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# Age Aspects of Cardiovascular Disease

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DEPARTMENT OF CLINICAL SCIENCES, MALMÖ | LUND UNIVERSITY 2017

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Klas Gränsbo

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Abstract

Cardiovascular disease is the leading cause of death in our part of the world. Cardiovascular risk is strongly associated with age. Atherosclerotic vascular disease is rather unusual in males below age of 35 years and females below age of 45 and the prevalence gradually increases thereafter. Identification of patients at risk for cardiovascular events in all age groups is very important in the efforts to improve prevention, prediction, diagnosis, and prognosis and thereby decrease the burden of disease and the related cost. The overall main theme of the thesis is to improve prediction of cardiovascular disease and it´s complications in different age settings and in particular groups of individuals and patients.I will be presenting four papers concerning age-related risk factors and relation to cardiovascular disease (three published and one manuscript).

In the first paper we wanted to test if traditional risk factor pattern in midlife differs between patients who develop early versus late myocardial infarction. Exposure to cholesterol and family history of MI in midlife more strongly predicts onset of MI at younger ages, suggesting that MI in younger subjects is preceded by a different risk factor pattern than MI presenting in older subjects.In the second paper we evaluated the proportion of cardiovascular disease (CVD) incidence that is explained by genetic variation at chromosome 9p21 and to test whether such variation adds incremental information with regard to CVD prediction, beyond traditional risk factors. We found that a) The variation of chromosome 9p21 has a high population attributable risk suggesting that future interventions interfering with downstream mechanisms of the genetic variation may affect CVD incidence over a broad range of ages. b) Variation of chromosome 9p21 alone does not add clinically meaningful information in terms of CVD prediction beyond traditional risk factors at any age.c) despite a decline in the proportion of CVD incidence attributable to the chromosome 9p21 with increasing age, the actual number of events increased with age, suggesting that any future intervention to inhibit the consequences of the chromosome 9p21 risk variant may in fact prevent more CVD events amongst older than younger individuals.In the third paper we wanted to determine whether statin treatment is effective and safe in very elderly (80 years and older) acute myocardial infarction (AMI) patients. In a large register based study in The “Register of Information and Knowledge about Swedish Heart Intensive care Admission”(RIKS-HIA) we found that statin treatment is associated with lower cardiovascular mortality in very elderly post-infarction patients without increasing the risk of the development of cancer. Along with an aging population heart failure is increasing worldwide. New tools and biomarkers are needed to identify individuals at risk. In paper four, we examine if plasma concentration of a C-terminal fragment of the Endothelin-1 precursor hormone (CT-proET-1) predicts heart failure among elderly patients without history of CAD (coronary artery disease) on top of established risk factors. The study shows that CT-proET-1 strongly and independently predicts HF development in an older population free of CAD. The association was independent of traditional CVD risk factors, natriuretic peptide level and renal function.



Key words Age, Myocardioal infarction, Cardiovascular disease, Risk factors, Cholesterol, Prevention, Endothelin-1

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# Age Aspects of Cardiovascular Disease

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**To my beloved family**

"It strikes him that once the measurement of time is waived, the past and the future are ever-present- like the river, which at one and the same moment exists not only where he sees it to be, but also at its source and at its mouth. The water which has yet to pass is tomorrow, but it already exists upstream; and that which has passed is yesterday, but it still exists, elsewhere, downstream."

From A Fortune-teller Told me by Tiziano Terzani

# Contents

# List of papers

This thesis is based on the following papers

I **Klas Gränsbo**, Peter Almgren, Peter M. Nilsson, Bo Hedblad, Gunnar Engström, Olle Melander. Risk factor exposure in individuals free from cardiovascular disease differs according to age at first myocardial infarction. European Heart Journal, 2016;37:1977-81.*

II **Klas Gränsbo**, Peter Almgren, Marketa Sjögren, J. Gustav Smith, Gunnar Engström, Bo Hedblad and Olle Melander. Chromosome 9p21 genetic variation explains 13% of cardiovascular disease incidence but does not improve risk prediction. Journal of Internal Medicine, 2013;274:233-40**

III **Klas Gränsbo**, Olle Melander, Lars Wallentin, Johan Lindbäck, Ulf Stenestrand, Jörg Carlsson and Jan Nilsson. Cardiovascular and Cancer Mortality in Very Elderly Post-Myocardial Infarction Patients Receiving Statin Treatment J Am Coll Cardiol, 2010; 55:1362-69***

IV **Klas Gränsbo**, Alan Maisel, Peter Almgren, Peter M Nilsson, Margaretha Persson, Olle Melander. C-terminal Endothelin-1 strongly predicts heart failure in a population without history of coronary artery disease. Manuscript

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# Summary in Swedish

## Svensk populärvetenskaplig sammanfattning

*Inledning*

Åldern är den starkaste riskfaktorn för hjärt-kärlsjukdom sjukdom. Åldersskillnaden mellan unga och gamla blir allt större och ställer därför större krav på forskning och medicinsk behandlingen inom området. Det övergripande huvudtemat i denna epidemiologiska avhandling är att belysa olika perspektiv som kan förbättra förståelsen för hjärt-kärlsjukdom och dess komplikationer i olika åldersgrupper. Epidemiologi är den grundläggande vetenskapen om folkhälsan och har sitt ursprung i Hippokrates mer än 2000 år gamla observation att faktorer i omgivningen och miljön kan påverka uppkomst av sjukdom. Flera epidemiologiska studier har spelat stor roll i att kunna förklara varför vissa faktorer orsakar hjärt-kärlsjukdom vilket i sin tur har lett till förbättrade preventiva åtgärder samt en förbättrad behandling. Befolkningsstudier som t.ex. Malmö Förebyggande Medicin och Malmö Kost-Cancer är och har varit viktiga för att kunna identifiera sjukdom och till att förtydliga samband mellan olika

riskfaktorer och utfall. Det är därför viktigt att resultaten i dessa studier bekräftas i andra befolkningar och i olika kliniska situationer.

## Hjärtkärlsjukdom

Hjärtkärlsjukdom är den vanligaste dödsorsaken i vår del av världen. I Europa dör mer än 4 miljoner människor varje år till följd av denna sjukdom. I Sverige beräknas ca 1,4 miljoner svenskar ha en känd hjärtkärlsjukdom. Identifiering av patienter som löper risk för kardiovaskulära händelser i alla åldersgrupper är mycket viktigt i arbetet med att förbättra prevention, diagnostik och prognos och därigenom minska sjukdomsbördan och vårdrelaterade kostnader. Hjärtkärlsjukdom inbegriper sjukdomar som hjärtinfarkt, stroke och sjukdomar i de större kärlen. Oftast avses sjukdomar som är starkt förknippade med ateroskleros som också brukar kallas åderförkalkning.

Åldern anses vara den viktigaste riskfaktorn för sjukdomen. Även om de flesta fall av sjukdom sker hos de äldre så börjar ateroskleros tidigt i livet och tecken på fettinlagring i kärlen kan noteras redan hos yngre individer som ej blivit sjuka. Kärlsjukdom p.g.a. ateroskleros är ganska ovanligt hos män yngre än 35 år och kvinnor under 45 år men förekomsten ökar gradvis därefter. Under de senaste decennierna har kunskapen om hjärtkärlsjukdom förbättrats avsevärt. Trots förbättring avseende förebyggande åtgärder, diagnostik och behandlingen så drabbas ca 30000 personer varje år i Sverige av hjärtinfarkt respektive stroke.

Personer i åldern 80 år eller äldre är den snabbast växande delen av befolkningen i höginkomstländerna. Ökningen i åldersspannet i befolkningen ställer nya krav på hjärtkärlforskning samt hälso- och sjukvårdssystemen. Det är viktigt att identifiera personer i riskzonen för att tidigt starta förebyggande strategier och vid behov inleda läkemedelsbehandling. Det finns inte bara ett behov av förbättrade riktlinjer avseende behandling av äldre utan också behov utav en mer individualiserad syn avseende förebyggande åtgärder hos yngre personer med hög risk för att utveckla hjärtkärlsjukdom.

## De stora befolkningsstudierna i Malmö MPP och MKC

Malmö Förebyggande Project (MPP) som startades på 1970-talet som ett allmänt brett primärpreventivt screeningprogram vid Medicinska kliniken i Malmö Allmänna Sjukhus (numera Skånes universitetssjukhus Malmö) under ledning av professor Bertil Hood. Projektet avsåg att identifiera riskfaktorer för hjärtkärlsjukdomar i den vuxna befolkningen för att kunna påbörja förebyggande insatser. Av alla som bjöds in så deltog ca 70 %. Mellan åren 1974-1992 inkluderades 33346 deltagare, totalt 22 444 män

(medelålder 46 år) och 10 902 kvinnor (medelålder 49 år) i projektet. Männen inkluderades framför allt i den första halvan av perioden och kvinnorna under den senare hälften vilket innebär olika uppföljningsperioder för män och kvinnor. Olika insatser i form av bl.a. livsstilsförändring och läkemedelsbehandling gjordes i ca 25 % av de screenade deltagarna. Projektet har legat till grund för ett flertal vetenskapliga artiklar och avhandlingar.

I början av 1990-talet tog Cancerfonden initiativ till en stor befolkningsstudie, Malmö Kost Cancer (MKC), med avsikt att studera orsaker till uppkomst av cancer och hjärtkärlsjukdom. Studien leddes initialt av professor Göran Berglund (professor i Internmedicin) och professor Lars Janzon (professor i epidemiologi). Studien är en prospektiv, populationsbaserad kohortstudie som omfattade 30 447 slumpvis utvalda män (födda mellan 1923 och 1945) och kvinnor (födda mellan 1923 och 1950) som genomgick en baslinje undersökning mellan 1991 och 1996. Medelåldern för män var 59,2 ± 7,0 år och för kvinnor 57,4 ± 7,9 och deltagandet var 41 %. Drygt 6000 av dessa individer blev dessutom inkluderade i en delstudie (den kardiovaskulära kohorten). Det ursprungliga syftet med studien var att identifiera och utforska sambandet mellan kost och utveckling av cancer. Studiedeltagarna lämnade blodprover inklusive DNA som kunnat sparas för senare biokemiska och genetiska analyser. Även denna befolkningsstudie är väl beskriven i ett flertal vetenskapliga artiklar och har legat till grund för ett flertal vetenskapliga avhandlingar. MKC är en del av EPIC - The European Prospective Investigation into Diet and Cancer. Detta samarbete involverar cirka 20 liknande befolkningsstudier i tio europeiska länder.

## Riks-HIA - Det stora hjärtinfarktregistret

RIKS-HIA är ett stort svenskt register för hjärtintensivvård som skapades 1991 av professor Lars Wallentin, Uppsala, och docent Ulf Stenestrand, Linköping. Registret utvecklades till ett nationellt kvalitetsregister från januari 1995. Genom åren har allt fler sjukhus har anslutit sig till registret och sedan 2008 deltar alla 74 svenska sjukhus som tar emot akut hjärtsjuka patienter i registreringen. Syftet med det svenska registret för hjärtintensivvård är att ”utveckla akut hjärtsjukvård genom kontinuerlig information om vårdbehov, behandling och behandlingsresultat samt förändringar såväl inom ett sjukhus som i jämförelse med andra sjukhus. Den långsiktiga målsättningen är att bidra till en minskad dödlighet och sjuklighet hos patienterna och att öka kostnadseffektiviteten i vården”. Registret stöds ekonomiskt av Sveriges Kommuner och Landsting, Socialstyrelsen, Hjärt-Lungfonden och är knutet till Svenska Kardiologföreningen.

## Riskfaktorer för hjärtkärlsjukdom

Få ämnen har fått så mycket uppmärksamhet i den kardiovaskulära litteraturen under de senaste åren som prediktion (förutsägelse) av framtida hjärtkärlhändelser. Begreppet "riskfaktor" användes ursprungligen av forskarna William Kannel och Thomas Dawber när de 1961 i en uppmärksammad artikel grundad på data ur The Framingham Heart Study beskrev riskfaktorer för att utveckla kranskärlssjukdom. En riskfaktor är en egenskap eller förhållande hos en individ eller befolkning som finns tidigt i livet och är associerad till en ökad risk att utveckla framtida sjukdom.

Man brukar dela in riskfaktorer i påverkningsbara och icke påverkningsbara (modifierbara och icke modifierbara) riskfaktorer. Exempel på icke påverkningsbara riskfaktorer är hög ålder, manligt kön och genetiska faktorer medan exempel på påverkningsbara riskfaktorer är högt blodtryck, rökning, diabetes, höga nivåer av kolesterol i blodet, fysisk inaktivitet/stillasittande och fetma. Tidigare studier har visat att riskfaktorerna är nära sammankopplade och ju fler riskfaktorer desto större möjlighet att utveckla framtida hjärt-kärlsjukdom. Patienter med högt blodtryck och höga nivåer av kolesterol i blodet och diabetes tenderar oftare att utveckla kranskärlssjukdom än patienter med normalt blodtryck och höga kolesterolnivåer.

Kardiovaskulär risk är sannolikheten för att en person utvecklar en dödlig eller icke-dödlig aterosklerotisk hjärtkärlhändelse under en definierad tidsperiod. Flera algoritmer eller scorer har skapats och används för att identifiera individer med hög risk att insjukna i hjärtkärlsjukdom på t ex. 5 och 10 års sikt. Riskberäkningarna är ofta baserade på statistiska analyser (s.k. multivariata regressionsanalyser) som härrör från stora befolkningsstudier. En av de mest kända och mest använda riskalgoritmerna härstammar från Framinghamkohorten där nivåerna av traditionella riskfaktorer (ålder, totalkolesterol, high-density-lipoprotein-kolesterol (HDL), systoliskt blodtryck och rökning) används för att förutspå sannolikheten att utveckla framtida kranskärlssjukdom separat för män och kvinnor. De europeiska riktlinjerna använder numer framförallt SCORE-algoritmen som utarbetas via europeisk kardiolog föreningen och som beräknar den 10-åriga summerande risken för en första aterosklerotisk händelse som tex hjärtinfarkt, stroke och plötslig hjärtdöd.

Dokumentationen avseende kolesterol som riskfaktor för åderförkalkning, ateroskleros, och utveckling av hjärtkärlsjukdom är gedigen. Ett flertal studier (både befolkningsstudier och läkemedelsstudier) har genom åren påvisat sambandet mellan höga kolesterolnivåer i blodet och risken att insjukna i hjärtkärlsjukdom. Vid hyperkolesterolemi föreligger förhöjda nivåer av kolesterol i plasma. Kolesterol transporteras i blodet av s.k. lipoproteiner. En stor del av dessa lipoproteiner utgörs av Low Density Lipoproteiner (LDL, eller i folkmun det ”onda” kolesterolet) och High Density Lipoproteiner (HDL, ”det goda” kolesterolet). Höga nivåer av LDL är

förknippat med ökad risk för hjärtkärlsjukdom medan höga nivåer av HDL är förknippat med minskad risk.

Kolesterolnivåerna i blodet påverkas till stor del av ärftlig reglering, så kallade genetiska faktorer, och av livsstilsrelaterade faktorer som kost och motion. Vid hyperkolesterolemi är det genetiska sambandet komplext med flera olika gener inblandade i processen. Undantag är dock familjär hyperkolesterolemi som är ett tillstånd som styrs av enstaka gener.

Synen på kolesterol och dess beståndsdelar som riskfaktor för hjärtkärlsjukdom har diskuterats intensivt under många år liksom nyttan av att sänka kolesterolnivåerna med läkemedel s.k. statiner. Av lipidsänkande läkemedel har statinerna den mest väldokumenterade effekten avseende kliniskt nytta för att kunna förebygga sjuklighet och död. I de flesta moderna guidelines för prevention och behandling av hjärtkärlsjukdom används LDL-nivån i blodet som mått. Gränsen för behandling relateras i de flesta fallen till den totala risken för insjuknande i hjärtkärlsjukdom som grundas traditionella riskfaktorer för hjärtkärlsjukdom som ålder, kön och blodtryck liksom den kliniska bilden.

## Ärftlighet och genetik

Det har sedan länge varit känt att risken att insjukna i stroke eller hjärtinfarkt ökar om sjukdomarna finns i familjen. På senare år har den genetiska forskningen lett till banbrytande resultat. Man har lyckats identifiera en stor mängd genetiska varianter som ökar risken för olika typer av hjärtkärlsjukdom. Flera av dessa genetiska varianter kan sammankopplas med traditionella riskfaktorer för hjärtkärlsjukdom som högt blodtryck och höga kolesterolnivåer men man har även funnit varianter som är sammankopplade med ökad risk för hjärtkärlsjukdom utan att man har kunskap om varför. 2007 kunde forskare konstatera att genetiska riskmarkörer för koronarsjukdom, d.v.s. sjukdomar i hjärtats kranskärl, finns lokaliserade på kromosom 9. Man fann även att genvariationerna inte bara ökar risken för hjärtinfarkt utan även ökar risken för stroke. Man har därefter kunnat kartlägga ytterligare ett stort antal genvarianter som är sammankopplade med hjärtinfarkt. Identifiering av nya gener kopplade till hjärtkärlsjukdom som verkar oberoende av idag redan kända riskfaktorer har lett till ny kunskap och ökad förståelse om mekanismer för uppkomst av sjukdomar som högt blodtryck och högt kolesterol. Att kunna identifiera orsakssambandet mellan genetisk variation med hjärtkärlsjukdom har varit av stor betydelse. Tack vare en djupare förståelse av de grundläggande genetiska mekanismerna och dess samband med olika proteiner kan förhoppningsvis nya och mer effektiva hjärt-kärlsskyddande läkemedel utvecklas.

Jag kommer att presentera fyra delarbeten om åldersrelaterade riskfaktorer och dess relation till hjärt-kärlsjukdom (tre publicerade och en inlämnad för publikation). I tre

av de fyra delarbetena utgår vi ifrån två sedan tidigare insamlade studiematerial dvs material från de stora befolkningsstudierna Malmö Förebyggande Medicin samt Malmö Kost Cancer. I delarbete 3 har vi använt material från det stora svenska hjärtinfarktregistret RIKS-HIA.

## Delarbete I

I det första arbetet som utgår från tillgängliga data från Malmö Förebyggande Medicin ville vi undersöka om traditionella riskfaktormönster för hjärtkärlsjukdom i medelåldern skiljer sig mellan patienter som utvecklar tidig kontra sen hjärtinfarkt. Under uppföljningstiden i studien som var drygt 22 år insjuknade 3687 individer med hjärtinfarkt. Studien visar att samtliga traditionella riskfaktorer som ingick i studien var kopplade till ökad risk för insjuknande i hjärtinfarkt. Efter att ha delat upp individerna som insjuknade i hjärtinfarkt i fyra grupper beroende på när man insjuknande i hjärtinfarkt så analyserades och jämfördes grupperna mot hjärtfriska individer. Studien visade att högt kolesterol i blodet förutspår ökad risk för uppkomsten av hjärtinfarkt i yngre åldrar jämfört med senare i livet. Sambandet är linjärt avtagande. Ett liknande mönster noterades för de deltagare som hade en familjehistoria avseende hjärtinfarkt i ung ålder. Rökning uppvisade ett U-format samband där ökad risk för att insjukna i hjärtinfarkt noterades i den yngsta och den äldsta gruppen. För övriga riskfaktorer noterades ingen skillnad avseende risk att insjukna tidigt eller sent vilket tyder på att dessa riskfaktorer bör beaktas oavsett ålder.

## Delarbete II

Kunskapsläget om hur risken av genvarianterna påverkas av åldern behöver förbättras. I det andra arbetet vill vi undersöka och utvärdera hur stor andel av hjärt-kärlsjukdomen som kan förklaras av genetisk variation på kromosom 9 (kromosom 9p21) samt försöka ta reda på om denna information kan (utöver traditionella riskfaktorer, såsom högt blodtryck, höga kolesterolnivåer, ärftlighet för hjärt-kärlsjukdom, rökning mm.) förbättra möjligheten att förutspå hjärt-kärlsjukdom.

I arbetet som utgår från Malmö Kost-Cancer studien kunde vi konstatera och bekräfta att genvariationen på kromosom 9p21 är vanlig i befolkningen. Risken för uppkomst av hjärtkärlsjukdom verkar vara av lika stor betydelse för kromosom 9p21 som andra viktiga traditionella riskfaktorer. Genvarianten påverkar förekomsten av hjärtkärlsjukdom i ett brett spektrum av åldrar. Med stigande ålder minskar andelen fall av hjärtkärlsjukdom som kan tillskrivas varianten på kromosom 9p21. Trots denna nedgång så ökar det faktiska antalet av händelser beroende på genvariationen med stigande ålder vilket tyder på att framtida insatser för att förhindra konsekvenserna av riskvarianten belägen på kromosom 9p21 i själva verket kan förhindra fler

hjärtkärlhändelser bland äldre än yngre individer. Studien visar dock att den genetiska tilläggsinformationen av variationen på kromosom 9p21 på toppen av traditionella riskfaktorer inte förbättrar möjligheten att förutspå av hjärt-kärlsjukdom.

## Delarbete III

Medellivslängden och antalet äldre i befolkningen ökar vilket leder till ökat antal fall av hjärtkärlsjukdom i denna åldersgrupp. Mer än 80 % av alla dödsfall hos patienter äldre än 65 år beror på kranskärlssjukdom. Som tidigare nämnts så har ett stort antal kliniska studier visat att behandling med kolesterolsänkande behandling med så kallade statiner signifikant minskar dödligheten efter hjärtinfarkt. Trots att äldre patienter som insjuknar med akut hjärtinfarkt löper större risk att avlida både på kort och på lång sikt så tillämpas denna behandling i lägre utsträckning för de äldre jämfört med de yngre patienterna. Att sambandet mellan kolesterol och risken för att insjukna i hjärtkärlsjukdom minskar med stigande ålder vilket kan vara en av förklaringarna till en lägre förskrivning av läkemedel till äldre liksom att de flesta läkemedelsstudierna avseende statiner som tidigare genomförts har uteslutit de äldre patienterna. En annan förklaring kan vara den rädsla som funnits för biverkningar och utveckling av allvarliga sjukdomar som t.ex. cancer. I tredje arbetet vi ville därför avgöra om statinbehandling är effektiv och säker hos patienter som är 80 år och äldre och som insjuknat och vårdats för akut hjärtinfarkt. I det stora svenska hjärtinfarktregistret, RIKS-HIA undersöktes och följdes mer än 21 000 patienter (80 år eller äldre) under åren 1999 and 2003 avseende sjuklighet och död beroende på om de hade fått statinbehandling i samband med utskrivning från hjärtkliniken.

Vi hade tillgång till kompletta data hos ca 15 000 patienter (studiepopulation A). För att minska risken för bias så exkluderades de patienter som dog inom 14 dagar(studiepopulation B) samt inom 365 dagar (studiepopulation C). Statistisk hänsyn togs även till skillnader i behandlingsgrupperna avseende bl.a. behandling och sjuklighet vid ankomst till sjukhuset genom att konstruera ett s.k. propensityscore. Resultatet i studien tyder på att statinbehandling är förknippad med lägre kardiovaskulär dödlighet hos äldre hjärtinfarktpatienter utan att öka risken för utvecklingen av cancer. Även om det finns flera felkällor i denna registerbaserade studie så är resultatet av intresse och kan ligga till grund för framtida studier.

## Delarbete IV

I en allt mer åldrad befolkning förväntas förekomsten av hjärtsvikt att öka. Nya och bättre verktyg behövs för att kunna diagnosticera sjukdomen i tid för att kunna påbörja tidig behandling. Medan patienter med etablerad hjärtkärlsjukdom rutinmässigt screenas och följs avseende sin hjärtsjukdom och eventuella komplikationer till denna

så utvecklar en stor del av patienter hjärtsvikt utan att ha någon tidigare känd hjärtsjukdom.

Endotelin-1 (ET-1) är ett kroppseget protein som har starkt kärlsammandragande egenskaper som bidrar till uppkomsten och utvecklingen av hjärtkärlsjukdom. ET-1 har visat sig ha effekter som höjer blodtryck, inducerar hjärtmuskeltillväxt samt kan påverka utsöndring av en rad andra hormoner kända för att ha skadliga långtidseffekter på hjärtmuskeln. Hos patienter med känd hjärtsvikt så har höga nivåer av ET-1 visat sig kunna förutspå dödlighet och återinläggning på sjukhus. Nivåerna av Endotelin-1 i blodet kan beräknas genom att mäta plasmakoncentration av en ”ny” biomarkör, ett C-terminalt fragment av endotelin-1 precursor hormon (CT-proET-1).

Syftet i denna epidemiologiska studie är att undersöka om plasmakoncentrationen av en CT-proET-1, kan förutspå framtida insjuknande i hjärtsvikt hos äldre patienter (69 år och äldre) utan känd historia av kranskärlssjukdom (CAD) utöver etablerade traditionella riskfaktorer. Det vi fann var att CT-proET-1 starkt och oberoende förutspådde utveckling av framtida insjuknande hjärtsvikt. Den fjärdedel av patienterna som hade den högsta koncentrationen av CT-proET-1 i plasma löpte 12,5 ggr högre risk att insjukna jämfört med den lägsta fjärdedelen. Även om resultatet i studien bör replikeras i fler framtida randomiserade kontrollerade studier så tyder resultaten på att äldre patienter utan tidigare känd hjärtsjukdom och som har hög koncentration av endotelin-1 i blodet har ökad risk för att utveckla hjärtsvikt och kan därför ha nytta av intensifierad blodtryckssänkande behandling inklusive inplaneras för regelbundna hälsokontroller.

# Sammanfattning


(Bild från Macrovector, Shutterstock)

Åldern är den starkaste riskfaktorn för kardiovaskulär sjukdom. Åldersskillnaden mellan unga och gamla blir allt större och ställer därför större krav på forskningen och på den medicinska behandlingen. Sammantaget syftar de fyra delarbetenas i avhandlingen till att belysa åldersfrågor utifrån flera olika perspektiv allt ifrån riskfaktor mönster för hjärtsjukdom hos friska medelålders vuxna till behandlingseffekter av kolesterolsänkande behandling hos hjärtinfarktpatienter som är 80 år eller äldre. Avhandlingen belyser även genetiska aspekter i form av kromosom 9p21:s betydelse i olika åldrar avseende risken att insjukna i hjärtsjukdom samt visa att man hjärtfriska äldre kan använda nya biomarkörer, som t.ex. Endotelin-1, för att prediktera framtida insjuknande i hjärtsvikt.

# Introduction

## General introduction

Age is regarded as the main risk factor for cardiovascular disease (CVD). People aged 80 or older are the fastest growing population in high-income countries. Even though most cases occur in the elderly, atherosclerosis starts early in life and asymptomatic atherosclerotic disease may be advanced in many younger individuals. It is of importance to identify persons at risk in order to start preventive strategies and if needed initiate pharmacological treatment. The increase in the age range of the population places new demands on health care systems and scientists in the in the cardiovascular research field. There is not only a need for guidelines including the very elderly but also a more individualized view regarding cardiovascular prevention of younger persons at high risk for developing CVD.

This thesis is primarily epidemiological. Epidemiology is the basic science of public health. It is originated in Hippocrates more than 2,000 years old observation that environmental factors affect the onset of disease. Epidemiology is important for finding new tools to prevent and predict risk of disease. Several epidemiological studies have played a major role in explicating factors that predispose to cardiovascular disease and thereby given opportunities for prevention of public health. Observational prospective population-based cohort studies as Malmö Preventive Project and Malmö Diet and Cancer study have been very important at identifying disease and to the clarification of the association between risk factors and outcome. Still it is of importance that the findings in these studies are confirmed in different cohorts and in different clinical settings.

Throughout this thesis I try to highlight different age aspects of cardiovascular disease; from different age aspects of primary prevention according to biological and genetic risk factors to secondary prevention in the very elderly.

## Cardiovascular disease

Cardiovascular diseases which include diseases of the heart, vascular diseases of the brain and diseases of the blood vessels is the leading cause of death in the world(1). CVD kills over 4 million people in Europe each year(2). Of all these deaths before 75 years 42% are due to CVD in women and 38% in men. According to the Swedish Heart and Lung Foundation 1,4 million Swedes live with CVD(3).

Even though people live longer due to improvement in economic circumstances, improved treatment of cancer and infectious diseases and technological advances such as coronary care units and development of new medicines the burden of CVD has increased with an aging population.

CVD mortality has declined since the 1980s, particularly in high-income regions, due to preventive measures including the success of smoking legislation and improved pharmacological treatment. However, many risk factors, particularly obesity and diabetes mellitus, have been increasing. Primary prevention is important and can be effective. According to WHO the elimination of health risk behaviours would make it possible to prevent at least 80% of CVDs and even 40% of cancers(4-6).

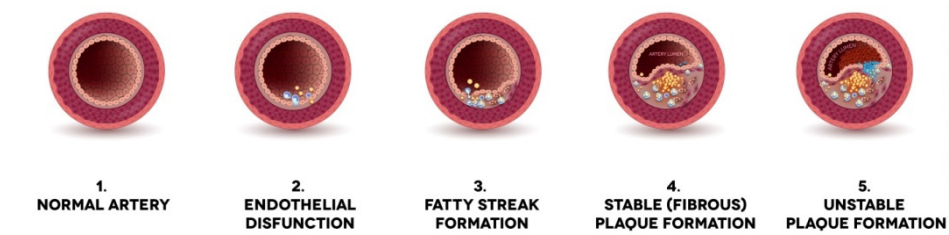
Cardiovascular risk is strongly associated with age. Atherosclerotic vascular disease is rather unusual in males below age of 35 years and females below age of 45 and the prevalence gradually increases thereafter. According to the Swedish Register of Information and Knowledge about Swedish Heart Intensive Care Admissions the mean age of patients suffering from myocardial infarction in Sweden has been stable since 2000, between 69 and 70 for men and 75 and 76 for women(7).

According to the European Guidelines on cardiovascular disease prevention in clinical practice 2016 prevention should be delivered (a) at the general population level by promoting healthy lifestyle behaviour and (b) at the individual level, i.e. in those subjects at moderate to high risk of CVD or patients with established CVD, by tackling unhealthy lifestyles (e.g. poor-quality diet, physical inactivity, smoking) and by optimising risk factors(4). It's well known that risk factors as hypertension, diabetes, abdominal obesity, smoking, psychosocial factors, consumption of fruits, vegetables, and alcohol, and regular physical activity account for most of the risk of myocardial infarction worldwide in both sexes and at all ages in all regions less is known about the influence of risk factors in different age groups(8) .

## Atherosclerosis and CVD

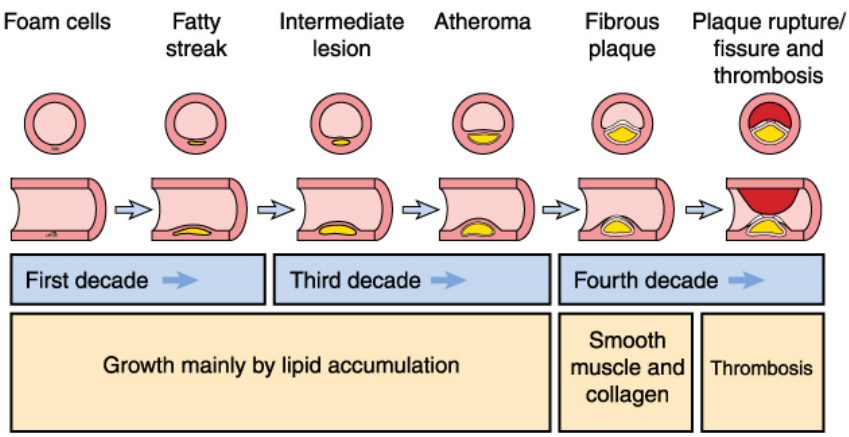
Atherosclerosis is the main contributor to myocardial infarction in adults but at the same time, up to 20% of MI patients below the age of 45 years do not display the evidence of atherosclerosis on coronary angiography(9). Atherosclerosis is a complex

process affecting different sites of the arterial tree. Through intimal inflammation, necrosis, fibrosis and calcification it develops over many years leading to thickening and hardening of the vessels and may lead to obstruction of the flow or suddenly cause life-threatening coronary thrombosis(10). The process is often silent during early stages and not noticed until it leads to ischemic heart disease, cerebrovascular disease (e.g. stroke), and diseases of the aorta and arteries including hypertension.




Picture above shows atherosclerosis formation (from Tefi, Shutterstock)

The atherosclerotic plaque is made of cholesterol, fatty substances, cellular waste products, calcium and fibrin (a clotting material in the blood). The process starts early in life and appears already in the fetal aorta while it appears in the coronary arteries in the second decade and in the cerebral arteries in the third decade(11). According to an earlier study atherosclerotic lesions are present in 1 of 6 teenagers(12). Onset of clinical symptoms is very individual due to different burdens of risk factors and differences in genetics. Figure below shows different stages of development of atherosclerosis.




The figure above shows the slow progression of atherosclerotic disease. (Blamb, Shutterstock)

## Acute Myocardial Infarction

Millions of people worldwide die from acute myocardial infarction each year(13). The term myocardial infarction is thought to reflect death of cardiac muscle cells, myocytes, due to prolonged ischaemia(14). A universal definition was presented 2012 by on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction (15)

According to the definition a myocardial infarction occurs if at least two of three criteria are fulfilled: typical ischaemic chest pain; raised concentrations of cardiac specific markers in serum in serum; and typical electrocardiographic findings, including development of pathological Q-waves(16).

Myocardial infarction can occur during the natural course of coronary atherosclerosis. Development of the disease might be clinically silent for years but in the long run, however, stenosis may become functionally important, and coronary artery disease becomes symptomatic.

The number of persons in Sweden who suffered from myocardial infarction in 2014 was estimated to around 27 000. Of those, more than 8500 people died before the end of the same year, of which 6700 with acute myocardial infarction as the underlying or contributing cause of death. In 5400 people myocardial infarction was specified as the underlying cause of death. Around 52% of all persons who suffered from myocardial infarction were 75 years or older and 25% of the cases were 85 years or older. Only 1.5% of the cases were below 45 years. See figure and table below.

The mortality after infarction, have dropped sharply in the past 20 years. In 1994, more than 40% of men and 44% of women died within 28 days after infarction. The corresponding values in 2004 were 30 and 33% respectively and in 2014, 25% and 29% respectively for men and women. The higher mortality rate for women is due to the greater the proportion of elderly among them. After age adjustment, men consistently have higher mortality rate than women(17). The dramatic increase in numbers of myocardial infarction cases from 45 years of age suggests that early CVD prevention is important. In my first article we want to examine if different risk factor patterns in mid-life can predict whether a person develops early or late myocardial infarction. 65% of the MI cases in Sweden were 70 years or older. The majority of earlier secondary prevention MI trials have excluded patients above 65 years of age. In the third article we therefore wanted to examine if statin treatment to very elderly patients with myocardial infarction gave cardiovascular protection.

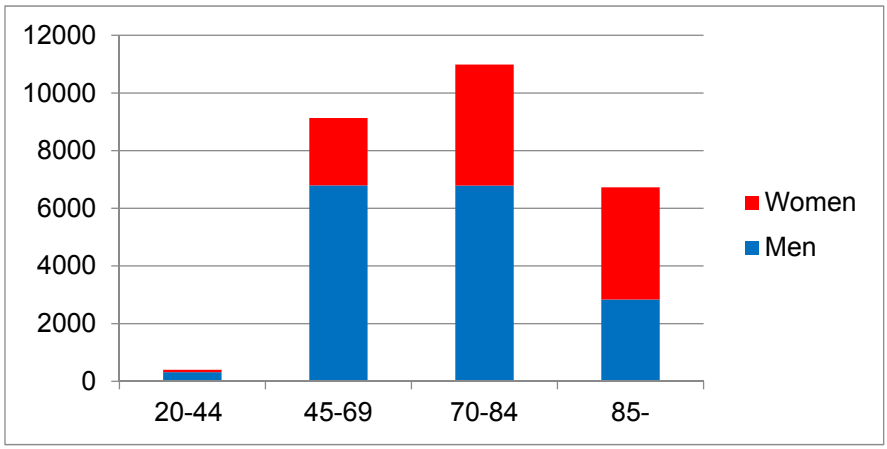



Figure shows number of people with at least one case per year of acute myocardial infarction by age and gender in Sweden 2014. Modified from Myocardial Infarctions in Sweden 1994–2014 http://www.socialstyrelsen.se/publikationer2015/2015-11-1

| Age (years) | Men | Women | Total |
|---|---|---|---|
| 20-44 | 317 | 83 | 400 |
| 45-69 | 6795 | 2334 | 9129 |
| 70-84 | 6791 | 4196 | 10987 |
| 85- | 2835 | 3891 | 6726 |
| **Total** | **16738** | **10504** | **27242** |

Table shows the number of people with at least one case of acute myocardial infarction by age and gender in Sweden 2014. Modified from Myocardial Infarctions in Sweden 1994–2014 http://www.socialstyrelsen.se/publikationer2015/2015-11-1

## Stroke

Stroke affects more than 15 million individuals worldwide each year leading to death in about one-third of the cases(18). The disease also causes severe disability in many of the survivors(19). Stroke is a heterogeneous disease including ischaemic and haemorrhagic events. The traditional definition of stroke is clinical and based on the sudden onset of loss of focal neurological function due to infarction or haemorrhage in the relevant part of the brain, retina, or spinal cord(20). An updated definition of stroke from the From the American Heart Association/American Stroke Association was published 2013 where the authors concluded that a stroke is an acute episode of focal dysfunction of the brain, retina, or spinal cord lasting longer than 24 h, or of any duration if imaging (CT or MRI) or autopsy show focal infarction or haemorrhage relevant to the symptoms (21). The definition includes subarachnoid haemorrhage.

Stroke is distinguished from transient ischemic attack (TIA) which similarly as a stroke, producing similar symptoms which do not persist more than 24h (usually last only a few minute) and doesn´t cause any irreversible damage and with no imaging evidence of infarction.

Ischemic stroke account for more than 80% of all stroke cases and is a contributor to death worldwide. Even though the incidence rates have been stable and the mortality rates have declined over the last two decades the number of incident strokes, prevalent stroke survivors and stroke-related deaths are increasing(22).The risk of having stroke increases significantly with age and because of an ageing population the burden of the disease remains high (20). Individuals 65 years and older are up to ten times more likely to suffer from a stroke than younger individuals in the age group of 18- to 44-years(23). The prevalence of stroke survivors is projected to increase, especially among elderly women(24). In contrast to many other diseases the average age of ischemic stroke onset is decreasing, owing to a rise in the incidence of stroke among individuals under 50 years of age and there seem to be a coincidence with an increasing prevalence of traditional risk factors in this younger age(25).

## Heart Failure

Heart failure (HF) among the elderly is not only common but also one of the leading causes of death and hospitalisation. In contrast to myocardial infarction, whose incidence has levelled off in parallel to the improvements of primary and secondary prevention, the incidence of HF and its associated comorbidities is increasing. The increasing burden of the disease might be due to a combination of an aging population, increasing incidence and improved treatment of CVD and its complications.

HF is defined as a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress(26).

HF is rare in persons younger than 50 years but in those older than 50 years there is a progressively increase in incidence and prevalence with age(27). Individuals aged ≥ 65 years account for more than 80% of the deaths and prevalent cases in the USA and Europe(28). About 250 000 people in Sweden suffer from symptomatic heart failure. The prevalence is approximately 2% of the adult population in Sweden, rising to ≥10% among people 80 years of age. The predominant causes are ischemic heart disease due to atherosclerosis and hypertension even though there are other causes as valvular disease, congenital heart disease, heart arrhythmia, inflammatory and infectious heart muscle disease, high alcohol consumption, cardio toxic drugs etc.(29).

Heart failure treatment has improved markedly in later years but the prognosis remains poor. The overall 1-year mortality for patients with heart failure registered in RiksSvikt is approximately 20%(29).

Even though prevention of onset of ischemic heart disease and hypertension is the key to reduce the burden of HF there is a need to find new tools for early detection and treatment of heart failure. In paper IV we address the issue to find out if screening elderly patients (mean age 69 years) free from heart failure and known ischemic heart disease with the biomarker C-terminal fragment of the Endothelin-1 precursor hormone (CT-proET-1) could predict future events of heart failure.

# Risk factors for cardiovascular disease

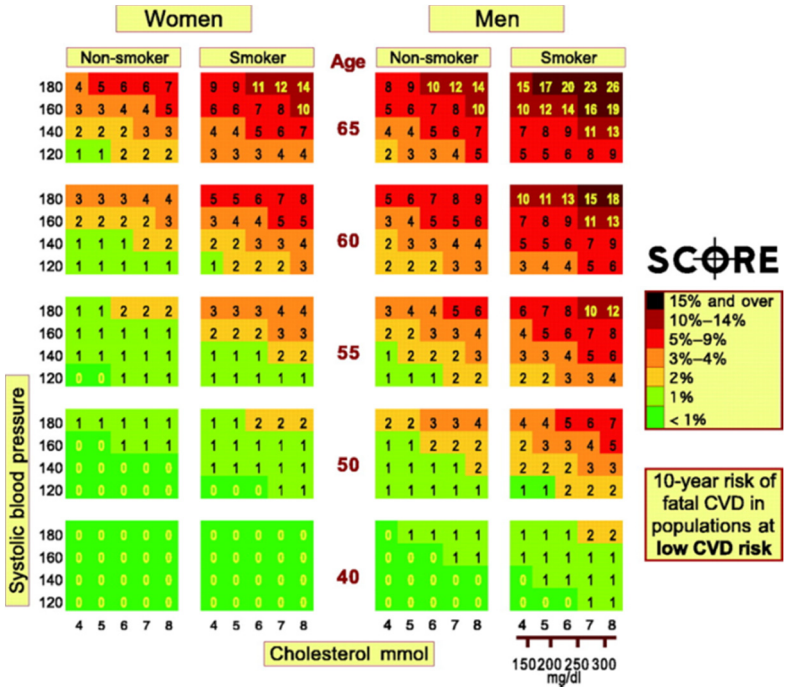
## Definition of Risk factors

A risk factor is a characteristic or feature of an individual or population that is present early in life and is associated to an increased risk of developing future disease. Today there are several well known risk factors for CVD. They are divided in modifiable and non-modifiable risk factors (30, 31). Risk factors including increasing age, male sex, genetics and family history of early onset of cardiovascular disease are considered to be non-modifiable. Modifiable risk factors include risk factors as hyperlipidaemia, smoking, hypertension, obesity, physical inactivity, and diabetes. The risk factors are used for to calculate CVD risk and make clinical assessment of patients to decide whether life-style intervention and pharmacological treatment is indicated.

## Risk scores and Risk Prediction

Few topics have received as much attention in the cardiovascular literature over the last years as risk prediction(32). The term 'risk factor' itself was used initially by the Framingham Heart Study investigators William Kannel and Thomas Dawber in their 1961 landmark paper 'Factors of risk in the development of coronary heart disease–six year follow-up experience'(33) Earlier studies have shown that risk factors are closely related. The more risk factors the greater possibility to develop future cardiovascular disease. Dzau et al described a chain of events precipitated by several cardiovascular risk factors which if left untreated ended up in heart failure and death. They called this chain the cardiovascular disease continuum. Hypertensive patients with hypercholesterolemia and impaired glucose tolerance or diabetes mellitus tend to develop coronary artery disease more often than normotensive patients(34). Cardiovascular risk mean the likelihood of a person developing a fatal or a non-fatal

atherosclerotic cardiovascular event over a defined period of time(2). Several algorithms have been created and used to identify individuals at high risk. Risk estimates (e.g. 5- and 10 years) are often based on multivariate regression analysis derived from large cohort studies. One of the most well-known and widely used risk prediction scores is derived from the Framingham cohort in which the levels of traditional risk factors (age, total cholesterol, high-density-lipoprotein cholesterol, systolic blood pressure, smoking status) are used to predict an absolute probability to develop future coronary heart disease (CHD) events separately for men and women(35, 36). The European guidelines use the algorithm of SCORE (37).The SCORE system estimates the 10-year cumulative risk of a first atherosclerotic event (heart attack, stroke, or other "occlusive" arterial disease, including sudden cardiac death).




The figure above from the SCORE-project (Ref. Conroy et al European Heart Journal (2003)24,987–1003) shows the 10-year risk of a fatal cardiovascular disease event for 400 combinations of risk factors for high low risk regions. There are two pairs of charts, one which shows cholesterol and one cholesterol/HDL cholesterol ratio. Risk is read by rounding the person's age to the nearest age shown on the chart, their cholesterol or cholesterol/HDL ratio to the nearest whole unit and their blood pressure to the nearest multiple of 20mmHg.

Other examples of available risk prediction scores are the Prospective Cardiovascular Munster (PROCAM) model(38) and the Reynolds risk score (women and men)(39, 40)

The newest scores include more risk factors as renal function (eGFR) and Diabetes Mellitus. The utility of the algorithms should be used with care and in the context of the clinical setting, the availability of safe and effective interventions to prevent disease and cost-benefit considerations of applying those therapies to the patients(32). Of note is that a single risk factor might not individually be able to produce an atherosclerotic lesion which leads to cardiovascular disease and, maybe more important, the primary care physician treats individual patients, and not populations.

## Hyperlipidaemia

Hyperlipidaemia which is an important risk factor for CVD is defined as an elevated amount of lipids (e.g. triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol or phospholipids) in the blood. It´s commonly referred as high cholesterol but other forms of elevated blood lipids may also be of importance. Hypertriglyceridemia (elevated levels of serum triglycerides) is not only associated with CVD but also with pancreatitis. Diet and lifestyle factors and genetic predisposition can all lead to hyperlipidaemia, but it is also seen in metabolic disorders as hypothyroidism (underactive thyroid), liver disease, kidney disease and diabetes.

Hypercholesterolemia is regarded as a main traditional risk factor for the development of coronary artery disease and cardiovascular events. The relationship between elevated serum cholesterol and incidence of cardiovascular disease is well known and documented. The association was established already in the 1960´s and confirmed later by several biochemical and epidemiological studies, as well as by clinical trials(41). Data from the Multiple Risk Factor Intervention Trial (MRFIT) showed that the risk for cardiac heart disease continuously increased with increasing serum cholesterol levels. However the association between plasma cholesterol and cardiovascular risk diminishes with increasing age and the correlation seems be weak after the age of 55 years (42-44).

Total cholesterol is made of low density lipoprotein-cholesterol (LDL) high density lipoprotein-cholesterol (HDL) and non-high density lipoprotein-cholesterol (nonHDL), where nonHDL-C reflects the cholesterol content of several pro atherogenic lipoprotein sub fractions (very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and chylomicron remnants). Cholesterol and fatty substances are main components involved in the process of developing atherosclerotic plaque. The process is complex and there are several sub fractions of lipoproteins involved in the development of atherosclerotic disease.

Circulating LDL is a well-known contributor for atherosclerosis. The LDL-cholesterol is a lipoprotein transporting cholesterol in the blood and contributes to the formation of atherosclerotic plaque and is therefore considered to be the “bad” cholesterol. The role of LDL-cholesterol is best exemplified by familial hypercholesterolemia (FH) which is a monogenetic disorder which leads to if untreated lifelong elevation of LDL-

cholesterol, which leads to early development of atherosclerotic lesions in the heart, brain, and peripheral arteries. Patients with homozygous familial hypercholesterolemia develop severe vascular disease, including myocardial infarction and ischemic stroke in childhood and rarely survives 20 years if untreated(45). In recent years, intensive lowering of low-density lipoprotein (LDL) cholesterol using statins has been established as an effective therapy to lower cardiovascular risk(46).

The Framingham study showed that raised HDL cholesterol levels offered protection against coronary heart disease and HDL is therefore often considered to be the "good" cholesterol. Low levels of HDL cholesterol have been shown to increase the risk of heart disease. Low levels of HDL-cholesterol are associated with an increased risk of coronary artery disease (CAD) and myocardial infarction, which has triggered the hypothesis that HDL, acts as an anti-atherogenic lipoprotein(47). At the same time, it seems as if the biological functions of HDL, i.e. endothelial-atheroprotective effects are highly heterogeneous and the properties are altered in patients with coronary disease or diabetes.

Serum lipid levels are related to age. Total cholesterol and LDL-cholesterol gradually increase after adolescence until the age of 60–65 in men and 70–75 in women, and there after start to decline. HDL-cholesterol changes less during adulthood. Existing data on the longitudinal changes of HDL-c in the elderly is rather inconsistent(48). Even though there are risk scores for total cholesterol and HDL-cholesterol the scores regarding risk-based intervention strategies suggest intervention strategies based on total CV risk and low-density lipoprotein cholesterol (LDL-C) level(2).

Interpretation of lipid levels in elderly patients is complex due to multiple illnesses, comorbidities and frailty (49). In the third paper we address the question in whether statin treatment is effective and safe in very elderly (80 years and older) acute myocardial infarction (AMI) patients.

## Family history and genetics

Family history of coronary artery disease could be defined as the occurrence of premature coronary disease in a first degree relative(9). Family history reflects both shared biological, environmental and genetic factors. It may be associated with accumulation of specific habits (as alcohol use and smoking) or traditional risk factors (as hypertension, diabetes mellitus and obesity) that may themselves have environmental and genetic contributors(50). Previous studies have shown that a positive family history of coronary artery disease is a major risk factor for myocardial infarction in young patients. First-degree relatives of a patient with myocardial infarction themselves have a substantial higher risk of myocardial infarction (51). Even though there often is familial aggregation of risk factors for example hypertension diabetes and hypercholesterolemia a history of premature cardiovascular disease family

history appears to predict cardiovascular mortality independent of these risk factors and the risk of cardiovascular disease associated with an apparently inherited predisposition appears to be affected by modifiable behaviour(52). It´s recently shown genetic risk measure was associated with coronary heart disease (CHD) independently of self-reported family history of CHD, as well as established risk factors(53), that is, self-reported family history is not a substitute for genetic risk assessment. In our first article family history we recognize that a positive family history of CVD is associated with early development of myocardial infarction together with hypercholesterolemia and smoking.

Molecular genetics and especially the sequencing of the human genome have made it possible to add genetic tests to the traditional diagnostic and predictive tools available for the management of cardiovascular disease. In contrast to classic mendelian genetic risk factors which usually are monogenetic the CVD genetic risk factors are more complex and the cause of disease is often polygenetic. The effect size of a specific gene may be small but widespread in the population or be large but only affect a small population(50). Certain genetic factors are affected due to environmental factors. Genome-wide association studies (GWAS) have been used successfully to identify a large number of common gene variants that increase risk of myocardial infarction and coronary artery disease (CAD)(54). Today there are more than 50 known gene variants associated with coronary heart disease. Genetic tests offer many advantages over traditional tests since they do not require invasive sampling, have high accuracy, and can be done at any time in life, whether or not symptoms of disease are present. It´s of great importance to emphasise that genetic testing can determine risk of disease, but does not guarantee that disease will become symptomatic(55).

In 2007 an important discovery was made in the genetics of cardiovascular diseases. Four independent genome wide association studies reported the association of the same locus on chromosome 9p21 with CAD and myocardial infarction (MI). In fact, of the hundreds of thousands single-nucleotide polymorphisms (SNPs) studied across the genome the same locus showed the strongest association with CAD in all four studies(56). The most robust genetic association was found to be located upstream of the genes coding for the cyclin-dependent kinase inhibitors 2A and 2B CDKN2A/CDKN2B locus on chromosome 9p21 and spanning a large cluster of SNPs in strong linkage disequilibrium with each other(57-60). We have therefore chosen to use this gene variant in our work.

## Smoking

Cigarette smoking accounts for several adverse health effects. Despite increasing awareness, more than 1 billion people worldwide smoke tobacco daily(61). Recent reports estimate that smoking accounts for nearly one of every five deaths each year in

the United States. Smokers are more likely to develop heart disease, stroke, and cancer. According to the Swedish Public Health Agency, the proportion of daily smokers, in Sweden in 2015, was 11 percent of women and 9 percent among men. Smoking is an age-dependent risk factor for coronary atherosclerosis(62). Cigarette smoking appears to be one of the most common risk factors in young MI patients(9). Smoking induces endothelial dysfunction and can precipitate coronary spasm and therefore it may contribute to early CVD in patients with minimal atherosclerosis. In an analysis by Mukherjee et al. of almost 16 000 patients with coronary artery disease who participated in trials of percutaneous coronary intervention (PCI), cigarette smoking was much more frequent in patients under the age of 40 years (59%) than in older patients (25%) (63). On the other hand, the late side effects of smoking seem to be closely related with coronary heart disease. In a study from the Honolulu Heart Program which included elderly persons between ages 65 and 74 show that cigarette smoking continues to be an independent predictor of CHD incidence in this cohort and that the effect is relatively undiminished compared with middle-aged group(64).

## Hypertension

Hypertension is largely prevalent worldwide and affects approximately 1 billion adults worldwide(65). Large studies have shown that hypertension is a main risk factor for cardiovascular disease (66, 67) and the benefit of antihypertensive treatment is well documented (68, 69). The Framingham Heart Study showed that cardiovascular risk is positively, continuously and independently associated with rising blood pressure(70) and a meta-analysis of individual data for one million adults in 61 prospective studies by Prospective Studies Collaboration showed age-specific relevance of usual blood pressure to vascular mortality (67). The Global Burden of Disease Study identified hypertension as the leading risk factor, among 67 studied, for death and disability-adjusted life-years lost during 2010(71).

Hypertension leads, if left untreated, to major seqelae such as coronary artery disease, cerebrovascular disease, renal disease and left ventricular hypertrophy of the heart(34).The prevalence of hypertension in Europe appears to be 30-45% in the general population(72) and in Sweden 2 million people are estimated to have hypertension(73). Hypertension guidelines recommend treatment based on blood pressure levels alone in contrast to CVD prevention guidelines which are based on risk.

Because risk is closely related with age the indication for preventive strategies increases markedly after the age of 60. According to international guidelines hypertension is defined as values ≥ 140 mmHg Systolic Blood Pressure (SBP) and/or ≥ 90 mmHg Diastolic blood pressure (DBP)(72).

The blood pressure targets for treatment are still under debate. If there should be differentiated targets according to age has yet to be defined. Even though hypertension

is more common among elderly individuals blood pressure lowering treatment seems to be equally important in all ages(74).

Previous studies have shown that antihypertensive treatment in middle-aged people and in hypertensive patients over 60 years has given beneficial effects, but whether treatment is beneficial in patients over 80 years old is less studied. In 1999 a meta-analysis of randomized controlled trials (RCTs) data found that treatment of hypertension in the very elderly group was associated with a reduction in major CV events and HF but no reduction no CV mortality and an increased risk of all-cause mortality(75). The Hypertension in the Very Elderly Trial (HYVET) trial was stopped early because it showed that among very elderly patients (≥80 years) with hypertension, defined as systolic blood pressure 160-199, treatment with a diuretic with or without an ACE inhibitor was associated with a reduction in all-cause mortality and cardiovascular outcomes. Goal blood pressure was < 150/80(76). Other trials as the SPRINT trial and ACCORD BP supports more intensive treatment with lower blood pressure targets (77, 78).

According to the Eighth Joint National Committee (JNC 8) there are "strong evidence to support treating hypertensive persons aged 60 years or older to a BP goal of less than 150/90mmHg and hypertensive persons 30 through 59 years of age to a diastolic goal of less than 90mmHg; however, there is insufficient evidence in hypertensive persons younger than 60 years for a systolic goal, or in those younger than 30 years for a diastolic goal, so the panel recommends a BP of less than 140/90mmHg for those groups based on expert opinion"(79).

## New plasma biomarkers and CVD risk

The US National Institutes of Health/Food and Drug Administration in 2001 defined a 'biomarker' as "a characteristic that is objectively measured and evaluated (usually in plasma or serum) as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (80). The term also covers traditional risk factors that are used for the above-mentioned risk algorithms.

The increasing burden of cardiovascular disease calls for better understanding of the underlying mechanisms and for better measures and prediction of disease. As mentioned above risk stratification in current guidelines is largely based on traditional Framingham risk factors. Early detection of disease is crucial for initiating preventive strategies and early treatment. Identification of a subset with high risk patients of CVD development with novel biomarkers might be a cost effective strategy to focus on with regular follow-ups in order to prevent, detect and treat CVD early. Biomarkers of inflammation, kidney disease, and stretch of the heart muscle reflect underlying mechanisms and can in combination with clinical evaluation be used in detecting disease and in some cases estimating future risk. In a very large study of people without

known cardiovascular disease assessment of the CRP or fibrinogen level in people at intermediate risk for a cardiovascular event could help prevent one additional event over a period of 10 years for every 400 to 500 people screened(81).

Markers of myocardial stress as B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) has been used for diagnose and clinical follow up of heart failure. The negative predictive values are high in both the non-acute and acute setting, but the positive predictive values are lower and therefore the guidelines recommend that the use of these biomarkers are used for ruling-out heart failure. ESC guidelines of acute and chronic heart failure recommends measurement of plasma natriuretic peptide level (BNP, NT-proBNP or MR-proANP) is in all patients with acute dyspnoea and suspected acute HF to help in the differentiation of acute heart failure from non-cardiac causes of acute dyspnoea(26). Heart failure diagnosis can be difficult due to comorbidity and atypical clinical presentation especially in elderly people. In the BACH trial the authors concluded that a biomarker reflecting stretch of the heart muscle, mid-region pro-atrial natriuretic peptide (MR-proANP), is as useful as BNP for acute heart failure diagnosis in dyspnoeic patients and may provide additional clinical utility when BNP is difficult to interpret. The authors also found that another biomarker, mid-region proadrenomedullin (MR-proADM) identifies patients with high 90-day mortality risk and adds prognostic value to BNP (82).

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide that has been implicated in the genesis and progression of several cardiovascular disease states(83). It has been shown to have inotropic effects, to increase blood pressure, induce cardiac hypertrophy and triggers secretion of a range of hormones known to have long-term adverse effects on the myocardium such as vasopressin, renin, aldosterone, epinephrine and norepinephrine(84). In patients with established HF, high levels of ET-1 have been shown to predict mortality and re-hospitalisation(85, 86) and has also shown evidence for being a predictor of all-cause death in a healthy population(87). However, measurement of the mature ET-1 hormone is technically difficult and its true concentration is difficult due to capture due to very short half-life in plasma both in vivo and ex vivo, low circulating concentrations and binding to plasma proteins(87). Therefore, an assay measuring a stable but biologically inactive C-terminal fragment of the precursor hormone, released in stoichiometric amounts with the mature hormone, has been developed(88). The stability of this C-terminal fragment of the ET-1 precursor hormone (CT-proET-1) and the advantages with the assay makes CT-proET-1 a more reliable marker of true secretion of the mature hormone than measurement of mature ET-1 itself. Interestingly, in patients with stable CAD, those with high CT-proET-1 were at increased risk of heart failure and death and also showed the greatest benefit from treatment with angiotensin-converting enzyme inhibitors(89).

In the fourth paper we tested weather CT-proET-1 (ET-1) can identify a subset of the elderly population without heart failure and CAD who have very high risk of

developing heart failure, yet cannot be pinpointed solely with traditional risk factors. We believe this is a relevant population to assess for high heart failure risk since they do not receive aggressive cardiovascular risk factor treatment unless the conventional risk factors, such as coronary artery disease, indicate high cardiovascular risk.

# Material, methods and design paper I-IV

## Patients

The material in the four papers was collected from 2 large population studies in Malmö (Malmö Preventive Project MPP and Malmö Diet and Cancer Study) and from The "Register of Information and Knowledge about Swedish Heart Intensive care Admission" (RIKS HIA).

Malmö is Sweden's third largest city located in the southern part of Sweden. The population has grown from 265 000 inhabitants in the early 1970s to around 313 000 inhabitants 2013.

### The Malmö Preventive Project (MPP)

Malmö Preventive Project (MPP) is a population screening programme for cardiovascular risk factors and alcohol abuse which started at the Department of Preventive Medicine, Malmö University Hospital in 1974(90). The aim was to screen large strata of the adult population in order to find high-risk individuals for preventive intervention. Subjects were invited to participate in a broad health-screening programme, including a physical examination and a panel of laboratory tests. Additionally, every participant filled in a self-administered questionnaire on lifestyle, and medical history. Between the years 1974-1992, a total of 22,444 men (mean age 46-years) and 10,902 women (mean age 49-years) attended the screening with an overall attendance rate of 71% (range 64-78%). Among these 33,346 subjects, men were mostly screened in the first half of the period (1974-1982), and women in the latter half (1981-1992), implying different follow-up time periods for men and women. Various interventions (lifestyle modification, drug therapy) engaged nearly 25% of the screened subjects. The invited men and women were fasting overnight prior to investigation. Height and weight were measured in light indoor clothing. A complete medication list was recorded for each participant. Blood pressure was measured in the supine position after 10 min rest. Fasting blood samples were collected and plasma

lipids (total cholesterol and triglycerides) were analysed by routine methods at the Department of Clinical Chemistry, Malmo University Hospital. Fasting plasma glucose was analysed with a hexokinase method. Diabetes was defined as fasting whole blood glucose 6.0 mmol/L or self-report of a physician diagnosis of diabetes or the use of anti-diabetic medication. Hypertension was defined as systolic or diastolic blood pressure ≥140/90 mmHg or the use of antihypertensive medication. Smoking was defined as being current smoker. A positive family history for MI was defined as reporting having at least one first degree relative with MI before the age of 60 years(91).

Between 2002-2006, a rescreening program of subjects within the MPP was performed at the Clinical Research Unit Medicine, University Hospital, Malmö. Of the 33,346 subjects investigated 1974-1992, 18,240 attended the re-examination in 2002-2006 providing data on cardiovascular risk factors as well as fasting plasma samples, which were stored in -80 degrees C for later analysis of biomarkers.

## The Malmö Diet and Cancer (MDC) study

The Malmö Diet and Cancer (MDC) study is a prospective, population-based epidemiological cohort study that included 30 447 randomly selected men (born between 1923 and 1945) and women (born between 1923 and 1950) who underwent a baseline examination between 1991 and 1996 (92). The study was initiated and planned by professor Göran Berglund (professor in Internal medicine) and professor Lars Janzon (professor in epidemiology) in collaboration with the International Agency for Research on Cancer (IARC), a World Health Organisation agency in Lyon, France, the Swedish Cancer Society and the city of Malmö. MDC is a part of EPIC - The European Prospective Investigation into Diet and Cancer. This collaboration involves about 20 similar population surveys in ten European countries(93).The mean age in the MDC was 59.2±7.0 years for men and 57.4±7.9 for women and the participation rate was 41%. The initial aim of the study was to identify and explore links between diets and development of cancer. Complete data (full set of data, blood samples, all questionnaires and physical examination) was eligible for 28449 individuals (and of these 28098 individuals had complete data, including dietary data, and were also participants in the EPIC Study).

## The "Register of Information and Knowledge about Swedish Heart Intensive care Admission" (RIKS-HIA)

The "Register of Information and Knowledge about Swedish Heart Intensive care Admission" (RIKS-HIA) which now has changed name to Swede Heart. RIKS-HIA includes all consecutive patients admitted to the coronary care units of all participating

Swedish hospitals. Data on about 100 different variables regarding baseline characteristics, examinations, interventions and complications in hospital, and discharge medication and diagnosis were reported in case record forms as has been described elsewhere(94). The variables in RIKS-HIA comply with the international Cardiology Audit and Registration Data Standards (CARDS). To ensure the validity of the information entered into the database a single specially trained monitor visited participating hospitals and compared information in the patient records, including ECG, with the information entered into the RIKS-HIA database in 30–40 randomly chosen patients for each hospital. Data quality was monitored in 5446 random records from all participating hospitals comprising 299,530 measurements there was a 94 % overall agreement between the registered information and patient records. Between 1999 and 2001 the number of participating hospitals increased from 65 to 72, out of all 74 Swedish hospitals, where it remained through 2003.

All patients for whom data were entered into RIKS-HIA were informed of their participation in the registry (patients could request to be excluded) and the long-term follow-up. The registry, and the merging with other registries, was approved by the National Board of Health and Welfare and the Swedish Data Inspection Board. The Ethics Committee of Uppsala University Hospital approved the study.

# Materials, methods and design paper I

## Clinical characteristics and assays in the MPP study

After exclusion of subjects with MI or stroke prior to baseline (n =163), 3687 individuals with complete data on cardiovascular risk factors developed a MI during follow-up (cases) and were divided into quartiles according to the age of their first MI (age at event). All cases were assigned at least one, and if possible two control subjects each who were free from MI and stroke prior to the baseline exam and during follow-up and were matched with cases for baseline age (± 3 years), gender, and date of baseline examination (±3 years). In addition, we required the control subjects to have at least as long follow-up time as the corresponding case. The baseline characteristics and final numbers of cases and matched controls used in our analyses and are shown in table 1 in paper I. The study design is shown in the figure below. The mean baseline age in cases ranged from 43 years in MI age quartile 1 to 52 years in MI age quartile 4. Study design is shown in figure below.

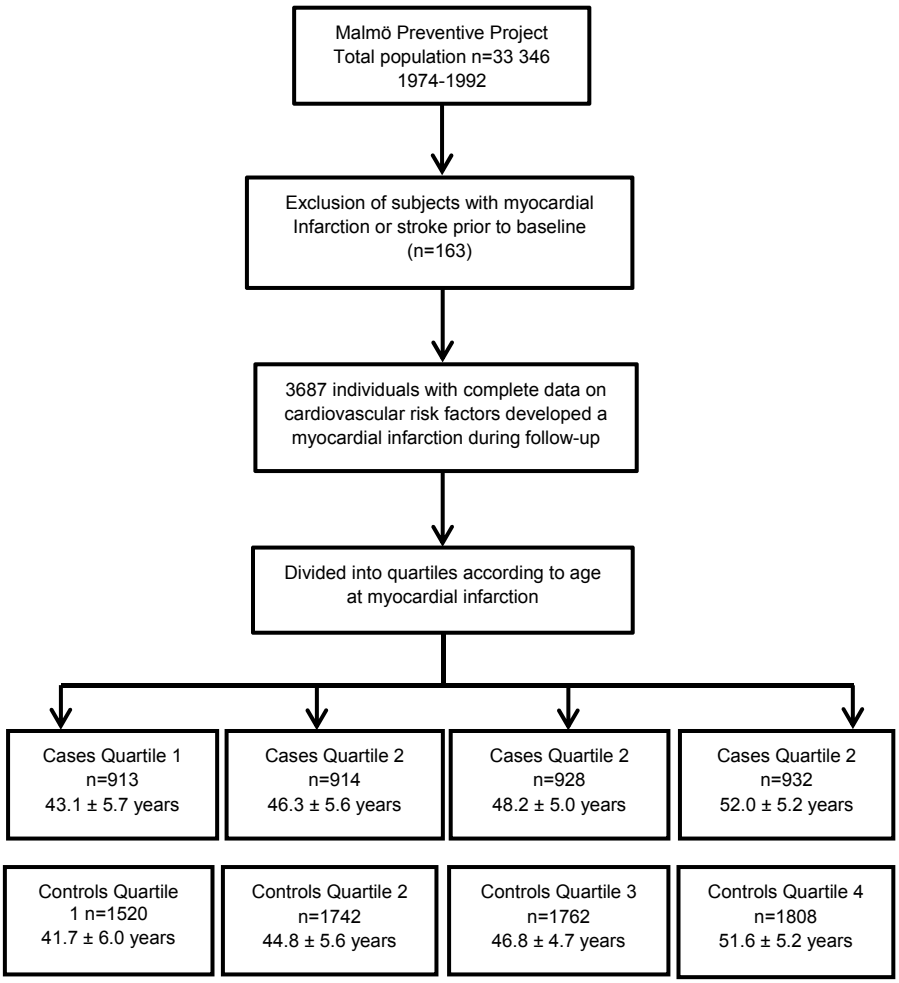



Figure shows Study Design for paper 1

## Endpoints in paper I

Myocardial infarction endpoints were retrieved through linkage of the unique 10-digit personal identification number with the Swedish National Hospital Discharge Register (SNHDR) and the Swedish National Cause of Death Register (SNCDR). Those who emigrated from Sweden before 2006 and had been event-free at the time of emigration (n = 66) received the date of emigration as the last follow-up date. The mean follow-up time was 22±7 years. We defined MI as fatal or non-fatal MI or death due to

coronary heart disease on the basis of the International Classification of Diseases 9th and 10th Revisions (ICD-9 and ICD-10) codes 410 and I21 in the SNHDR, respectively, and codes 410, 412, and 414 (ICD-9) or I21-I23 and I25 (ICD-10) in the SNCDR. The register-based diagnosis of MI in the SNHDR has been found to be highly valid(95).

# Material, methods and design in paper II

## Clinical characteristics and assays in the MDC study

Patients were admitted 1991-1996. The chromosome 9p21 rs4977574 polymorphism was successfully genotyped in 27 885 of the 28 449 participants in the MDC study. Amongst these subjects, there were incomplete data (which overlapped to a certain extent) for blood pressure (n = 47), BMI (n = 47), smoking (n = 1705) and diabetes (n = 2117). In addition, subjects with CVD prior to the baseline examination were excluded (n = 1018); overall, 24 777 subjects without history of CVD and with complete data for all covariates were included in this study. Written informed consent was obtained from all participants, and the study was approved by the local ethics committee. The study design is shown in figure below.

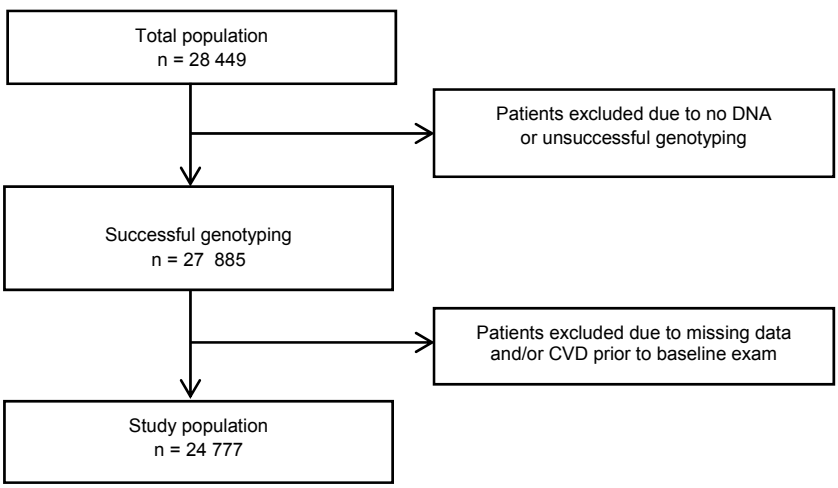



Figure shows the study design of paper II

Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or the use of antihypertensive medications. Diabetes was defined as self-reported history of physician diagnosed diabetes or current antidiabetic treatment. Smoking was defined as having ever smoked and lipid-lowering therapy was defined as current use of lipid-lowering medications. Body mass index (BMI) was measured as weight in kilograms divided by the square of height in metres. DNA was extracted from frozen granulocyte or buffy coat samples from blood collected at the baseline examination (1991–1996) using QIAamp 96 spin blood kits (QIAGEN, VWR, Gaithersburg, MD, USA). The baseline characteristics of the study subjects are presented in the article. The mean age was 58 years (±7.7 years) and the follow-up time was 11.7 years.

## Endpoints in paper II

The study end-point was a composite of coronary artery disease (CAD) and fatal or nonfatal ischaemic stroke, whichever came first (CVD events). The procedure for ascertaining outcome events has been described previously(96, 97).CAD was defined as fatal or nonfatal myocardial infarction, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG) or death due to ischaemic heart disease, whichever came first. CVD events were identified through record linkage of the 10-digit personal identification number of each Swedish citizen with four registries: the Swedish Hospital Discharge Registry, the Swedish Cause of Death Registry, the Stroke in Malmö Registry and the Swedish Coronary Angiography and Angioplasty Registry (SCAAR). The registers have been previously described and validated for classification of outcomes (98-100). Myocardial infarction was defined on the basis of the International Classification of Diseases 9th (ICD9) and 10th revision (ICD10) codes 410 and I21, respectively. Death due to ischaemic heart disease was defined as codes 412 and 414 (ICD9) or I22, I23 and I25 (ICD10). CABG was identified from national classification of surgical procedures, KKA from 1963 until 1989 and Op6 thereafter, and defined as procedure code 3065, 3066, 3068, 3080, 3092, 3105, 3127, 3158 (Op6) or FN (KKA97). PCI was defined as operation codes FNG05 and FNG02. Fatal and nonfatal ischaemic stroke was defined as codes 434 and 436 (ICD9) and I63 and I64 (ICD10). Patients were followed for outcomes until 1 January 2007.

# Materials, methods and design paper III

## Clinical characteristics and assays in the RIKS-HIA study

Between 1999 and 2001, the number of participating hospitals increased from 65 to 72, out of all 74 Swedish hospitals, where it remained through 2003. All patients for whom data were entered into RIKS-HIA were informed of their participation in the registry (patients could request to be excluded) and the long-term follow-up. The registry and the merging with other registries were approved by the National Board of Health and Welfare and the Swedish Data Inspection Board. The Ethics Committee of Uppsala University Hospital approved the study. Study population. We included all patients 80 years of age and older who were admitted with the diagnosis of AMI in the RIKS-HIA between January 1, 1999, and December 31, 2003 (n= 21,410). To be included in the end point analyses, we required complete data on all covariates that were adjusted for and specific cause of death in those who died during follow-up, leaving 14,907 patients for survival analyses (study population A) (see table 1 in paper III). Furthermore, to limit the bias related to effects of short life expectancy and comorbidity on physicians' choice of treatment, we excluded patients who died within 14 days from baseline (study population B) and all patients who died within 365 days (study population C) (Table 1 paper III). The study design is summarized in figure below. Cardiovascular drug therapies were entered in a structured formula on admission and at discharge. We used data from the Swedish National Patient Register to record a diagnosis of stroke, kidney failure, chronic obstructive pulmonary disease, dementia, congestive heart failure, myocardial infarction, peripheral artery disease, and cancer before the registration in RIKS-HIA.

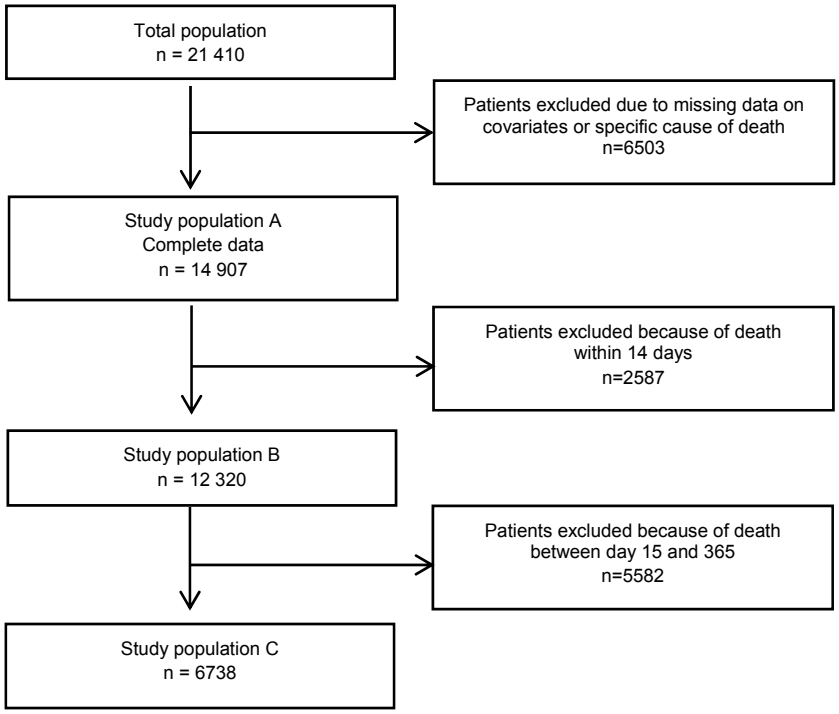



Figure shows the study design of paper III

## Endpoints in paper III

Patients were followed for end points with a median follow-up time of 296 days (interquartile range: 44 to 738 days, maximum of 5 years) by linking the Swedish 10-digit personal number with the Swedish National Cause of Death Register and the National Patient Register from baseline until the time of first event, death, or until December 31, 2003. End points were defined according to the International Classification of Diseases 10th Revision. Mortality end points were retrieved from the Swedish National Cause of Death Register with codes I21-I22 defining AMI mortality, codes I00-I99 defining cardiovascular mortality, and codes C00-D48 defining cancer mortality. In analyses of fatal and nonfatal AMI, end points were defined as codes I21-I22 in the National Patient Register or Swedish National Cause of Death Register. The date of hospital discharge was defined as the baseline.

# Materials, methods and design paper IV

## Clinical characteristics and assays

The current study population consists of a random sample of 5386 the 18240 re-examined individuals, the only exception from a complete random sample being that the selected individuals had not participated in another population survey in Malmö, i.e. the Malmö Diet and Cancer study (101). Of these 5386 subjects, we excluded (1) subjects with missing data on any of the cardiovascular risk factors (2) subjects who had a history of heart failure (HF) and (3) subjects with a history of coronary artery disease (CAD) (i.e. myocardial infarction or revascularization procedures as detailed below). This left 4819 subjects for analysis who were followed prospectively from the new baseline examination between 2002-2006, during a mean follow-up time of 5.6 years. The baseline characteristics are shown in table 1 shown in paper IV. Study design is shown below.

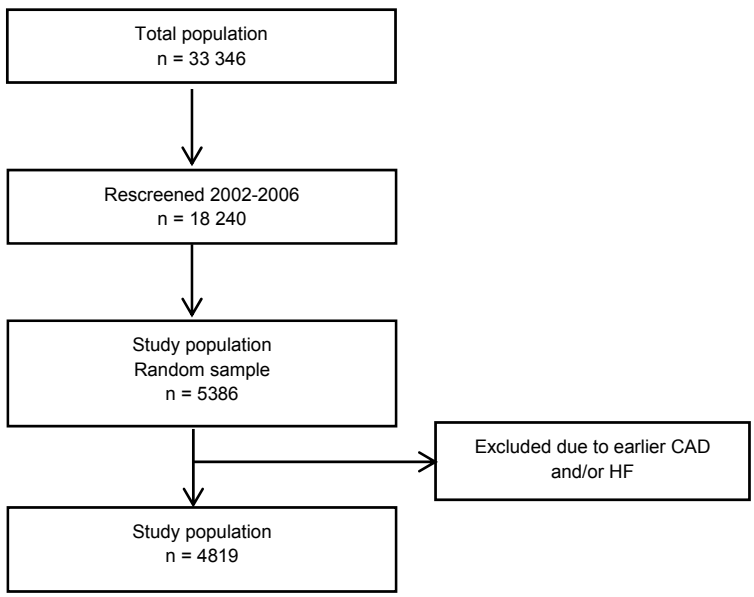



Figure shows the study design of paper II

At the baseline of the current study (2002-2006) participants underwent a medical history, physical examination and laboratory assessment. Blood pressure was measured using an oscillometric device twice after 10 minutes of rest in the supine position. Diabetes mellitus was defined as fasting plasma glucose 7.0 mmol/L or above, a self-

reported physician diagnosis of diabetes, or use of anti-diabetic medication. Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any use within the past year. Measurements of fasting serum total cholesterol, HDL cholesterol, and triglycerides were made according to standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. LDL cholesterol was calculated according to Friedewald's formula. The mid-regional fragments of pro-atrial natriuretic peptide (MR-ANP) and plasma concentration of a C-terminal fragment of the Endothelin-1 (CT-proET-1) were measured with sandwich immunoluminometric assays as described previously (BRAHMS, Berlin, Germany)(88, 102). Serum creatinine was measured using the Beckman Coulter modified Jaffe procedure (103).

## Endpoints paper IV

Cardiac disease end points and mortality were ascertained by linkage of Swedish personal identification numbers to the national Swedish registers (Swedish Hospital Discharge Register, Swedish Cause of Death Register) maintained by the Swedish National Board of Health and Welfare. High case validity in these registers has been previously found for heart failure(103). Heart failure was ascertained from the Swedish Hospital Discharge Register and the Swedish Cause of Death Registry using diagnosis codes 427.00, 427.10, and 428.99 for International Classification of Diseases-8th Revision (ICD-8), 428 for the 9th Revision (ICD-9), and I50 and I11.0 for the 10th Revision (ICD-10) as primary diagnosis as in previous studies (104). Coronary artery disease (CAD) was defined as coronary revascularization, fatal or nonfatal myocardial infarction, or death due to ischemic heart disease. Myocardial infarction was defined on the basis of ICD-9 code 410 or ICD-10 code I21. Death attributable to ischemic heart disease was defined as ICD-9 codes 412 and 414 or ICD-10 codes I22, I23 or I25. Coronary artery bypass surgery was identified from national Swedish classification systems of surgical procedures and defined as procedure codes 3065, 3066, 3068, 3080, 3092, 3105, 3127, or 3158 (the Op6 system) or procedure code FN (the KKÅ97 system). Percutaneous intervention was identified from the SCAAR (105).

# Statistics

## Paper I

After exclusion of subjects with myocardial infarction (MI) or stroke prior to baseline (n=163), 3687 individuals with complete data on cardiovascular risk factors developed a MI during follow-up (cases) and were divided into quartiles according to the age of their first MI (age at event). All cases were assigned at least one, and if possible two control subjects each who were free from MI and stroke prior to the baseline exam and during follow-up and were matched with cases for baseline age (±3 years), gender, and date of baseline examination (±3 years). In addition, we required the control subjects to have at least as long follow-up time as the corresponding case. The final numbers of cases and matched controls used in our analyses are shown in the table shown above.

Multivariate conditional logistic regression (all models adjusted for baseline cholesterol, smoking, diabetes, body mass index, hypertension, family history of myocardial infarction (MI), triglycerides, and baseline age) was used to assess relationship between risk factors (cholesterol, smoking, diabetes, body mass index, hypertension, family history of MI, and triglycerides) and MI case–control status within each age at event quartile. Odds ratios of continuous risk factors were expressed as per 1 SD increment whereas dichotomous risk factors were expressed as presence of the risk factor in question. We then tested if there was a significant deviation from equality between the β-coefficients of each risk factor obtained within each age at event quartile using seemingly unrelated estimation (suest) as implemented in Stata. All tests were performed using Stata version 12 (Stata Corp, College Station, TX, USA) and a two-sided P-value of ,0.05 was considered significant.

## Paper II

Multivariate Cox proportional hazard models were constructed, for the whole cohort and within quartiles of baseline age, to test the association between chromosome 9p21 (rs4977574) and incident CVD (n=2668) with adjustment for traditional risk factors (age, sex, hypertension, lipid-lowering therapy, diabetes, smoking and BMI). The follow-up time was 11.7 years

The proportional hazards assumption was checked using Schoenfeld residuals (i.e. a test of nonzero slope in a generalized linear regression of the scaled Schoenfeld residuals versus time). To assess model discrimination, the c-statistic was calculated for models including conventional risk factors with and without rs4977574 (106).

The ability of biomarkers to reclassify risk was also evaluated using NRI and IDI, according to previously described methods(39, 107).

Using multivariate risk models with the traditional risk factors as defined above, participants were initially classified into four categories of predicted 10-year risk of a CVD event (<5%, 5–10%, 10–20% and 20%). Participants could then be reclassified into different categories with the addition of the rs4977574 data.

We assessed the number of participants reclassified and also calculated net reclassification improvement (NRI) and integrated discrimination improvement (IDI)(107).

Population attributable risk (PAR) was calculated as:

(X–1)/X, where $X = (1–f)^2 + 2f(1–f)\ \gamma + f2(\gamma + \gamma\text{-}1)$, $\gamma$ is the estimated genotype relative risk (in this case between 1.12 and 1.26) and f is the frequency of the risk allele (in this case ~45%) and an additive model was assumed(108).

To test whether the strength of the association between rs4977574 and incident CVD differed between quartiles of baseline age, we performed multivariate logistic regression adjusted for the traditional risk factors. The equality of coefficients across the four models [quartile (Q)1 to Q4] was tested using the Stata seemingly unrelated regression (suest) command (StataCorp, College Station, TX, USA). All tests were two-sided and a P-value of <0.05 was considered statistically significant. Analyses were performed with the use of SPSS Statistics 19.0 (IBM SPSS Statistics, Chicago, IL, USA) or Stata software version 11.

## Paper III

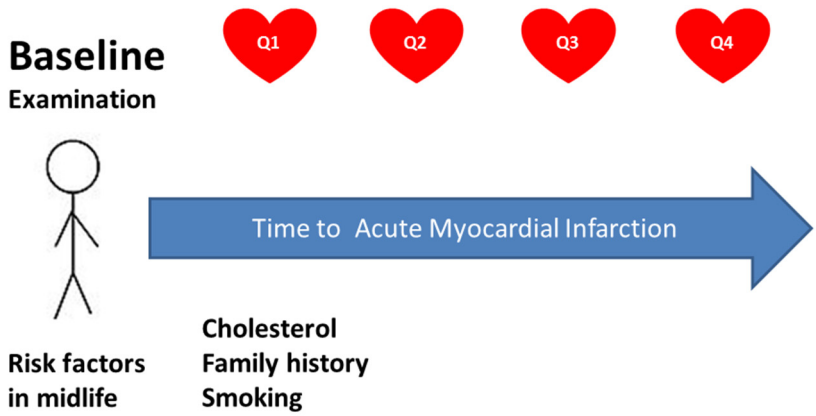
Apart from exclusion of patients with short survival time (i.e., restricting the study population to study populations B and C), we attempted to further decrease bias related to comorbidities and the physicians' probability to prescribe statins at discharge by creating and adjusting for a propensity score(109). The propensity score was estimated using a logistic regression model including the baseline variables, including cardiovascular medications at admission, as presented in a table above. We used Cox regression models to establish the relationship between statin treatment at the time of discharge and time to event. The models included other cardiovascular medications at discharge (beta-blockers, acetylsalicylic acid, other platelet inhibitors, and angiotensin-converting enzyme inhibitors), statin treatment on admission, the propensity score, and year of admission. The results are presented as relative risk (RR) and 95% confidence interval (CI). All statistical analyses were done using R version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria).

## Paper IV

Association between CT-proET-1 and incidence of HF and mortality was tested in multivariate Cox proportional hazard models where we adjusted for age, sex, systolic blood pressure, antihypertensive treatment, body mass index (BMI), current smoking, diabetes mellitus, and LDL- and HDL cholesterol. On top of adjustment for these traditional cardiovascular risk factors, we also made additional adjustments for MR-proANP and serum creatinine. Due to right skewed distributions, CTproET-1 and MR-proANP were log transformed prior to analysis and effect estimates were reported as hazard ratios (HR) and 95% confidence intervals (95%CI) per one standard deviation (SD) increment. We also analyzed CT-proET-1 as quartiles in crude Kaplan-Meier plots and in multivariate adjusted Cox proportional hazards models, with the bottom quartile defined as reference group. The proportionality of hazards assumption was confirmed using Schoenfeld's global test. In order to evaluate clinical utility we made analyses of discrimination and reclassification using C-statistics and continuous Net Reclassification Improvement (cNRI)(110, 111).

# Results

## Paper I




In the first paper, published in European Heart Journal 2016, "Risk factor exposure in individuals free from cardiovascular disease differs according to age at first myocardial infarction", we performed a matched case-control study in the population-based Malmö Preventive Project (n=33 346), where 3687 individuals developed MI during 22,7 years of follow-up (=cases). We used conditional logistic regression to assess relationship between risk factors at baseline and case status within quartiles of age at incident MI. The overall median age of first MI in the case group was 64 years (range 37–84 years) and when divided in to quartiles of age at incident MI the median age of MI in cases ranged from 52 years (range 37-57 years) in MI age quartile 1 to 74 years (range 70-84 years) in MI age quartile 4. All risk factors were significantly associated with myocardial infarction in the entire material ($P<0.05$ for all) (see table below).

**Relationship between risk factors and incident myocardial infarction in the entire cohort**

| | **OR (95% CI)** | **P-value** |
|---|---|---|
| Cholesterol | 1.34 (1.27-1.41) | <0.0001 |
| Triglycerides | 1.18 (1.12-1.24) | <0.0001 |
| Diabetes | 1.33 (1.11-1.60) | 0.002 |
| Hypertension | 1.33 (1.21-1.46) | <0.0001 |
| Body mass index | 1.06 (1.01-1.11) | 0.025 |
| Smoking | 1.52 (1.38-1.67) | <0.0001 |
| Family history | 1.56 (1.41-1.72) | <0.0001 |

OR=Odds Ratio; 95% CI=95% confidence interval. All risk factors shown as well as age were entered simultaneously in the model. All listed variables were mutually adjusted and in addition baseline age was included as covariate. Odds ratios of continuous risk factors were expressed as per 1 SD increment whereas dichotomous risk factors were expressed as presence of the risk factor in question.

**The relationship between risk factors and incident myocardial infarction in different age at event groups is shown in table below.**

| | OR (95% CI) | | | | P-value equality for β |
|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | |
| Cholesterol | 1.68 (1.50-1.87) | 1.43 (1.28-1.61) | 1.27 (1.14-1.42) | 1.08 (0.98-1.19) | <0.0001 |
| Triglycerides | 1.17 (1.05-1.31) | 1.28 (1.13-1.43) | 1.17 (1.04-1.31) | 1.12 (1.01-1.24) | 0.413 |
| Diabetes | 1.12 (0.79-1.59) | 1.09 (0.73-1.62) | 1.50 (1.01-2.23) | 1.63 (1.15-2.31) | 0.357 |
| Hypertension | 1.31 (1.08-1.60) | 1.28 (1.03-1.57) | 1.19 (0.98-1.46) | 1.48 (1.24-1.76) | 0.453 |
| Body mass index | 1.10 (1.00-1.23) | 0.97 (0.87-1.08) | 1.07 (0.97-1.19) | 1.09 (0.99-1.20) | 0.322 |
| Smoking | 1.87 (1.52-2.31) | 1.30 (1.06-1.59) | 1.23 (1.01-1.50) | 1.69 (1.42-2.02) | 0.008 |
| Family history | 1.85 (1.50-2.28) | 1.93 (1.54-2.42) | 1.43 (1.16-1.77) | 1.33 (1.10-1.61) | 0.028 |

OR=Odds Ratio; 95% CI=95% confidence interval. All risk factors shown as well as age were entered simultaneously in the model. All listed variables were mutually adjusted and in addition baseline age was included as covariate. Odds ratios of continuous risk factors were expressed as per 1 SD increment whereas dichotomous risk factors were expressed as presence of the risk factor in question.

The odds ratio (95% CI) for incident myocardial infarction was associated with 1 SD increase of baseline cholesterol decreased with age of MI and was 1.68 (1.50-1.87) for Q1, 1.43 (1.28-1.61) for Q2, 1.27 (1.14-1.42) for Q3 and 1.08 (0.98-1.19) for Q4. Similarly, family history of MI had a stronger relationship with myocardial infarction in Q1 than with myocardial infarction in Q4 of myocardial age, whereas smoking displayed a U-shaped relationship (see figure below).

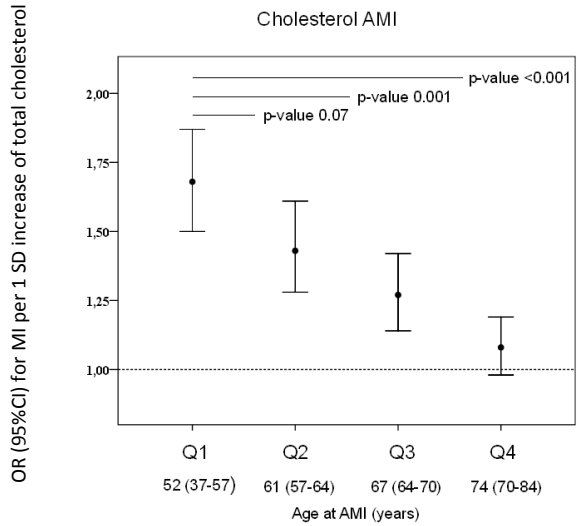



Figure 1 Odds ratio and 95% confidence intervals for myocardial infarction per each 1 standard deviation increment of cholesterol within quartiles 1–4 (Q1–Q4) of myocardial infarction age. All analyses were multivariate adjusted.

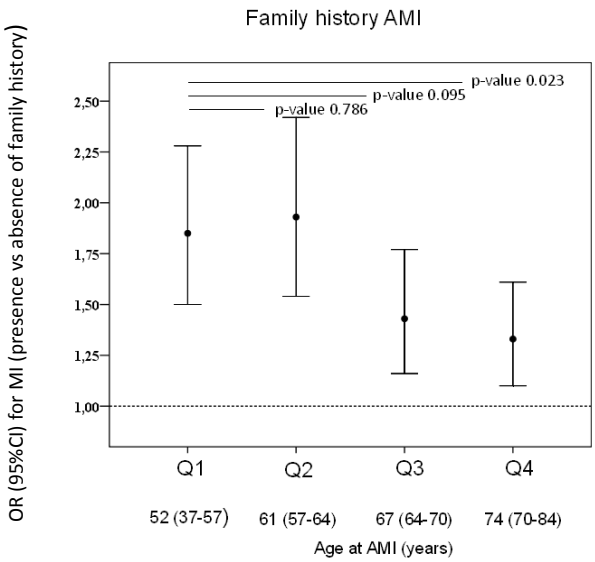



Figure 2 Odds ratio and 95% confidence intervals for myocardial infarction in subjects with vs. without family history of myocardial infarction within quartiles 1–4 (Q1–Q4) of myocardial infarction age. All analyses were multivariate adjusted.

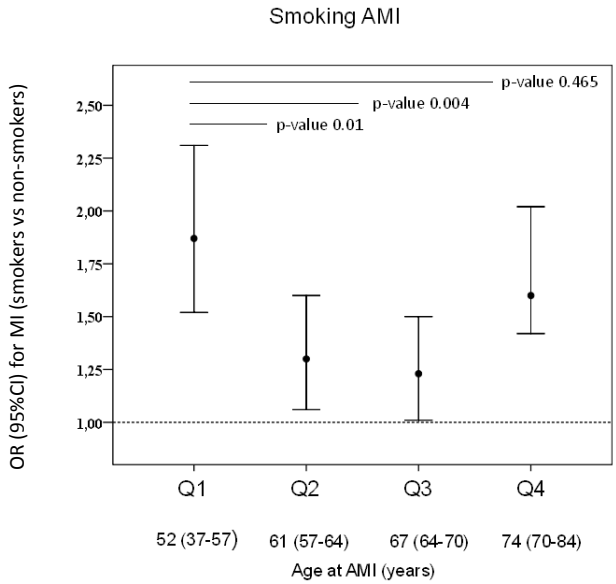



Figure 3 Odds ratio and 95% confidence intervals for myocardial infarction in smokers vs. non-smokers within quartiles 1–4 (Q1–Q4) of myocardial infarction age. All analyses were multivariate adjusted.

When comparing effect sizes between case control pairs with age of myocardial infarction above and below the median, results were similar and significant for cholesterol and family history, whereas there was no significant difference in effect size for smoking, which was expected due to its U-shaped relationship in age of myocardial infarction quartiles.

**Relationship between risk factors and incident myocardial infarction in different age at myocardial infarction groups**

| | OR (95% CI) | | P-value equality for β |
|---|---|---|---|
| | Below median age at myocardial infarction | Above median age at myocardial infarction | |
| Cholesterol | 1.56 (1.44-1.68) | 1.17 (1.08-1.25) | <0.0001 |
| Triglycerides | 1.22 (1.13-1.32) | 1.14 (1.06-1.23) | 0.225 |
| Diabetes | 1.12 (0.86-1.45) | 1.56 (1.21-2.03) | 0.088 |
| Hypertension | 1.30 (1.13-1.50) | 1.35 (1.18-1.53) | 0.759 |
| Body mass index | 1.03 (0.95-1.11) | 1.09 (1.02-1.17) | 0.266 |
| Smoking | 1.59 (1.38-1.83) | 1.46 (1.28-1.66) | 0.383 |
| Family history | 1.88 (1.61-2.19) | 1.35 (1.17-1.55) | 0.002 |

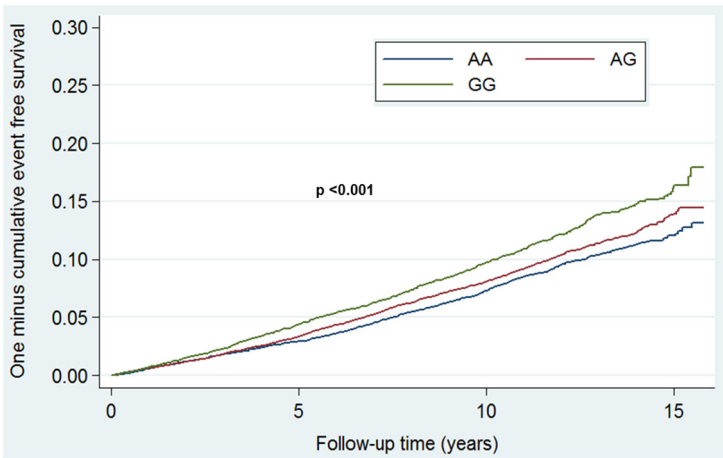
OR=Odds Ratio; 95% CI=95% confidence interval; β=beta coefficient. Odds ratios of continuous risk factors were expressed as per 1 SD increment whereas dichotomous risk factors were expressed as presence of the risk factor in question.

Exposure to the remaining risk factors did not differentially affect myocardial infarction occurring at different age spans. The conclusion was that exposure to cholesterol and family history of MI more strongly predicts onset of MI at younger ages, suggesting that MI in younger subjects is preceded by a different risk factor pattern than MI presenting in older subjects.

# Paper II

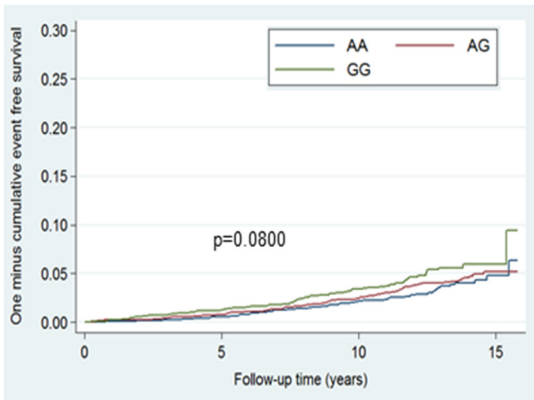
In the second paper we wanted evaluate the proportion of cardiovascular disease (CVD) incidence that is explained by genetic variation at chromosome 9p21 and to test whether such variation adds incremental information with regard to CVD prediction, beyond traditional risk factors. rs4977574 on chromosome 9p21 was genotyped in 24 777 subjects from the Malmö Diet and Cancer study who were free from CVD prior to the baseline examination. Association between genotype and incident CVD (n = 2668) during a median follow-up of 11.7 years was evaluated in multivariate Cox proportional hazard models. Analyses were performed in quartiles of baseline age, and linear trends in effect size across age groups were estimated in logistic regression models. The baseline characteristics in the whole study population and within quartiles of age at baseline are shown above (see methods).

Overall, there were 2668 incident CVD events (Table 2). The proportional hazards assumption was met in all analyses, and there was no evidence of multicollinearity between covariates in any of the analyses. There was no significant deviation from Hardy–Weinberg equilibrium (P = 0.482). There was significant association between the G allele of rs4977574 and incident CVD in crude additive models in the whole cohort as well as in each of the quartiles of baseline age (see figure below).

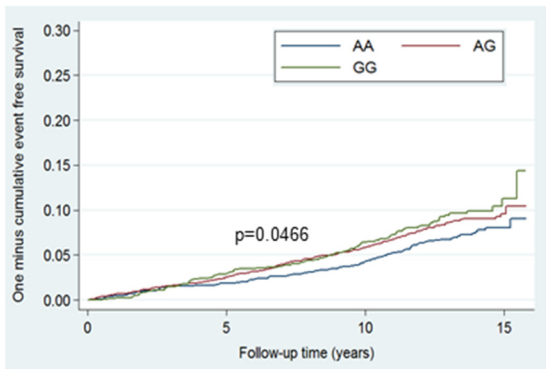


**Total population (n=24777)**

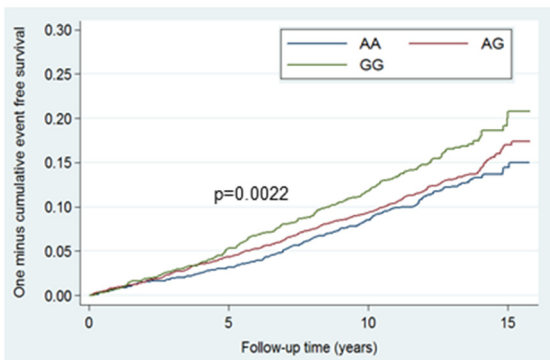
Q1(n=6199)

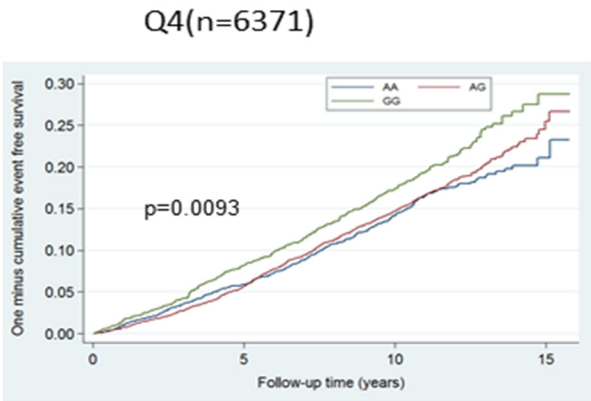



Q2(n=6026)




Q3(n=6181)






Similarly, after adjustment for traditional risk factors, the G allele was significantly associated with incident CVD in additive models both in the whole cohort an

d in subgroups according to quartile of age at baseline (Table 2, below). Although point estimates of the hazard ratios decreased from Q1 to Q4 of baseline age, these differences were not significantly different between quartiles (P = 0.713).

**Table 2** **Multivariate adjusted relationship between chromosome 9p21 (rs4977574) and incident cardiovascular disease (CVD) events and its population attributable risk (PAR) in the entire population and in quartiles of baseline age (Q1-Q4)**

| | Age, median (IQR) | No of CVD events | Age at CVD event, median (IQR) | AA (ref) | AG HR (95% CI) | GG HR (95% CI) | Additive HR (95% CI) | P* | PAR |
|---|---|---|---|---|---|---|---|---|---|
| Total population (n=24777) | 57.6 (51.1-64.1) | 2668 | 69.6 (63.7-74.7) | 1.0 | 1.12 (1.02-1.22) | 1.37 (1.23-1.52) | 1.17 (1. 11-1.23) | <0.001 | 0.13 |
| Q1 (n=6199) | 48.8 (47.4-49.8) | 231 | 57.5 (54.4-59.9) | 1.0 | 1.18 (0.87 -1.62) | 1.52 (1. 06-2.17) | 1.23 (1.03-1.48) | 0.023 | 0.17 |
| Q2 (n=6026) | 54.1 (52.6-55.7) | 475 | 62.1 (58.8-65.5) | 1.0 | 1.24 (1.00-1.54) | 1.42 (1. 09-1.84) | 1.19 (1.05-1.36) | 0.008 | 0.15 |
| Q3 (n=6181) | 60.9 (59.1-62.3) | 826 | 68.4 (64.9-71.6) | 1.0 | 1.11 (0.94-1.31) | 1.37 (1.13-1.66) | 1.17 (1.06-1.29) | 0.002 | 0.13 |
| Q4 (n=6371) | 67.3 (65.4-71.0) | 1136 | 75.1 (71.7-78.2) | 1.0 | 1.06 (0.92-1.22) | 1.31 (1.11 -1.54) | 1.14 (1.05-1.24) | 0.002 | 0.11 |

P-value refers to additive model. PAR=Population attributable risk. All models are adjusted for age, gender, hypertension, smoking, diabetes, body mass index, use of lipid lowering medication.

In additive multivariate-adjusted models, the PAR of rs4977574 with regard to incident CVD was 13% in the total population and showed a decreasing trend across increasing quartiles of baseline age: 17% in Q1, 15% in Q2, 13% in Q3 and 11% in Q4 (Table 2). However, as a result of a substantially greater incidence of CVD in older subjects, the number of CVD events attributable to rs4977574 increased with baseline age. In contrast to the large PAR estimates related to rs4977574, the effect of this SNP on discrimination in addition to traditional risk factors, as assessed by the c-statistic, was only marginal (0.1% in the total population and between 0.1 and 0.3% in the different quartiles of baseline age) (Table 3).

**Table 3 Metrics of discrimination and reclassification for incident cardiovascular disease (CVD) when adding rs4977574 on top of traditional risk factors**

| | C-statistic | IDI | NRI (%) |
|---|---|---|---|
| **Total population (n=24801)** | | | |
| Traditional risk factors | 0.750 | - | - |
| Chr9p21 (rs4977574)* | 0.751 | 0.001 (P<0.001) | 1.2% (P=0.043) |
| **Q1 (n=6199)** | | | |
| Traditional risk factors | 0.741 | - | - |
| Chr9p21 (rs4977574)* | 0.744 | 0.001 (P=0.18) | 1.3% (P=0.45) |
| **Q2 (n=6026)** | | | |
| Traditional risk factors | 0.744 | - | - |
| Chr9p21 (rs4977574)* | 0.746 | 0.001 (P=0.08) | 3.0% (P=0.07) |
| **Q3 (n=6181)** | | | |
| Traditional risk factors | 0.689 | - | - |
| Chr9p21 (rs4977574)* | 0.692 | 0.001 (P=0.02) | 0.0% (P=0.99) |
| **Q4 (n=6371)** | | | |
| Traditional risk factors | 0.659 | - | - |
| Chr9p21 (rs4977574)* | 0.660 | 0.002 (P=0.001) | 1.7% (P=0.03) |

Traditional risk factors = age, sex, hypertension, diabetes, lipid lowering therapy ever smoked, and BMI. The genetic model includes both traditional risk factors and genotype.

*Additive model

The impact of rs4977574 on reclassification across categories of CVD risk was significant compared with traditional risk factors in the total population. However, the proportion of the population correctly reclassified was only 1.2% (Table 3). The NRI was also small in the four age quartiles (Table 3). The SNP conferred a significant increase in IDI in the total population and in Q3 and Q4 of baseline age, but the magnitude of the effect was small (Table 3). There was no interaction between gender and rs4977574 in incidence of CVD (P = 0.374).

Lack of gender difference in the association between rs4977574 and CVD incidence was further supported by gender-stratified analyses showing significant relationships in both genders: hazard ratio(HR) 1.14, 95% confidence interval (CI) 1.06–1.22, P < 0.001 in men; HR 1.21, 95% CI 1.10–1.33, P < 0.001 in women.

Finally, the significance of the association between rs4977574 and CAD was slightly stronger than between the SNP and ischaemic stroke; however, both associations were significant (Table 2 A and B).

**Table 2A** **Multivariate adjusted relationship between chromosome 9p21 (rs4977574) and incident coronary artery disease (CAD) events and its population attributable risk (PAR) in the entire population and in quartiles of base line age (Q1-Q4)**

| | Age, median (IQR) | No of CAD events | Age at CAD event, median (IQR) | AA (ref) | AG HR(95%CI) | GG HR(95%CI) | Additive HR(95%CI) | P* | PAR |
|---|---|---|---|---|---|---|---|---|---|
| Total Population (n=24777) | 57.6 (51.1-64.1) | 1839 | 69.1(63.2-74.4) | 1.0 | 1.14 (1.02-1.27) | 1.36 (1.20-1.55) | 1.17 (1.09-1.24) | <0.001 | 0.13 |
| Q1 (n=6199) | 48.8 (47.4-49.8) | 172 | 57.6 (54.4-59.9) | 1.0 | 1.33 (0.91-1.93) | 1.74 (1.15-2.65) | 1.32 (1.07-1.63) | 0.009 | 0.22 |
| Q2 (n=6026) | 54.4 (52.6-55.7) | 351 | 62.0 (58.7-65.3) | 1.0 | 1.13 (0.88-1.45) | 1.29 (0.96-1.75) | 1.14 (0.98-1.32) | 0.095 | 0.11 |
| Q3 (n=6181) | 60.9 (59.1-62.3) | 574 | 68.5 (64.9-71.7) | 1.0 | 1.11 (0.92-1.36) | 1.35 (1.07-1.70) | 1.16 (1.03-1.30) | 0.012 | 0.13 |
| Q4 (n=6371) | 67.3 (65.4-71.0) | 742 | 74.9 (71.6-78.0) | 1.0 | 1.11 (0.94-1.32) | 1.32 (1.08-1.61) | 1.15 (1.04-1.27) | 0.009 | 0.12 |

P-value refers to additive model. PAR=Population attributable risk. All models are adjusted for age, gender, hypertension, smoking, diabetes, body mass index, use of lipid lowering medication.

**Table 2B** **Multivariate adjusted relationship between chromosome 9p21 (rs4977574) and incident ischemic stroke (IS) events and its population attributable risk (PAR) in the entire population and in quartiles of base line age (Q1-Q4)**

| | Age, median (IQR) | No of CAD events | Age at CAD event, median (IQR) | AA (ref) | AG HR(95%CI) | GG HR(95%CI) | Additive HR(95%CI) | P* | PAR |
|---|---|---|---|---|---|---|---|---|---|
| Total Population (n=24777) | 57.6 (51.1-64.1) | 1002 | 71.0 (65.4-75.7) | 1.0 | 1.05 (0.91-1.22) | 1.35 (1.13-1.60) | 1.16 (1.06-1.26) | 0.001 | 0.13 |
| Q1 (n=6199) | 48.8 (47.4-49.8) | 66 | 57.4 (54.7-60.4) | 1.0 | 0.82 (0.47-1.42) | 1.00 (0.52-1.92) | 0.98 (0.70-1.37) | 0.906 | 0.00 |
| Q2 (n=6026) | 54.4 (52.6-55.7) | 147 | 63.0 (59.8-66.5) | 1.0 | 1.59 (1.05-2.41) | 1.85 (1.14-2.99) | 1.35 (1.07-1.70) | 0.011 | 0.24 |
| Q3 (n=6181) | 60.9 (59.1-62.3) | 313 | 68.8 (65.3-72.0) | 1.0 | 1.06 (0.81-1.38) | 1.46 (1.08-1.98) | 1.21 (1.03-1.41) | 0.019 | 0.16 |
| Q4 (n=6371) | 67.3 (65.4-71.0) | 476 | 75.7 (72.5-78.7) | 1.0 | 0.97 (0.79-1.20) | 1.22 (0.96-1.57) | 1.10 (0.97-1.24) | 0.155 | 0.08 |

P-value refers to additive model. PAR=Population attributable risk. All models are adjusted for age, gender, hypertension, smoking, diabetes, body mass index, use of lipid lowering medication.

# Paper III

The third paper for the PhD thesis "Cardiovascular and Cancer Mortality in Very Elderly Post-Myocardial Infarction Patients Receiving Statin Treatment". The aim of this work was to determine whether statin treatment is effective and safe in very elderly (80 years and older) acute myocardial infarction (AMI) patients. We included all patients 80 years and older who were admitted with the diagnosis of AMI in the Register of Information and Knowledge About Swedish Heart Intensive Care Admissions between 1999 and 2003 (n = 21,410). Of these, complete covariate and follow-up data were available for 14,907 patients (study population A).

Of the total number of patients in study population A (n=14,907), 8,817 (59.1%) patients died during follow-up. Of those who died, 6,929 (78.6%) patients died of cardiovascular causes (myocardial infarction, other ischemic heart diseases, congestive heart failure, stroke, cardiac arrhythmias, and other cardiac causes), 4,423 (50.2%) patients died of myocardial infarction, and 477 (5.4%) patients died of cancer. All-cause mortality was markedly lower in patients receiving statin treatment at discharge in study populations A, B, and C (Table 2, Fig. 2).

**Table 2Mortality Outcome in Elderly Post-MI Patients Discharged With or Without Statins**

| | | Events | | Time at risk* | | Crude† | Cox-regression‡ | | |
|---|---|---|---|---|---|---|---|---|---|
| Mortalitet | Study population** | No Statin | Statin | No Statin | Statin | RR | RR | LCL | UCL |
| Total | A | 7718 | 1099 | 13.96 | 4.63 | 0.43 | 0.55 | 0.51 | 0.59 |
| | B | 5392 | 926 | 13.94 | 4.63 | 0.52 | 0.62 | 0.57 | 0.67 |
| | C | 2198 | 374 | 12.27 | 3.99 | 0.52 | 0.64 | 0.57 | 0.73 |
| CVD | A | 6070 | 859 | 13.96 | 4.63 | 0.43 | 0.55 | 0.51 | 0.60 |
| | B | 3945 | 702 | 13.94 | 4.63 | 0.54 | 0.64 | 0.58 | 0.70 |
| | C | 1478 | 244 | 12.27 | 3.99 | 0.51 | 0.61 | 0.52 | 0.72 |
| AMI | A | 3910 | 513 | 13.96 | 4.63 | 0.40 | 0.53 | 0.48 | 0.59 |
| | B | 1901 | 375 | 13.94 | 4.63 | 0.59 | 0.67 | 0.59 | 0.77 |
| | C | 627 | 105 | 12.27 | 3.99 | 0.52 | 0.62 | 0.49 | 0.79 |
| Cancer | A | 399 | 78 | 13.96 | 4.63 | 0.59 | 0.62 | 0.47 | 0.82 |
| | B | 385 | 77 | 13.94 | 4.63 | 0.60 | 0.63 | 0.47 | 0.83 |
| | C | 203 | 49 | 12.27 | 3.99 | 0.74 | 0.83 | 0.59 | 1.19 |

RR=Relative risk; LCL=lower 95% confidence interval; UCL=upper 95% confidence interval; CVD=cardiovascular disease; AMI=acute myocardial infarction.*Expressed as multiples of 1000 person-years. †Crude RR is calculated as the ratio of "events per 1000 person years" between the Statin and the No statin treatment groups. ‡All Cox regression models were adjusted for cardiovascular medications other that statins at discharge (beta-blockers, acetyl salicylic acid, other thrombocyte inhibitors and angiotensin converting enzyme inhibitors), statin treatment upon admission, the propensity score and year of admission. **Study population A refers to entire study population; Study population B refers to patients who survived at least 14 days after discharge; Study population C refers to patients who survived at least 365 days after discharge.

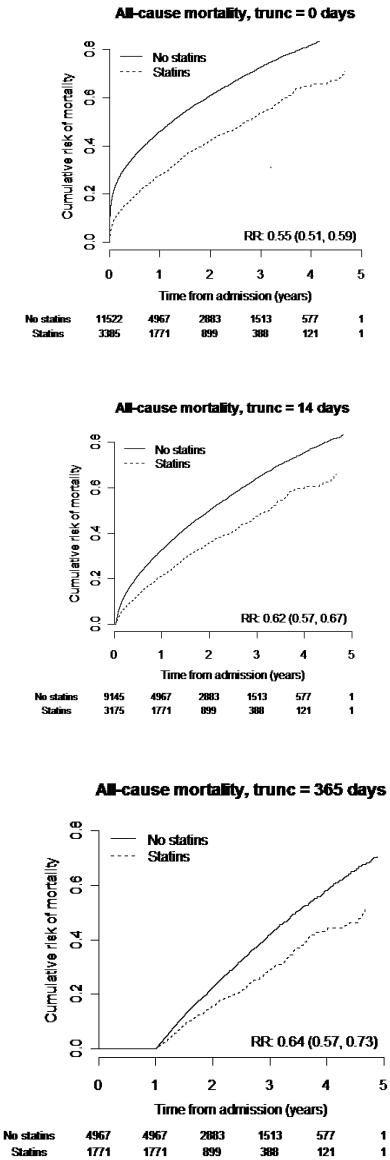



**Figure 2. Adjusted Cumulative Risk of All-Cause Mortality Estimated at the Mean of Each Covariate of the Model**
RR=relative risk; trunc=truncation time; trunc=0 days=all patients included in analysis; trunc=14 days=patients who died within 14 days after discharge were excluded from analysis; trunc=365 days= patients who died in 365 days after discharge were excluded from analysis

However, the relative risk (RR) for mortality associated with statin treatment was clearly dependent on whether patients who died early after discharge were excluded (Fig. 3). The RR reduction for mortality in statin treated compared with non–statin-treated patients seemed to be less pronounced in study population B than in study population A, and it decreased even further as we excluded patients who died during the first 180 days from baseline in a stepwise manner. This suggested that part of the statistical relationship between statin treatment and mortality was attributed to bias related to comorbidities and the physicians' inclination to prescribe statins at discharge. Exclusion of all patients who died during the first year from baseline did not seem to further influence RR for mortality in statin-treated versus nonstatin-treated patients, suggesting that such bias was of less importance in study population C compared with study populations A and B (Fig. 3).

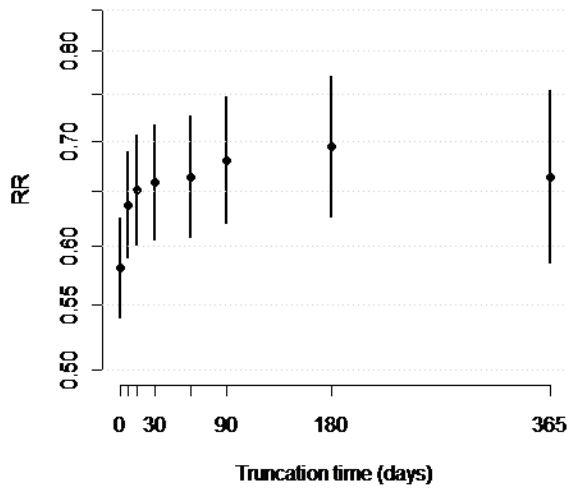



**Figure 3 Relative risk of All-Cause Mortality after Stepwise Exclusion of Patients Who Died Early after Discharge**
Relative risk (RR) (with 95% confidence intervals as vertical bars) of all-cause mortality for patients discharged with statins after truncation of the study population at 0, 7, 14, 30, 60, 90, 180 and 365 days after discharge. Only patients who survived at least as long as the respective truncation time are included in the analysis. Adjustments were done using the propensity score.

We subsequently performed stratified analyses in patients belonging to different quartiles of the propensity score, in those with and without myocardial infarction or congestive heart failure before admission, and by sex in study population C. The lower risk of all-cause mortality in patients treated with statins compared with those not treated with statins was significant in all these subgroups except in the lowest quartile of the propensity score (Table 3).

**Table 3**
Relative risk of All-Cause Mortality for Different Subgroups in Population C*

| Variable | RR | LCL | UCL |
|---|---|---|---|
| Hist of heart failure | | | |
| No | 0.65 | 0.56 | 0.77 |
| Yes | 0.60 | 0.46 | 0.78 |
| Hist of AMI | | | |
| No | 0.63 | 0.53 | 0.74 |
| Yes | 0.66 | 0.53 | 0.82 |
| Sex | | | |
| Male | 0.61 | 0.51 | 0.73 |
| Female | 0.69 | 0.57 | 0.82 |
| Propensity group | | | |
| Q1 | 0.93 | 0.67 | 1.29 |
| Q2 | 0.61 | 0.46 | 0.79 |
| Q3 | 0.55 | 0.44 | 0.70 |
| Q4 | 0.70 | 0.55 | 0.89 |

* Study population C refers to patients who survived at least 365 days after discharge. RR=Relative Risk; LCL=Lower control limit; UCL= Upper control limit; AMI=Acute myocardial infarction; Q1= first quartile; Q2=second quartile; Q3=third quartile; Q4= fourth quartile

To limit the bias related comorbidity on statin therapy, we also performed analyses excluding patients who died within 14 days of the acute event (study population B) and all patients who died within 365 days (study population C). A propensity score was used to adjust for initial differences between treatment groups.

In study population C, the RR of cardiovascular mortality as well as AMI mortality was markedly lower in patients treated with statins compared with patients not treated with statins at discharge. Results were similar in study populations A and B (Table 2). The RR for the combination of fatal and nonfatal AMI during follow-up was reduced to a somewhat lesser degree compared with the RR for AMI mortality in study population C (RR: 0.69; 95% CI: 0.56 to 0.84), study population B (RR: 0.84; 95% CI: 0.76 to 0.92), and study population A (RR: 0.70; 95% CI: 0.65 to 0.76).

There was no increase in cancer mortality in statin treated compared with non–statin-treated patients regardless of whether patients who died at different times during the first year from baseline were excluded, and it was even lower in statin-treated patients in study populations B and A (Table 2). The RR for cancer mortality was similar in statin-treated and nonstatin-treated patients in study population C (Table 2, Fig. 6).

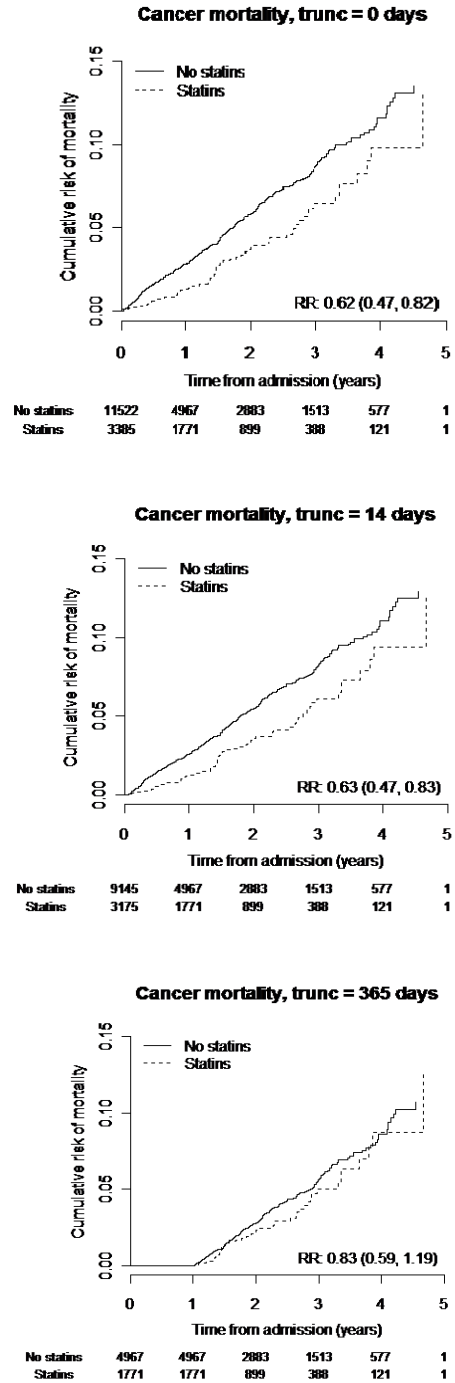

Cancer mortality, trunc = 0 days
No statins
Statins
Cumulative riek of mortality
0.00
0.05
0.10
0.15
RR: 0.62 (0.47, 0.82)
0
1
2
3
4
5
Time from admission (years)
No statins 11522 4967 2883 1513 577 1
Statins 3385 1771 899 388 121 1
Cancer mortality, trunc = 14 days
No statins
Statins
Cumulative riek of mortality
0.00
0.05
0.10
0.15
RR: 0.63 (0.47, 0.83)
0
1
2
3
4
5
Time from admission (years)
No statins 9145 4967 2883 1513 577 1
Statins 3175 1771 899 388 121 1
Cancer mortality, trunc = 365 days
No statins
Statins
Cumulative riek of mortality
0.00
0.05
0.10
0.15
RR: 0.83 (0.59, 1.19)
0
1
2
3
4
5
Time from admission (years)
No statins 4967 4967 2883 1513 577 1
Statins 1771 1771 899 388 121 1

Figure 6

# Paper IV

The fourth paper is a manuscript sent for submission. Between 2002-2006 4819 subjects without history of HF as well as CAD underwent a health screening within the community-based study "Malmö Preventive Project Rescreening Programme". The mean age was 69.4±6.2 years. We related plasma concentration of CT-proET-1 at the baseline exam to incidence of a first HF event in models adjusted for traditional cardiovascular risk factors using multivariate Cox proportional hazard models.

During a mean follow up time of 5.6 years, 121 subjects were diagnosed with heart failure. The proportionality of hazards assumption was met in all analyses. There was a strong relationship between CT-proET-1 and incidence of heart failure. Each standard deviation (SD) increment of log-transformed CT-proET-1 was associated with a hazard ratio (HR) (95% Confidence Interval) of 1.77 (1.56-2.00) (P<0.0001), which was independent of traditional risk factors (see table below). Quartile analyses revealed a graded increase in HF risk with a multivariate adjusted 12.6-fold increased risk in the top versus the bottom quartile of CT-proET-1(Figure and Table below).

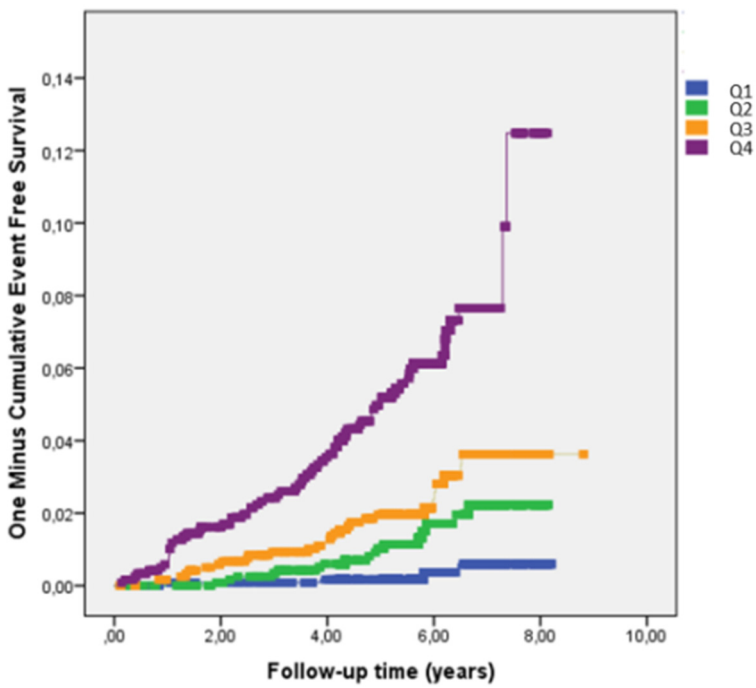



**Figure 1 Kaplan Meier plot of one minus cumulative heart failure free survival in quartile 1-4 of C-terminal Endothelin-1 plasma concentration**
Quartiles 1 (blue), 2 (green), 3 (orange) and 4 (purple) of baseline plasma concentration of C-terminal Endothelin-1 plasma concentration in relation to heart failure incidence (one-minus heart failure free survival)

**Table 2 Risk of incident heart failure over quartiles* of CT-proET-1 (quartile 1 as the reference)**

| | Number of events | CT-proET-1 Kryptor (pmol/L) Median (min-max) | HR | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|---|
| CT-proET-1, quartile 1 (n=1204) | 4 | 54,58 (3,96 - 59,23) | REF (1.0) | - | - | - |
| CT-proET-1, quartile 2 (n=1205) | 17 | 63,14 (59,24 - 67,12) | 3.81 | 1.28 | 11.35 | 0.016 |
| CT-proET-1, quartile 3 (n=1205) | 29 | 71,34 (67,13 - 76,89) | 5.99 | 2.09 | 17.18 | 0.001 |
| CT-proET-1, quartile 4 (n=1205) | 71 | 86,38 (76,89 - 432,42) | 12.61 | 4.48 | 35.47 | <0.0001 |

CT-proET-1=C-terminal pro-Endothelin 1; HR=Hazard Ratio; 95% CI=95% Confidence Interval *P for trend over quartile 1-4 <0.0001

In a basic model relating cardiovascular risk factors to incidence of HF the C-statistic (95% confidence interval) was 0.72 (0.68-0.77). After addition of CT-proET-1 to the model, the C-statistic rose to 0.79 (0.76-0.83). Comparing the same basic model with addition of CT-proET-1 resulted in an improvement of the cNRI with 56 % (P<0.0001).

After additional adjustment for MR-proANP, on top of cardiovascular risk factors, CT-pro-ET1 remained significant with each standard deviation increment of log-transformed ET-1 being associated with a hazard ratio of 1.44 (95% CI 1.24-1.68, p<0.0001). When adding MR-proANP on top of cardiovascular risk factors and CT-pro-ET1, the c-statistics further improved to 0.82 (95% CI 0.78-0.85); however, the cNRI was nominally lower with the addition of both CT-proET1 and MR-proANP (54%; p-value=0.0003) than with CT-proET-1 only.

During the study there were 605 deaths. CT-pro-ET1 predicted total mortality independently of CVD risk factors. Each standard deviation increment of log-transformed CT-pro-ET1 was associated with a hazard ratio of 1.35 (95% Confidence Interval) (1.26-1.46) (P<0.0001) for total mortality. This relationship was independent of additional adjustment for MR-proANP (1.28; 95% CI 1.18-1.39, p<0.0001). When subjects were divided into quartiles of CT-pro-ET1 and quartile 1 was defined as the reference (hazard ratio= 1.0), the hazard ratios in quartiles 2-4 were 1.08 (95% CI 0.80-1.44, p=0.624), 1.18 (95% CI 0.89-1.58, p=0.245) and 2.08 (95% CI 1.59-2.72, p<0.0001) (P for trend <0.0001).

Finally, we tested if our findings were dependent of renal function by additionally adjusting models with cardiovascular risk factors and CT-proET-1 for e-GFR using the MDRD formula, however the relationships between CT-proET-1 and both heart failure (HR per SD increment=1.72; 95% CI 1.49-1.99, p<0.0001) and mortality (HR per SD increment 1.37; 95% CI 1.26-1.49, p<0.0001) were virtually unchanged. As the incidence of HF and CT-proET-1 concentration both increase with age, we tested whether there was any significant interaction between CT-proET-1 and age on incidence of HF. There was no interaction between CT-proET-1 and age, both with respect to outcome of HF (p=0.07), and subsequent mortality (P=0.28).

# Discussion

## Paper I

The first paper highlights the importance of the risk factor exposure for developing cardiovascular disease at different age. It´s worth high lightening that the risk factors were obtained at baseline examination prior to incident myocardial infarction. All traditional risk factors included in the study were significantly associated with MI in the entire material ($P<0.05$ for all) supporting the fact that all traditional risk factors included in the model are important regarding ischemic heart disease. The study shows that exposure to cholesterol and family history of MI in midlife more strongly predicts onset of MI at younger ages.

### Age and cumulative risk - The age related paradox

Given the potential pathophysiological differences between early and late-onset of MI, we here tested whether the relationship between risk factor exposure in individuals free from cardiovascular disease at baseline and incident MI differs between strata of age at MI onset in a large population-based prospective cohort. We enrolled 33 346 individuals with 3687 incident cases of MI who were followed regarding to endpoints for an average of 22 years. We wanted examine if there was a difference regarding risk factors in midlife and later development of early versus late myocardial infarction.

It is likely that age-related comorbidities and other yet unrecognized age-related factors have a larger impact on late-onset MI than in MI presenting early in life. At the same time, up to 20% of MI patients below the age of 45 years do not display the evidence of atherosclerosis on coronary angiography (11). Whatever explains age-related pathophysiological differences little is known about the role of risk factor exposure in this context. Importantly, even if impact of risk factors for myocardial infarction has been studied in several different large prospective population-based cohorts with various baseline age ranges, much less is known as to whether risk factor pattern in mid-life differs between in individuals who later develop MI early and late in life. If comorbidities play a major role for late-onset MI, it can be assumed that certain risk factors would have relatively larger impact for early myocardial infarction events. On

the other hand, the impact of exposure to the risk factors would be higher in individuals suffering a MI late in life.

In contrast to hypertension guidelines which recommend initiation of therapy based on defined blood pressure levels primary prevention guidelines today recommend using risk scores to decide whether the patient will benefit from pharmacological treatment regarding lipid lowering therapy. Risk scores are to a great extent driven by age and therefore it can lead to that older individuals with a lower burden of a risk factor seems to be of greater need of pharmacological treatment compared to a younger individual with a higher burden of the same risk factor.

It seems as there are certain risk factors which are more related to early development of cardiovascular disease compared to late. In our study hypercholesterolemia and smoking seem to be important in younger ages but on the other hand the same risk factors accumulate over the years and time exposure may therefore be regarded in risk assessment according to developing cardiovascular disease later in life. An interesting way of looking at age is "time of exposure or cumulative exposure" to certain risk factors. Navarr-Boggan and co-authors published a very interesting article in Circulation 2015 where they had studied the future risk of coronary heart disease according to duration of hyperlipidaemia in almost 1500 participants free of CVD at age 55 in the Framingham Offspring Cohort. The authors concluded that a prolonged exposure to even moderate elevations of non-LDL-cholesterol was associated with an increased future risk suggesting that there is an indication for earlier and more aggressive primary prevention strategy(112). Familial hypercholesterolemia is good example on how severity and duration of exposure of a risk factor may affect future event of CVD (thus an example of age related risk. Patients with the monogenetic disorder homozygous familial hypercholesterolemia are born with extremely high LDL-cholesterol levels and develop severe atherosclerotic disease in early years and rarely survive to the age of 20 if untreated(62). In contrast, individuals with extremely low LDL-cholesterol levels due to genetic polymorphisms are exposed to low levels of LDL-cholesterol from birth and therefore have significantly lower risk than average developing future cardiovascular disease(113).

Cumulative exposure should be relevant for several other risk factors. Today clinicians use tobacco pack-years when estimating risk due to smoking. Even though it will require new studies it would be of interest to integrate the cumulative exposure concept in future risk scores and prediction models. Cholesterol-years, hypertension-years and diabetes-years may be of more value than single measurements of the risk factors at different times(114).

## Cholesterol

When the baseline visit of the study was performed in the MPP study, total cholesterol was the standard measurement and thus we are limited in not having low- and high-density lipoprotein levels in our cohort. As mentioned earlier total cholesterol is an established risk factor for cardiovascular disease (42) and it could be used in risk prediction models without having information of LDL-cholesterol levels. The authors in the Emerging Risk Factors Collaboration concluded in a study of individuals without known CVD published in JAMA 2012 that addition of information on the combination of other lipoproteins (apolipoprotein B and A-I, lipoprotein(a), or lipoprotein-associated phospholipase A2 mass) to risk scores containing total cholesterol and HDL-C only led to slight improvement in CVD prediction(115).

Earlier studies have shown the relationship between hypercholesterolemia and risk of developing CVD diminish according to higher age (42, 43). Our study focus on whether baseline risk factors in midlife can predict early onset versus late onset of myocardial infarction. Total cholesterol had a significantly stronger relationship within Q1, i.e. the quartile with the youngest myocardial infarction cases, when compared with the relationship within Q4, i.e. the quartile with the oldest MI cases.

The results from our first study show the importance of high total cholesterol as a risk factor for developing early myocardial infarction. The odds ratio for cholesterol in relation to MI decreased in a linear fashion with quartile of age of myocardial infarction. Of note the linear attenuation of the relationship between midlife cholesterol and risk of suffering from MI across Q1 to Q4 of myocardial infarction age should not be mixed up with the fact that total cholesterol in older people shows a weaker relationship with MI than in younger individuals. Potential explanations to our findings may thus be that cholesterol and genetic factors are more strongly related to early development of atherosclerosis, whereas hypertension seems to affect atherosclerosis development over a broader age range. One can speculate that the stronger association between e.g. baseline cholesterol and myocardial infarction in the younger case group may result from survival bias, which clearly is a possibility. On the other hand, other risk factors which affect mortality, such as hypertension and diabetes, did not differ in strength according to age at first myocardial infarction, suggesting that survival bias is not the sole explanation of our results.

The result supports earlier findings in the importance of highlighting cholesterol as a risk factor for early CVD. Coronary atherosclerosis is a chronic progressive disease that begins early in life and develops slowly before becoming clinically manifest. The authors of a large meta-analysis combining data from more than 300 000 participants concluded that prolonged exposure to low LDL-cholesterol beginning early in life was associated with a great reduction in the risk of coronary heart disease(116). It is

therefore tempting to draw the conclusion that lowering cholesterol early in life may be more effective in preventing early MI as compared to late onset MI.

### Family history

It´s well known for physicians and researchers that ischemic heart disease tends to aggregate or cluster in certain families. Previous studies have shown that a positive family history of coronary artery disease is a major risk factor for myocardial infarction in young patients (52). In contrast to genetic testing family history reflects both shared biological, environmental and genetic factors. Our result supports earlier findings. The stronger association between family history for myocardial infarction and events among the quartiles with the youngest cases underlines the importance of family history for risk stratification of primarily early events.

### Smoking

The finding that smoking exposure displayed a U-shaped relationship with age of MI is more intriguing. It is well known that early coronary atherosclerosis and MI is strongly linked to cigarette smoking(117). The association between smoking and MI in quartiles of older cases may be related to a different pathophysiology caused by long-term exposure to cigarette smoking, namely large artery stiffness. Indeed, smoking has previously been shown to be a strong risk factor in elderly subjects with systolic hypertension and to be a stronger risk factor for coronary heart disease in older as compared to middle aged subjects. Thus we suggest that the higher smoking related risk in the earliest and latest events are explained by two different pathophysiological mechanisms.

## Paper II

### Can our genes predict future risk for cardiovascular disease?

As mentioned earlier family history of premature coronary heart disease and genetic factors are more strongly related to early development of atherosclerosis and development of early CVD (58, 118). Genetics has been a part of cardiovascular medicine for many years. Genetic testing is routine especially when it comes to identifying rare inherited diseases as severe cardiomyopathies, disorders leading to sudden cardiac death and severe lipid disorders. Genome-wide association studies have

been used successfully to identify a large number of common gene variants that increase risk of myocardial infarction and coronary artery disease (CAD). Genetic risk score composed of 50 common gene variants has been shown to be associated with CAD independently of self-reported family history of coronary heart disease (53).

The most robust myocardial infarction and CAD genetic association was found to be located upstream of the genes coding for the cyclin-dependent kinase inhibitors 2A and 2B CDKN2A/CDKN2B locus on chromosome 9p21 and spanning a large cluster of single-nucleotide polymorphisms (SNPs) in strong linkage disequilibrium with each other(57-60).The same locus is also associated with ischaemic stroke with similar effect size as compared with myocardial infarction and CAD(119, 120). Thus, the chromosome 9p21 locus is highly relevant for cardiovascular disease (CVD), and studies are warranted to evaluate its clinical meaning in the general population.

In our study the genetic variation of the chromosome 9p21 locus explained as much as 13% of the population attributable risk (PAR) in the total population. The results of the INTERHEART trial, a large case–control study including patients with coronary heart disease (CHD) and control subjects from 52 countries worldwide, showed that nine modifiable risk factors explained more than 90% of the PAR of CHD(8). Although it is not easy to compare individual PAR estimates for different risk factors both within and between studies, the PAR of genetic variation of the chromosome 9p21 locus for CVD was similar to that of diabetes for coronary heart disease in men in the INTERHEART study.

Despite the substantial risk attributable to genetic variation of the chromosome 9p21 locus, the incremental value when added to traditional risk factors for CVD prediction, assessed by the c-statistic, IDI and NRI, was negligible. The benefit of general CVD screening in individuals for single gene variants associated with CVD seem to be low since the effect of each genetic polymorphism is small.

Even though the genetic variance of chromosome 9p21 is the most robust common CVD susceptibility gene variants known, several gene variants for CHD and myocardial infarction have recently been reported(54). Prior studies have used multiple loci or genetic scores (GRS) to demonstrate the genetic effect in contrast of using single genetic variants for risk prediction to(53). In an article published in Lancet 2015 Mega et al showed that a genetic score based on 27 genetic variants could identify individuals at increased risk for incident and recurrent coronary heart disease events. The authors also concluded that individuals with the highest burden of genetic risk derived the largest relative and absolute clinical benefit from statin therapy which is a good example of how genetics already today could be used in a clinical setting(121).

### Genetic variation and age

In the current study, we also compared the relative risks, PAR and incremental value for risk prediction associated with carrying the risk allele in different subgroups of baseline age. Although the relative risk of CVD associated with the 9p21 locus, which was significant in all quartiles of baseline age, tended to decrease with increasing age (see Figure and Table in result section above), this decline in the magnitude of risk with age was not statistically significant.

Interestingly, it was shown in a meta-analysis of a large number of case–control samples that age at diagnosis was inversely related to the magnitude of the odds ratio associated with the chromosome 9p21 risk allele(122). This result is inconsistent with our finding of no significant effect of age on the magnitude of relative risk; however, we assessed baseline age in one prospective cohort study compared with assessment of age at case diagnosis in several different case–control studies. In addition, despite a decline in the proportion of CVD incidence attributable to the chromosome 9p21 with increasing age, the actual number of events increased with age, suggesting that any future intervention to inhibit the consequences of the chromosome 9p21 risk variant may in fact prevent more CVD events amongst older than younger individuals. Altogether, our findings do not provide statistical support for a greater impact of the chromosome 9p21 risk variant in younger than in older individuals.

Our conclusion is that chromosome 9p21 has a high population attributable risk suggesting that future interventions interfering with downstream mechanisms of the genetic variation may affect CVD incidence over a broad range of ages. However, variation of chromosome 9p21 alone does not add clinically meaningful information in terms of CVD prediction beyond traditional risk factors at any age. It would be interesting to investigate if use of genetics together with our traditional risk assessment be able to find patients with higher and lower risk in order to improve primary prevention and treatment. However, the value of genetics in general CVD risk prediction needs to be further evaluated. These studies need to be replicated and confirmed in different clinical settings.

## Paper III

### Statins to the elderly?

Our aim was to study if statin treatment is effective and safe in very elderly (≥ 80 years) patients admitted to hospital for acute myocardial infarction. To examine if cholesterol lowering therapy by using statins is associated with lower cardiovascular mortality in

very elderly post-infarction patients we performed a register based study in The "Register of Information and Knowledge about Swedish Heart Intensive care Admission"(RIKS-HIA) where we included all patients (n=21,410) 80 years and older who were admitted to the coronary care units of all participating Swedish hospitals with the diagnosis of AMI and registered between 1999 and 2003.

The proportion of patients above 80 years of age is growing rapidly in the western countries. Beneficial clinical effects of treatment with lipid-lowering statins have been shown in middle-aged patients and patients over 60 years old but whether treatment is beneficial in patients over 80 years old is not known. The value of statin treatment to very old patients remains controversial. Elderly individuals constitute an increasing percentage of patients admitted to hospitals for acute myocardial infarction. Even though elderly patients with acute coronary syndromes have a higher short- and long-term mortality risk, the application of evidence-based medicine remains much lower than for younger patients (123-127). A large number of clinical trials have established that treatment with lipid-lowering statins significantly reduces cardiovascular mortality in post-myocardial infarction patients (46). The generalizability of these findings to the broader elderly population are questioned due to the fact that patients at high age are underrepresented in these studies or tend to be relatively healthy with few comorbidities(128, 129).

Statin prescription at discharge after acute coronary disease seems to be lower to the very elderly. Data from observational studies such as GRACE (Global Registry of Acute Coronary Events)(130) and the Euro Heart Survey on ACS(130) suggest that 40% of myocardial infarction patients older than 75 years are prescribed statins at discharge. Several circumstances may to contribute to a lower use of statins in elderly post myocardial infarction patients. The association between plasma cholesterol and cardiovascular risk diminishes with increasing age (43, 44), and most lipid trials have excluded older patients. With increasing age the amount of comorbidity is increasing which can influence the clinicians choice of pharmacological treatment of the elderly patients despite the fact that multi morbidity is associated with substantially lower life expectancy(132).

There are several limitations of the present study that needs to be considered. Even though the study shows real life data from patients included in RIKS-HIA 1999-2003 the generalizability could be questioned. The inherent limitations of a nonrandomized, registry study should be acknowledged. The study is observational and should reflect how the physicians treat the patient in contrast to a clinical study where the physicians are told how to treat a patient. Despite appropriate statistical adjustments, unknown confounders may have affected the results and therefore it´s difficult to draw any conclusions. A propensity score was used to statistically control for the possible influence of baseline factors associated with increased probability to receive statin prescription. It would have been possible to include other discharge medication

variables in the propensity score but conceptually it is easier to think of the propensity score as capturing all available information that can affect the treatment assignment decision. The other discharge treatment variables can instead be entered together with the propensity score in the final models.

Although our analyses are adjusted for confounding it is likely that the lower incidence of CVD mortality among patients given statins in some way reflects a bias in not prescribing statins to patients with decreased life expectancy. Furthermore, we see that the probability of receiving statin treatment at discharge is highly dependent on whether the patient was on statin treatment at admission or not.

However, the fact that statin-treatment remained significantly associated with lower cardiovascular mortality risk also when all subjects that died during the first year were excluded argues against this possibility.

Another limiting factor of this study is that data regarding drug treatment is based solely on hospital discharge records. Accordingly, it can be assumed that part of the patients prescribed statins stopped taking the medication during that follow-up period and also that some patients discharged without statins began taking the medication at a later stage. However, assuming that this is correct, such a bias is likely to reduce the difference in cardiovascular mortality between the groups.

Finally, and not the least, a large proportion of very elderly patients attending hospitals is treated in general medical wards due to multiple illness and comorbidities and therefore not included in the registry.

## Are statins dangerous to the elderly?

The potential of increased risk of cancer by cholesterol-lowering treatment was widely debated in the pre-statin era. Although meta-analysis of long-term statin trials have revealed no support for an increased cancer risk(133) the observation of a higher incidence of cancer in the pravastatin group of the PROSPER trial raised concerns that(134) elderly patients could be at particular risk. Reports of inverse associations between plasma cholesterol and cancer rates in older persons(43) have also argued for precautions in treating elderly patients with statins. Using propensity analysis, we demonstrated a significant reduction in all-cause mortality without any increase in cancer mortality. These data are particularly interesting as it points to the benefit of statin treatment without increased cancer mortality in an elderly population with a high risk of cancer. Contrarily, in analysis including the entire population of post-AMI patients for whom complete data were available (study population A; n=14,907) we observed a decreased incidence of cancer mortality among subjects receiving statin treatment (RR=0.65; 95% CI 0.49-0.86). However, no reduction of cancer mortality rates was observed in statin treated patients if the study population was restricted to

subjects surviving at least one year after the acute event. Although our analyses included controlling for prevalence of cancer at the original admission it is likely that the lower incidence of cancer mortality among patients given statins in study population A in some way reflects a bias in not prescribing preventive treatment to patients with decreased life expectancy. In spite of these limitations, the present study shows that lipid-lowering therapy for the elderly, unless, of course, they have a life-threatening comorbidity or are statin intolerant could be considered(62). Still there is a need for randomized controlled trials or at least a randomized multicentre registry based studies before we apply the results in guidelines.

# Paper IV

## Need for better prediction

The increasing burden of cardiovascular disease calls for better understanding of the underlying mechanisms and for better measures and prediction of disease. Early detection of disease is crucial for initiating preventive strategies and early treatment. Heart failure among the elderly is not only common but also one of the leading causes of death and hospitalisation. In contrast to myocardial infarction, whose incidence has levelled off in parallel to the improvements of primary and secondary prevention, the incidence of heart failure and its associated comorbidities is increasing(135).

Since early detection of left ventricular dysfunction enables early treatment, which is important for survival and well-being (136-139) it is crucial to access simplified tools to identify individuals at risk (140, 141). Biomarkers may be useful in specific subgroups, but further studies are required. Biomarkers may be cost-effective and lead to improved clinical results when integrated into clinical routines and laboratories. In later years a large amount of "new" biomarkers have been discovered which calls for large epidemiologic population studies to evaluate the value of this new information. There is also a need to understand underlying mechanisms and evaluate if there are any differences in pathophysiology in different age spans. Still not all potentially useful circulatory and urinary biomarkers have undergone state-of-the-art assessment of their added value in cardiovascular risk prediction on top of conventional risk factors. Current guidelines do not, however, recommend testing any biomarkers beyond lipids, creatinine, glycaemia and glycated haemoglobin, adding the organ specific BNP or NT-proBNP only if presence of heart failure is suspected(26).

## Endothelin-1

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide that has been implicated in the genesis and progression of several cardiovascular disease states(83). It has been shown to have inotropic effects, to increase blood pressure, induce cardiac hypertrophy and

triggers secretion of a range of hormones known to have long-term adverse effects on the myocardium such as vasopressin, renin, aldosterone, epinephrine and norepinephrine(83).

The key findings of this study is that the stable C-terminal endothelin-1 precursor hormone fragment CT-proET-1, strongly predicts development of new-onset heart failure (HF) in elderly subjects with no history of CAD. The association was independent of traditional CVD risk factors, natriuretic peptide level and renal function.

This is the first observational study examining the gain in heart failure prediction by measuring CT-proET-1 in subjects without CAD, a group that constitutes a rapidly increasing part of the population. Although the study is observational and results need to be replicated in other populations with similar inclusion criteria the findings may be of importance especially among individuals with high concentration of CT-proET-1. The present study demonstrates that the effect size for HF prediction was large, with a 13-fold increased risk in the top versus bottom quartile of CT-proET-1 and substantial improvement of both the C-statistic and cNRI.

Even though ET-1 antagonists have failed to prove any benefit in heart failure treatment due to undesirable effects (142) the understanding of the endothelin system may be of importance for prevention and novel targets of pharmacological treatment of heart failure. Earlier studies have demonstrated that life-style intervention, i.e. increased physical activity, affects the vascular tone induced by endothelin-1 and that antihypertensive treatment with ACE-inhibitors reduces the levels of ET-1(143-145). Finally, it can be speculated that newer drugs interfering with the endothelin system could be useful in heart failure prevention in subjects with high CT-proET-1.

However, even though our conclusion is that CT-proET-1 strongly and independently predicts HF development in an older population free from CAD, there are several issues that need to be addressed in this observational study. We acknowledge a number of limitations of our study. First, the findings in this Caucasian, middle-aged population may not be applicable to other settings. Further only 121 patients out of 4819 developed heart failure which is a low number and the heart failure diagnose is based on hospital records and not on re-examination with echocardiography and clinical follow-up. It´s also important to consider the competing risks problem since we evaluate heart failure onset in elderly patients. Individuals studied are at increased risk for not just HF but also mortality. The study is observational and not a randomized trial and therefore it is important to replicate the results in other settings. We want to emphasize the lack of evidence regarding if biomarker-guided preventive strategies improve outcome and that use of CT-proET-1 would be in the context of overall risk (taking traditional risk factors into account rather than just CT-pro-ET-1). Further studies using Mendelian randomisation (146) could be useful to evaluate whether or not ET-1 is causally related to heart failure.

# Limitations and future considerations

The studies in the thesis are observational and reflecting a Caucasian population. We acknowledge the low participation rate, only 41%, in the MDC-study which makes the results in paper II less certain if they are applicable in a bigger general population.

The studies were performed several years ago and risk factor exposure at baseline may to some extent be different today. The most obvious example of this is smoking habits, which are much lower today than in the study. Current smoking is much lower today than in the 70´s and obesity is much more common in this part of the world.

In agreement with earlier studies we acknowledge the importance and strength of traditional risk factors in predicting future events of cardiovascular disease. However different risk factor patterns at different age seem to predispose cardiovascular disease. More research is needed to guide therapy in a more individual way. To combine traditional information and risk factor patterns with genetic information in different age groups would be of importance for scientists and clinicians in their future research and work.

Since most pharmacological studies have excluded the very elderly there is a great need for evaluating several of the drugs used for CVD treatment. Since there are difficulties in performing randomized controlled statin trials in the very elderly, and the interest probably is low, randomised trials with registry follow-up might be an option.

# Conclusions

**Paper I** Exposure to cholesterol and family history of MI in midlife more strongly predicts onset of MI at younger ages, suggesting that MI in younger subjects is preceded by a different risk factor pattern than MI presenting in older subjects.

**Paper II** a) The variation of chromosome 9p21 has a high population attributable risk suggesting that future interventions interfering with downstream mechanisms of the genetic variation may affect CVD incidence over a broad range of ages.

b) Variation of chromosome 9p21 alone does not add clinically meaningful information in terms of CVD prediction beyond traditional risk factors at any age.

c) In addition, despite a decline in the proportion of CVD incidence attributable to the chromosome 9p21 with increasing age, the actual number of events increased with age, suggesting that any future intervention to inhibit the consequences of the chromosome 9p21 risk variant may in fact prevent more CVD events amongst older than younger individuals.

**Paper III** Statin treatment is associated with lower cardiovascular mortality in very elderly post-infarction patients without increasing the risk of the development of cancer.

**Paper IV** CT-proET-1 strongly and independently predicts HF development in an older population free of CAD. The association was independent of traditional CVD risk factors, natriuretic peptide level and renal function.

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The overall main theme of the thesis is to improve prediction of cardiovascular disease and it´s complications in different age settings and in particular groups of individuals and patients. Four papers concerning age-related risk factors and relation to cardiovascular disease will be presented in this summary.

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