.25)

Presented as hazard ratios (95% CI) adjusted for age and sex or in a model including all risk factors in the table.
SD indicates standard deviation.

Table 3
Incidence of first atrial fibrillation hospitalization in relation to sex-specific quartiles of intima-media thickness in the common carotid artery (CCA-IMT).

MDC ($n = 4846$)	Sex-specific quartiles of IMT				HR per SD[a]
	Q1	Q2	Q3	Q4	
N (men/women)	497/689	466/812	498/731	483/670	
Atrial fibrillation, *n* (%)	50 (4.2)	89 (7.0)	86 (7.0)	128 (11.1)	
Per 1000 person years	2.7	4.5	4.6	7.6	
Age and sex adjusted HR	1.00	1.46 (1.03–2.07)	1.26 (0.88–1.79)	1.82 (1.30–2.55)	1.21 (1.09–1.35)
+Risk factors[b]	1.00	1.39 (0.98–1.97)	1.17 (0.82–1.66)	1.61 (1.14–2.27)	1.15 (1.03–1.29)
+Carotid plaque[c]	1.00	1.36 (0.96–1.93)	1.12 (0.79–1.61)	1.52 (1.08–2.16)	1.12 (1.00–1.25)

[a] Hazard ratios per 1 standard deviation increase of log-IMT.
[b] Risk factors: age, sex, smoking, diabetes, waist circumference, systolic blood pressure, anti-hypertensive medication, LDL, HDL, education, physical activity and CRP (log-transformed).
[c] Model adjusted for all risk factors including carotid plaque.

significant interaction was observed between IMT (in CCA or bifurcation) and sex with respect to incident AF.

CCA-IMT is a risk predictor for MI and HF in the present cohort. We therefore performed a secondary analysis, in which all cases with MI or HF during the follow-up period were censored at the time of the event. A total of 297 individuals had incident AF without previous or concomitant MI or HF. The age- and sex-adjusted relationship between CCA-IMT and incident AF was essentially unchanged. Comparing the 4th vs. 1st quartile, HR was 1.68 (1.16–2.43), and the risk factor adjusted HR was 1.50 (1.02–2.19).

4. Discussion

This prospective cohort study found that carotid IMT, a measure of arterial thickening, is associated with incidence of AF hospitalizations among middle-aged subjects, especially among men. The risk estimates remained significant after adjustment for cardiovascular risk factors. In a multivariate analysis including both CCA-IMT and carotid plaque, both were significantly associated with incidence of first AF hospitalization.

Previous studies have shown that carotid IMT can predict future cardiovascular events [26]. Large epidemiological studies have shown strong relationships between IMT and incident MI, coronary heart disease and stroke [7,8,10,27–29]. Few studies have focused on the potential association between carotid IMT and AF, and the results from previous studies are contradictory. The CHS found no association between subclinical atherosclerosis (measured as either carotid IMT, carotid stenosis or ankle-arm index) and incidence of AF [11,12]. The Rotterdam Study showed a significant association between carotid IMT and incident AF, especially among women [13]. Even though all these studies are population-based, there are important differences between the studies that might explain the different conclusions. The CHS included self-reported AF while the Rotterdam Study, like the present study, only included patients with a physician's diagnosis of AF. Mean age also differed significantly between studies, being lowest in the present study and highest in the CHS. Since AF is strongly associated with increasing age [4,30], it is possible that death is a competing risk that reduces the associations between atherosclerosis and AF in older age groups. This could also be a possible explanation why the present study found a stronger association among men, while the Rotterdam Study found the association to be stronger among women. It should however be pointed out that no significant interaction was observed between sex and IMT in the present study or in the Rotterdam study. Thus, it is possible that the observed sex-differences are simply due to chance.

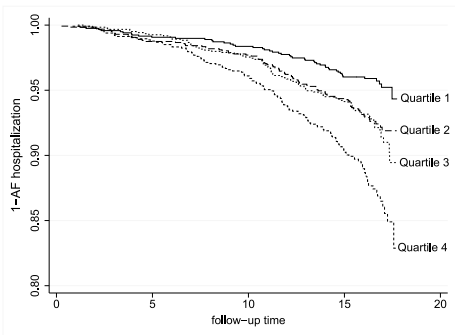

Fig. 1. Incidence of first atrial fibrillation hospitalization during a mean follow-up of 15.3 years, in relation to quartiles of CCA-IMT.

Incidence rates of AF in the present study and the Rotterdam study [13] were comparable with the rates reported in the Framingham Study [31], while the CHS reported twice as high incidence rates. Differences in age distributions as well as case report methods could possibly explain the different results.

Most of the common risk factors for atherosclerosis, such as age, hypertension, diabetes and obesity, have also been reported as risk factors for AF [1,32]. It has however been debated whether IMT could be used as a marker of atherosclerosis or not. Plaque area or plaque volume has been suggested as more accurate measures of atherosclerosis than IMT [33–35]. In the present study both CCA-IMT and carotid plaque were significantly associated with AF independently from one another, suggesting that CCA-IMT and plaque might affect AF through partly different mechanisms. The results are in concordance with previous studies from the present cohort showing that CCA-IMT was significantly associated with incident stroke even in the absence of carotid plaque [8]. Hence, it is possible that IMT and plaque might reflect different biological aspects of atherogenesis.

Plaque occurrence shows, compared to IMT, stronger associations with hyperlipidemia and smoking and is a stronger predictor of MI, while CCA-IMT shows stronger associations with hypertension and incident ischaemic stroke [33,36]. IMT likely reflects hypertensive medial hypertrophy and it is possible that IMT could be seen as a marker of the cumulative effect of hypertension or a physiological adaptation to changes in blood flow and wall tension [23]. A substantial portion of the hypertensive population also suffers from left-atrial enlargement, which may predispose to AF [37]. It is notable, though, that carotid IMT remained significantly associated with AF in the present study even after adjustment for blood pressure and anti-hypertensive medication.

Table 4
Incidence of first atrial fibrillation hospitalization in relation to sex-specific quartiles of intima-media-thickness (IMT) in the bifurcation.

MDC ($n = 3347$)	Sex-specific quartiles of IMT in the bifurcation				HR per SD[a]
	Q1	Q2	Q3	Q4	
N (men/women)	326/422	340/535	361/531	346/486	
Atrial fibrillation, *n* (%)	33 (4.4)	63 (7.2)	57 (6.4)	80 (9.6)	
Per 1000 person years	2.8	4.7	4.3	6.6	
Age and sex adjusted HR	1.00	1.18 (0.78–1.78)	0.89 (0.58–1.37)	2.00 (1.37–2.91)	1.27 (1.12–1.44)
+Risk factors[b]	1.00	1.19 (0.79–1.80)	0.87 (0.56–1.34)	1.90 (1.29–2.79)	1.24 (1.09–1.41)
+Carotid plaque[c]	1.00	1.17 (0.78–1.78)	0.83 (0.53–1.29)	1.66 (1.06–2.61)	1.25 (0.99–1.34)

[a] Hazard ratios per 1 standard deviation increase of log-IMT in the bifurcation.
[b] Risk factors: age, sex, smoking, diabetes, waist circumference, systolic blood pressure, anti-hypertensive medication, LDL, HDL, education, physical activity and hs-CRP (log-transformed).
[c] Model adjusted for all risk factors including carotid plaque.

Previous studies have shown associations between different markers of systemic inflammation and AF [25]. It has also been shown that the joint exposure to high CCA-IMT and high levels of hs-CRP substantially increases the risk of HF [9]. In the present study, censoring incident cases of HF and MI during follow-up did not have any significant effects on the association between IMT and AF. Leukocyte levels were not associated with IMT, and adjustment for hs-CRP did not influence the observed HR between IMT and AF. Low-graded chronic inflammation seems to be less important for the risk of AF compared to MI or HF.

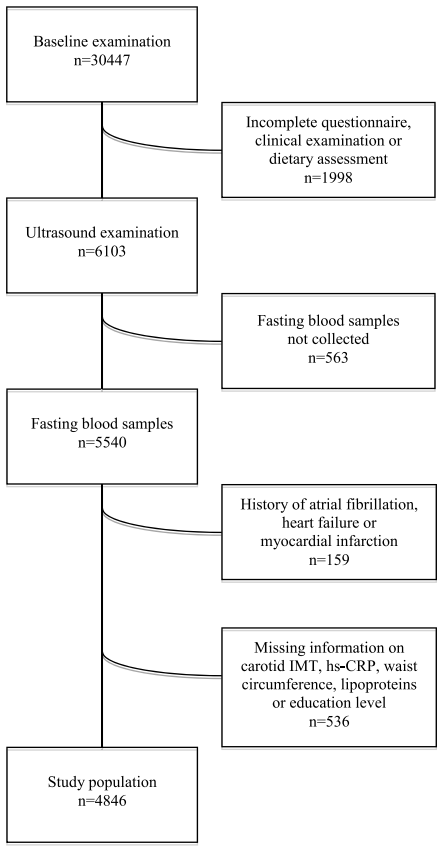

Fig. 2. Study population.

Some potential limitations need to be considered. History of AF, HF and MI at baseline and incidence of end-points were retrieved using the Swedish Hospital Discharge Register and the Swedish Cause of Death Register. Validation studies of the Swedish Hospital Discharge Register have shown a high validity of AF, MI and HF [24,38–40]. Some cases of AF are only handled in primary health care and are never treated in hospital. A main limitation is that this group is not included in the present study. Furthermore, no 12-lead electrocardiogram was performed at baseline, suggesting that some cases might have had AF already when entering the study. However, AF is unusual in this age group and a recent study of AF in the MDC cohort reported estimates of prevalence (about 1%) and incidence (4.3 per 1000 person-years), which are largely comparable with estimates from population-based studies in US, Italy and the Netherlands [4,13,30].

Another shortcoming is that baseline exposures in terms of lifestyle, medical treatment, socio-economic circumstances etc., were obtained from a self-administered questionnaire. The reliability and validity on such data may be questioned. In addition, blood pressure was only measured on a single occasion. Blood pressure is a powerful risk predictor for incident CVD (e.g., coronary events, stroke, HF, etc.) in this cohort [41,42], which should strengthen its internal validity. But we cannot rule out that multiple measurements at baseline or up-dated information on blood pressure could have weakened the observed relationship between carotid IMT and incident AF. We also lack information on incident HF outside hospital. However, the Swedish HF registry has shown that 90% of HF diagnoses are established following a hospital visit and only 10% following a visit in a primary health care setting [43]. Furthermore, the incidence of HF in the age group 45–68 years (as in the present cohort) is very low.

IMT was measured only in the right carotid artery, whereas many other studies calculated the mean value from both sides [10,23]. This is another potential limitation. The reproducibility of the IMT measurements and prediction of cardiovascular events in the present study is however comparable with the results from other large population-based cohort studies, which scanned both sides [10,23,44]. It is still possible that measuring IMT on both sides could further improve the prognostic value of IMT.

In conclusion, carotid IMT was independently associated with incidence of hospitalized AF in this study of middle-aged subjects from the general population. The results suggest that arterial thickening can predict future AF.

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Disclosures

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2014.01.050.

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