New frontiers of biomarkers in prediction of cardiovascular disease

**Time:** June 7–8\textsuperscript{th} 2013

**Venue:** “Jubileumsaulan” (*Main Hall*), Skåne University Hospital, Jan Waldenströms gata 1–5, Malmö, Sweden

**Organizing secretariat:** Peter M Nilsson, Olle Melander, Camilla Key

**Organizers:** Lund University, Sweden, and a Danish-Swedish EU-Interreg IV project for cardiovascular prevention, in collaboration with the European Society of Hypertension (ESH).

**Registration:** Research administrator Camilla Key, e-mail: Camilla.Key@med.lu.se, phone +46 40 33 23 01. No registration fee, but registration needed for the symposium dinner.

**Posters:** Abstracts for poster presentation should be sent by e-mail no later than May 26\textsuperscript{th}, 2013.

**Transportation:** From the Copenhagen Airport, a local train to Malmö-Triangeln underground railway station (located 200 meter from symposium venue) runs 3 times/hour and takes only 15–20 minutes.

**Accommodation:** List of local hotels will be provided for self-registration.
Welcome to the ESH Satellite Symposium in Malmö, Sweden, on 7–8th June 2013

During recent years new biomarkers and imaging biomarkers of cardiovascular disease and target organ damage has emerged based on discoveries in genetics, circulating biomarkers and the new imaging technologies of cardiovascular function. These issues will be further discussed at a satellite symposium to the ESH 23rd Meeting in Milan, 14–17th June, 2013, to be held in Malmö, Sweden, on 7–8th June 2013 with the ambition to further present and discuss these new biomarkers in relation to existing biomarkers and guidelines for cardiovascular prevention.

We have managed to attract a number of excellent lecturers from many countries, also from North America, to join us in Malmö. This will take place at a season in early June when Scandinavia normally offers very nice and pleasant weather conditions. Malmö is easily accessible by flights and also close to nearby Copenhagen with many touristic attractions.

The symposium is jointly organized by the Lund University and a Danish-Swedish research network dealing with cardiovascular epidemiology and prevention within the EU-Interreg IV regional programme. We hope that you will find the programme attractive and encourage you to participate. A summary of the proceedings will later be published on the ESH official web-site www.eshonline.org

Welcome!

Peter M Nilsson, MD, PhD
Professor, Lund University

Olle Melander, MD, PhD
Professor, Lund University
Programme

Friday June 7\textsuperscript{th} 2013

13:00–13:05  Introduction, welcome \textit{Peter M Nilsson, Olle Melander}

13:05–15:05  \textbf{Session 1. Genetic biomarkers}
\textbf{Chair: Olle Melander, Malmö, Sweden}

Common and rare genetic variants for risk of coronary heart disease
\textit{Jeanette Erdmann, Lübeck, Germany}

Can we use genetics to predict treatment response?
\textit{Sandosh Padmanabhan, Glasgow, UK}

Biomarkers in the beta-cell and diabetes development
\textit{Anders Rosengren, Malmö, Sweden}

Gene-environment interactions in type 2 diabetes and cardiovascular disease: genetic biomarkers defined by environment?
\textit{Marju Orho-Melander, Malmö, Sweden}

15:05–15:30  \textit{Coffee and posters}

15:30–16:00  \textbf{State of the Art 1}
\textbf{Chair: Olle Melander, Malmö, Sweden}

Omics: What’s at the clinical horizon?
\textit{Anna Dominiczak, Glasgow, UK}
16:00–18:00 **Session 2. Imaging and circulating biomarkers**

**Chair:** Peter M Nilsson, Malmö, Sweden

Vascular structure and function as imaging biomarker: Beyond hypertension  
**Stéphane Laurent,** Paris, France

Biomarkers in the primary preventive setting  
**Adam Butterworth,** Cambridge, UK

Lessons from the REFINE Reykjavik Study  
**Vilmundur Gudnason,** Reykjavik, Iceland

The Asklepios study on vascular ageing  
**Ernst Rietzschel,** Ghent, Belgium

18:00–18:30 **State of the Art 2**

**Chair:** Peter M Nilsson, Malmö, Sweden

Cardiovascular gene therapy – an update 2013  
**Seppo Ylä-Herttuala,** Kuopio, Finland

18:45–20:30 Symposium buffet dinner at the Restaurant located at the Clinical Research Center (CRC), Skåne University Hospital, Malmö
Programme

Saturday June 8\textsuperscript{th} 2013

08:30–10:30  \textbf{Session 3. Mechanisms – new understanding of risk}

\textbf{Chair: Anna Dominiczak, Glasgow, UK}

Hypertension: is there a role for hypertonicity in the interstitium?
\textit{Jens Titze}, Erlangen, Germany, and Nashville, USA

Fetal programming—part of the heritability of cardiovascular disease?
\textit{Johan Eriksson}, Helsinki, Finland

Fluid biomarkers in Alzheimer’s disease – current concept
\textit{Oskar Hansson}, Malmö, Sweden

What can we learn from Mendellan randomisation about causal inference?
\textit{Sarah Lewis}, Bristol, UK

10:30–11:00  \textbf{State-of-the-Art 3}

\textbf{Chair: Anna Dominiczak, Glasgow, UK}

Integration of genetics and plasma biomarkers for understanding of cardiovascular disease
\textit{Olle Melander}, Malmö, Sweden

11:00–11:30  \textit{Coffee and posters}
Session 4. Treatment aspects

Chair: Stephane Laurent, Paris, France

Risk prediction in hypertensive patients
Michael H. Olsen, Odense, Denmark

Screening for vascular ageing and possible interventions
Pedro Cunha, Guimarães, Portugal

Genomics of blood pressure – time for translation
Mark Caulfield, London, UK

New guidelines in cardiovascular prevention
Peter M Nilsson, Malmö, Sweden

13:30–13:40 Summary and conclusions
Stéphane Laurent, Paris, France

13:40 Farewell  Peter M Nilsson, Malmö, Sweden
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Biomarkers in the primary preventive setting

Using the Emerging Risk Factors Collaboration to assess the additional benefit of adding novel biomarkers to standard cardiovascular risk prediction tools

Numerous algorithms exist that aim to identify those individuals at highest risk of cardiovascular disease, so that high-risk groups can be targeted with appropriate interventions. Algorithms such as the Framingham Risk Score, SCORE and QRisk have become widely used in clinical practice. However, many novel biomarkers have been shown to be associated with incident cardiovascular disease, often independently of traditional vascular risk factors, suggesting that they may provide additional benefit if added to existing scores.

Using data from the Emerging Risk Factors Collaboration, which involves >130 prospective cohorts and >2 million participants, a framework for evaluating the benefit of novel biomarkers has been developed, including traditional measures of risk prediction (eg, C-index and NRI), as well as public health modelling to explore potential impact. Examples relating to lipids, lipoproteins and inflammatory markers will be considered.
Cardiovascular disease is now the number 1 global cause of death and by 2025 there will be a 1.5 billion people worldwide hypertension. Although evidence-based interventions have transformed cardiovascular disease (CVD) epidemiology, a more sophisticated understanding of pathogenesis coincides with a decline in licensing of new medicines, and increased development costs due to high rates of late-stage failure. A major reason is that pre-clinical drug target validation is often sub-optimal and the definitive experiment (the randomized controlled trial) comes at the end of the drug development pipeline. A potential way to address is to harness the potential of genomics to select and validate drug targets employing approaches such as Mendelian Randomisation. By integrating expertise in this area alongside high throughput human cellular assays with deeper phenotyping of appropriate model organisms, experimental medicine, pharmacology and medicinal chemistry we can strengthen target validation. If to this we then add the untapped potential of the electronic health record for multi-omics research, accelerated clinical trial delivery, recall by genotype studies and ‘real-world’ evaluation of clinical effectiveness then there is a new opportunity to populate lean pipelines for cardiovascular therapeutic intervention.
Screening for vascular ageing and possible interventions

If detected, abnormal vascular ageing presents to the clinician as an opportunity to program early intervention and cardiovascular disease prevention. As a developing concept, much debate has been carried on about the best way to define it and to detect it. Should the same strategy be used in different populations? Are there different aspects linked with age that should point out to the preferential use of different screening strategies?

From the public health authority’s point of view, it seems crucial to know the population’s characteristics and understand early exposure to risk factors that accelerate and promote arterial ageing. This knowledge is crucial to set up tighter screening strategies, more intensive clinical follow-up justifying the use of auxiliary evaluations that are nowadays fundamental but not available for widespread use – as is the case of pulse wave velocity (the currently accepted gold-standard measure of arterial stiffness) – and more aggressive coordinated public health interventions.

Observing several cardiovascular risk factors as continuous variables and understanding that minimum risk exposure is, most of the times, quite different from the established treatment cut-off values, is one of the relevant issues to analyse and promote early multidisciplinary interventions and abnormal vascular ageing screening strategies both at a population and an individual level.

From the clinician standpoint, identifying subjects at risk of developing abnormal vascular ageing is clearly a challenging issue of modern individualized cardiovascular risk reduction. Several aspects of an individual relevant medical and family history are currently and progressively being used as warning signs that should prompt early vascular evaluation.

Currently, treatment of abnormal vascular ageing is directed at early changes towards healthy lifestyle behaviour and aggressive control of existing risk factors, but new approach strategies are being researched, including the use of new drug therapies, stemming from the increasing comprehension of mechanisms that lead to structural and functional vascular changes.
Anna Dominiczak, Professor
anna.dominiczak@clinmed.gla.ac.uk

Regius Professor of Medicine, Vice-Principal and Head of College of Medical, Veterinary and Life Sciences, University of Glasgow

Omics: What’s at the clinical horizon?
Common and rare genetic variants for risk of coronary heart disease

Myocardial infarction (MI), a leading cause of death in the Western world, usually marks the acute clinical manifestation of the silent process of atherosclerosis in the coronary arteries (coronary heart disease, CHD).

CHD is promoted by several risk factors, including age, arterial hypertension, hypercholesterolemia, diabetes mellitus, smoking, and a positive family history. MI itself occurs in the majority of cases when the fibrous cap overlying an atherosclerotic plaque in a coronary artery ruptures resulting in exposure of blood to the atherosclerotic material and consequent thrombus formation which occludes the artery.

The importance of genetic predisposition to MI/CHD is best documented by genome-wide association studies (GWAS) with more than 45 loci tagged by common variants identified so far.

However, there is ample evidence for „missing heritability“, which may result from currently not considered rare variants or gene-gene or gene-environment interactions.

We recently analyzed large-scale data after 1000G imputation (>16,000 cases and controls), from ExomeArray (>25,000 cases and controls), as well as whole exome-sequencing in unrelated cases and in myocardial infarction (MI) families.

We now have abundant preliminary evidence for multiple further loci with strong effects on CAD risk. As a proof-of-concept study, sequencing of a severely affected MI family revealed digenic mutations in GUCY1A3 and CCT7 (LOD 5.68), which cause a highly penetrant MI risk (25 affected individuals) by impaired sGC dependent NO signaling and accelerated thrombus formation.
Fetal programming – part of the heritability of cardiovascular disease?

The global burden of non-communicable diseases, including cardiovascular disease, is rapidly increasing. Much of the risk of these disorders is not explained by traditional risk factors and thus remains outside the scope of etiologically based prevention. The concept of Developmental Origins of Health and Disease (DOHaD) proposes that early life environmental adversities, e.g. materno-fetal metabolic disturbances and psychosocial stress, may, in part through changes in epigenomic patterns and gene expression, alter tissue and organ function, resulting in phenotypic differences, predisposing to later disease.

An accumulating body of evidence suggests that maternal CVD risk factors and lifestyle, before and during pregnancy, determine not only her own cardiovascular health but also the cardiovascular structural and functional development during fetal life and subsequently determine CVD risk of her offspring.

Numerous studies have identified prenatal life as a period of development when an organism is particularly vulnerable to environmental insults. Slow growth both during fetal life and infancy is associated with an increased risk of coronary heart disease and type 2 diabetes later in life. This early pattern of growth is associated with an increased disease risk especially when followed by a relative gain in body size later in childhood. This can lead to increased risk of metabolic disorders including insulin resistance, obesity and cardiovascular dysfunction.

Our extensive previous work, capitalizing on the Helsinki Birth Cohort Study (HBCS) has been in the world forefront in supporting the DOHaD concept. While previous findings have been of utmost importance in underlining that many common diseases have early life origins, a consensus within the DOHaD-field is that further epidemiological studies and follow-ups of the existing cohorts will not be sufficient to advance understanding of disease etiology and to develop novel ways to prevent disease. More focus is needed on underlying mechanisms explaining these associations. The DOHaD concept may provide a base to understand disease etiology and prevention better.
Lessons from the REFINE Reykjavik Study

Background…

The bulk of coronary heart disease (CHD) events (CE) occur in individuals who are calculated to have a moderate or low risk of suffering a CE, such as myocardial infarct (MI) in the next 10 years by conventional risk calculators, such as the European SCORE risk calculator. This is due to the fact that majority of the population belongs to that low or moderate riks fraction. It is a challenge to identify, at the population level those who are with low or moderate risk and who will suffer CE. Atherosclerosis is the underlying cause for most CE and can be detected by imaging, such as by CT of the coronary arteries for coronary artery calcium assessment or by ultrasound of the carotids for intima media thickness (IMT) or for a clearer manifestation of atherosclerosis by detection of arterial plaque. Atherosclerosis is a systemic disease and atherosclerosis in one arterial bed increases the likelihood of atherosclerosis in another vascular bed. For this reason, manifest atherosclerosis in the carotid arteries has been used as a surrogate marker for coronary atherosclerosis. By detecting atherosclerosis in an individual who is calculated by conventional risk assessment to have a low or moderate risk it is possible to reclassify that individual into a higher category of risk of CHD and focus on addressing his or her modifyable risk factors in a more aggressive way than previously.

The REFINE Reykjavik study

The Icelandic Heart Association set out to address the use of carotid ultrasound in an attempt to develop methods to detect subclinical atherosclerosis to reclassify the individual’s risk of atherosclerosis and development of CHD. In this study called REFINE Reykjavik study, more than 7000 individuals, men and women between the age of 25 and 70 years were recruited by random selection from the national rostrum between 2006 and 2012. In addition to measurement and assessment of conventional risk factors for CHD they all underwent an ultrasound of the carotid arteries for the assessment of IMT and plaque. The results from this study will be described.
Fluid biomarkers in Alzheimer’s disease – current concepts

The diagnostic guidelines of Alzheimer’s disease (AD) have recently been updated to include brain imaging and cerebrospinal fluid (CSF) biomarkers, which will increase the certainty whether a patient has an ongoing AD neuropathologic process or not. The CSF biomarkers total tau (T-tau), hyperphosphorylated tau (P-tau) and the 42 amino acid isoform of amyloid β (Aβ42) reflect the core pathologic features of AD, which are neuronal loss, intracellular neurofibrillary tangles and extracellular senile plaques. Since the pathologic processes of AD start decades before the first symptoms are noticed, measurement of these biomarkers may provide means of early disease detection. The updated guidelines identify three different stages of AD: preclinical AD, MCI due to AD and AD with dementia. I will aim to summarize the CSF biomarker data available for each of these stages. I will also present the results from blood biomarker studies. In summary, the core AD CSF biomarkers have a relatively high diagnostic accuracy and can also with relatively high sensitivity and specificity predict incipient AD in patients with mild cognitive impairment. Longitudinal studies on healthy elderly and recent cross-sectional studies on patients with dominantly inherited AD mutations have also found biomarker changes in cognitively normal at-risk individuals. This will be important if disease-modifying treatment becomes available, given that treatment will probably be most effective early in the disease. An important prerequisite for this is trustworthy analyses. Since measurements vary between studies and laboratories, standardization of analytical as well as pre-analytical procedures will be essential. This process is already initiated. Apart from filling diagnostic roles, biomarkers may also be utilized for prognosis, disease progression, development of new treatments, monitoring treatment effects and for increasing the knowledge about pathologic processes coupled to the disease. Hence, the search for new biomarkers continues. Several candidate biomarkers have been found in CSF, and although biomarkers in blood have been harder to find, some recent studies have presented encouraging results. But before drawing any major conclusions, these results need to be verified in independent studies.
**Vascular structure and function as imaging biomarker: Beyond hypertension**

According to the *Biomarkers Definition Working Group* of the *National Institute of Health* (NIH), a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.” Parameters of vascular structure and function, accessible to non-invasive evaluation, such as carotid intima-media thickness (cIMT) or aortic stiffness (carotid-femoral pulse wave velocity, cfPWV) are “imaging” biomarkers. A surrogate endpoint is a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiological, or other scientific evidence. In order for a biomarker to be considered as a “surrogate endpoint” of CV events, several steps should be completed, according to international guidelines.

In the case of arterial stiffness, the first four steps have already been completed. Step 1 - Proof of concept: Do novel marker levels differ between subjects with and without outcome? Yes, since several diseases have been associated with an increase in arterial stiffness. Step 2 - Prospective validation: Does the novel marker predict development of future outcomes in a prospective cohort or nested case-cohort study? Yes, since the predictive value of arterial stiffness has been largely demonstrated. Step 3 - Incremental value: Does the novel marker add predictive information to established, standard risk markers? Yes, since the independent predictive value of aortic stiffness has been demonstrated after adjustment to classical cardiovascular risk factors, including brachial PP. This indicates that aortic stiffness has a better predictive value than each of classical risk factors. Step 4 - Clinical utility: Does the novel risk marker change predicted risk sufficiently to change recommended therapy? Yes, since at least three studies showed that patients at intermediate risk could be reclassified into a higher or a lower CV risk, when arterial stiffness was measured. Step 5 - Clinical outcomes: Does use of the novel risk marker improve clinical outcomes, especially when tested in a randomized clinical trial? No study has yet been done, although it is crucial to determine whether a reduction in arterial stiffness is a desirable therapeutic goal in terms of hard clinical endpoints such as morbidity, mortality. This is the objective of the SPARTE study (*Laurent et al. Hypertension 2012*). Step 6 - Cost-effectiveness:
does use of the novel risk marker improve clinical outcomes sufficiently to justify the additional costs? This question remains unanswered.

In the case of carotid IMT, only three steps have been completed, among six. Step 1 - Proof of concept: Yes, several diseases have been associated with an increased cIMT. Step 2 - Prospective validation: Yes, the predictive value of cIMT has been largely demonstrated. Step 3 - Incremental value: Yes, the independent predictive value of cIMT has been demonstrated after adjustment to classical cardiovascular risk factors, although eth additive value was quite limited. Step 4 - Clinical utility: No direct study has yet been done, as indicated above. Indirectly, in a meta-analysis of 41 randomized trials, the reduction in cIMT was not reported to be associated with a reduction in CV morbidity-mortality. We will analyse the various reasons why (a) cIMT is lacking ability to reclassify individuals in higher risk group, (b) cIMT regression is lacking predictive value for the reduction in CV events, and (c) cIMT progression is lacking predictive value for CV events, and focus on methodological issues. Particularly, the precision for measuring cIMT and detection of carotid plaque will be compared.

In conclusion, increased aortic stiffness and cIMT, which reflect subclinical organ damage, can be considered as “imaging biomarkers”. Their measurement is recommended by the 2007 and the 2013 ESH-ESC Guidelines for the management of hypertension. However, some levels of evidence are low for cIMT measured with classical imaging systems. Particularly, cIMT may NOT be useful for the risk stratification of individuals in the general population. cIMT should be accurately measured using high resolution echotracking (ET) systems (ET-IMT). PWV and ET-IMT should be measured in outcome trials, in order to demonstrate that they are true surrogate endpoints.
What can we learn from Mendelian randomisation about causal inference?

The field of epidemiology has struggled to make headway in determining whether exposures are causal factors for complex diseases, including cardiovascular disease. This is largely because of the problems of confounding, reverse causation, and bias. To overcome the problems inherent in observational studies, many epidemiologists are now using genetic variants as proxies for exposures. The idea is to exploit genetic variants that influence exposure propensity or are involved in metabolism, transport, or cellular uptake of the exposure or are otherwise associated with exposure levels. The advantage of using genetic variants is that because of the random assignment of alleles with respect to subsequent lifestyle factors, they are considered independent of factors that may confound epidemiological studies. In recent years, findings based on several thousand participants and using genetic variants which are robustly associated with exposures of interest, have provided strong evidence that; alcohol intake is positively and causally associated with blood pressure, that the interleukin-6 pathway is a causal related to heart disease risk and that, contrary to expectation, CRP and HDL-cholesterol are unlikely to play causal roles in the disease. Mendelian randomization is not without limitations, such as lack of suitable variants, pleiotropy and confounding by population structure. However, thanks to recent advances in genetics it is now possible to overcome many of these problems.
Integration of genetics and plasma biomarkers for understanding of cardiovascular disease

During the last years the knowledge of genetic determinants of cardiometabolic disease in general has exploded thanks to the success of genome wide association studies. Here a number of examples of how the genetic discoveries can be taken forward to highlight novel pathophysiological mechanisms, as well as drug and life-style modifiable targets of cardiometabolic disease, will be presented. In this context, there will be particular emphasis on the interface between diabetes and cardiovascular disease. From a methodological point of view, a blend consisting of Mendelian Randomization studies and experimental interventions in both animals and humans is utilized. Recent data on four systems of potential importance in cardiometabolic disease will be shown; those being sortillin-1/neurotensin, natriuretic peptides, vasopressin and specific metabolomics signatures along with preliminary data on how to potentially modify these systems by life style and drugs.
New guidelines in cardiovascular prevention

During the period 2011 to 2013 a number of important international guidelines for cardiovascular prevention have been presented. The first was the ESC-EAS guidelines on treatment of dyslipidaemia (2011) [1] to be followed by the Joint European guidelines on cardiovascular prevention, headed by the ESC in collaboration with eight other societies (2012) [2]. In the US the American Diabetes Association (ADA) presented its “Standard of medical care” in early 2013 with recommendations for the treatment of patients with type 2 diabetes and risk factor control [3]. A thorough meta-analysis of the effects of statins in primary prevention of cardiovascular disease was presented as a Cochrane meta-analysis in early 2013 [4], concluding that statin therapy might even reduce total mortality by a relative risk reduction of 14 percent.

New guidelines are awaited in the near future. In June 2013 the new ESH-ESC Guidelines on arterial hypertension will be presented at the ESH 23rd Meeting in Milan, Italy, and in September will be presented the new ESC-EASD Guidelines on risk factor control in diabetes, first at the ESC meeting in Amsterdam and soon after at the EASD meeting in Barcelona. Finally, the revised hypertension guidelines in the US will probably be presented by the end of 2013, the so called Joint National Committee (JNC-8) document. This is said to focus on a few questions related to the treatment of hypertension and will not provide a full set of guidelines.

In general, the trend in all these new guidelines is to analyse absolute cardiovascular risk as a basis for decision-making on treatment, but also to use a more individualized strategy for defining treatment goals in the individual patient when background factor, co-morbidities, tolerability and acceptance are of increasing importance. For hypertension, the new technical interventions such as renal nerve ablation and baroreceptor stimulation are supposed to become more widely used. The World Health Organisation (WHO) has recently declared hypertension to be the most important risk factor globally.

References
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Risk Prediction in Hypertensive Patients

Traditional cardiovascular (CV) risk factors are inadequate to predict CV events in individuals. This is due to the fact that development of CV disease from CV risk factors takes several years and is dependent on the susceptibility of the individual subject for the harmful effects of the CV risk factors. This makes targeted primary prevention difficult. Subclinical target organ damage (TOD), which predicts CV death independently of SCORE, might be a good marker for individual risk factor susceptibility. However, measuring TOD in unselected subjects does not improve individual risk prediction significantly compared to SCORE alone. Therefore, we need more research on the additive prognostic value of TOD in different groups of subjects/patients as well as more information on the possible additive prognostic information of measuring different markers of TOD together. Therefore, ESH has in corporation with ESH Centre of Excellence initiated GIRO-TOPIC: Importance of Genes and Interactions of Risk Factors for Target Organ Damage and Prognosis in a Prospective Intervention Cohort. The multicenter study started last fall but due to lack of funding inclusion of centres and patients has been very slow.
Gene-environment interactions in type 2 diabetes and cardiovascular disease: genetic biomarkers defined by environment?

Although type 2 diabetes (T2D) and cardiovascular disease (CVD) are known to result from interplay between genetic predisposition and unfavorable environment, very little is known about such interactions. During the recent years GWAS studies have learned us a lot about common variants that increase the risk for T2D, CVD and related traits, however only a minor part of heritability of these diseases and traits can be explained by the so far identified genetic variants. We hypothesize that part of the unexplained heritability is hidden in interactions between genetic factors and environment, in particular diet. To test this hypothesis we are studying the population based prospective Malmö Diet and Cancer Study (MDCS) of ~30,000 individuals with diet data collected at baseline (1991-1996) using a modified diet history method including an extensive food frequency questionnaire, a 7-day food diary and an interview, and with 2,860 incident T2D and 2,921 CVD cases recorded during a mean follow up period of 14 years. Our studies so far indicate that our genetic make-up modifies how environmental factors affect our susceptibility to obesity, T2D and CVD i.e. that environmental factors modify (accentuate or diminish) the genetic susceptibility.
Biomarkers in the beta-cell and diabetes development

Type 2 diabetes (T2D) is an escalating health problem of enormous proportions. Current clinical management and treatment are insufficient and are not able to prevent the complications in kidneys, eyes and the cardiovascular system. T2D results from a combination of defective insulin secretion from the pancreatic beta-cells and insulin resistance of target cells. The clinical manifestation of T2D is highly variable, which is likely to reflect clinical subgroups with different pathophysiology. Today we have limited knowledge of the characteristics of T2D subgroups and the underlying disease mechanisms, which is a severe hindrance to more effective treatment of the disease. Here I will present data on genetic and serum biomarkers for T2D that have been coupled to specific disease mechanisms and discuss how this information may enable more targeted interventions to T2D.

In 2010 we identified a risk variant for T2D in the *ADRA2A* gene (rs553668), which leads to overexpression of the alpha2A-adrenergic receptor, increased adrenergic tone, and reduced glucose-stimulated insulin secretion (Rosengren et al., Science 2010). Interestingly, the ADRA2A antagonist yohimbine normalized the defective insulin secretion in human pancreatic islets from risk allele carriers for rs553668. We have recently translated these findings to clinical settings by conducting a double-blind randomized trial in which T2D patients have received yohimbine or placebo followed by an OGTT. The study represents one of the first attempts to personalized treatment for T2D based on genotype. We will discuss the results of this study, which was completed in March 2013.

We have also used a network-based approach to explore the pathophysiology of T2D by analysing gene co-expression networks in donated human pancreatic islets. SFRP4, which encodes secreted frizzled-related protein 4, was identified as a highly connected “hub” gene that is overexpressed in T2D (Mahdi et al., Cell Metabolism, 2012). Release of SFRP4 from islets was stimulated by interleukin-1beta, and elevated SFRP4 caused reduced expression of Ca2+ channels and suppressed insulin secretion. SFRP4 silencing with siRNA improved insulin secretion in vitro. Interestingly, SFRP4 was increased in serum from T2D patients several years before diagnosis (OR 3.32), suggesting that the protein could be a potential biomarker for T2D. SFRP4 provides a previously unknown link between inflammation and islet dysfunction.
Can we use genetics to predict treatment response?

Despite availability of many effective antihypertensive drugs, only about 40% of treated hypertensive has their blood pressure (BP) controlled to target. Pharmacotherapy is the mainstay of HTN management, but initial therapy is often selected empirically, and low response rates to any particular antihypertensive drug suggest the current approach to therapy selection and hypertension management is not optimal. Five drug classes are the main first line agents for HTN, but response rates to any given drug are only about 50%. This lecture will discuss the following areas in predicting antihypertensive treatment response – common or rare variants? Personalisation or stratification? New pathways to new drugs and early results from the NORDIL study.
The Asklepios study on vascular ageing

The ASKLEPIOS study consists of a representative, random population sample of >2500 apparently healthy Belgian subjects, examined initially in 2002-2004 and currently undergoing re-examination after a 10-year interval. The study has a medium-term goal of providing better understanding into the interplay between ageing, hemodynamics (and non-hemodynamic stresses) on the development of cardiovascular disease. The long-term goal is to focus on better risk prevention strategies.

One of the important research foci has been to study the parallels in cardiac and vascular ageing/stiffening. Stiffening of the arterial tree occurs in parallel to cardiac stiffening and remodeling. This parallel stiffening has potentially major implications as it simultaneously limits the reserve of the arterial tree (exacerbating the hypertensive response to exercise) and the cardiac reserve to effectively cope with this increase in afterload (leading to elevated filling pressures). This mutually deleterious interaction underscores the importance of considering the integral interrelationship between the heart and the peripheral vasculature and the feasibility of assessing ventricular-vascular interactions noninvasively. Better characterization and understanding of these heart-vessel interactions is possible with time domain analyses using time-resolved left ventricular wall dimensions (and derived wall-stress), and time-resolved pressure and flow, derived from arterial tonometry, Doppler echocardiography, and speckle tracking echocardiography. Using these techniques, it becomes apparent that different arterial properties have selective effects on time-resolved ejection-phase myocardial wall stress, which are not apparent from single-time point measurements.
Hypertension: is there a role for hypertonicity in the interstitium?

Evidence from genetically targeted mice

Renal control of blood composition by urinary electrolyte and water excretion is considered sufficient for maintaining the internal environment of the interstitial space. Recent evidence, however, suggests that the interstitium of the skin comprises a separate, locally-regulated compartment, where cells of the mononuclear phagocyte system (MPS) sense local interstitial hypertonicity and actively modulate the internal environment by expressing vascular endothelial growth factor C (VEGF-C) in response to local osmotic stress. Interfering with this MPS/VEGF-C-driven homeostatic response results in salt-sensitive hypertension. Whether MPS cells exert their blood pressure regulatory activity via VEGF-C/VEGFR-2-mediated increases in eNOS expression, or whether VEGF-C/VEGFR-3-driven hyperplasia of the skin lymph capillary network is mechanistically involved is unclear. We show that selective blockade of MPS-driven VEGF-C/VEGFR-3-mediated lymph capillary hyperplasia in the skin results in salt-sensitive hypertension, despite increased eNOS expression via the intact VEGF-C/VEGFR-2 regulatory pathway. This salt-sensitive hypertension in response to experimental blockade of physiologic lymph capillary hyperplasia, was paralleled by increased Cl⁻ retention in the skin. We conclude that MPS cells deploy homeostatic and blood pressure regulatory activity in response to local hypertonic electrolyte accumulation in the skin. The immune cells apparently organize local interstitial Cl⁻ clearance from the skin via VEGF-C/VEGFR-3-driven hyperplasia of cutaneous lymph vessels.

Asleep at the switch?

While recent experiments in animals provide consistent evidence that considerable amounts of Na⁺ and Cl⁻ can be stored in skin and muscle and modify immune function, the physiological interpretation of these findings is controversial. Questions such as how extracellular sodium ions may escape equilibrium or how intracellular sodium concentrations could increase without deleterious effects on membrane potential are unanswered.

In addition to these basic research questions, a burning question for clinician-scientists is whether this storage phenomenon is an animal-research curiosity or whether it exists in humans. To address this question, we have implemented 23NaMRI technology to quantitatively and noninvasively visualize Na⁺ reservoirs in humans. We find Na⁺
storage in muscle and skin, which increases with age, is more pronounced in men than in women and is directly associated with blood pressure levels. This association leads to the hypothesis that tissue Na\(^+\) storage characterizes a disruption of internal environment composition, which may be causally linked to essential hypertension.

The emerging concept of Na\(^+\) storage opens new questions for basic researchers and clinician-scientists. Some straightforward clinical questions are whether humans with increased Na\(^+\) storage are at risk for developing cardiovascular disease and whether tissue Na\(^+\) content can be modified by life-style changes or medical treatment. Seeing the Na\(^+\) in humans is a new conceptual approach to provide answers.
Cardiovascular gene therapy – an update 2013

Therapeutic angiogenesis is a potentially useful therapeutic strategy for ischemic heart disease or peripheral arterial occlusive disease. Arteriogenesis is a process caused by increased sheer stress at the arteriolar level resulting in the formation of large conduit vessels from pre-existing small vessels. Angiogenesis involves growth and sprouting of capillaries and smaller vessels in ischemic tissues. Most commonly used growth factors for therapeutic angiogenesis are vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF). Some other cytokines and growth factors may also have angiogenic effects in vivo. Improved perfusion can be achieved by angiogenesis and arteriogenesis. However, best delivery methods and growth factors causing potentially useful effects in man remain to be determined.

References:


### Poster Abstracts

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

Aims
Inflammatory diseases and inflammatory markers secreted from the liver, including C-reactive protein (CRP) and ceruloplasmin, have been associated with incident atrial fibrillation. Genetic studies have not supported a causal relationship of CRP with atrial fibrillation, but have not studied ceruloplasmin.

The purpose of this Mendelian Randomization study was to explore whether genetic polymorphisms in the gene encoding ceruloplasmin are associated with elevated ceruloplasmin levels, and whether such genetic polymorphisms also are associated with incidence of atrial fibrillation.

Methods & results
Genetic polymorphisms in the Ceruloplasmin gene (CP) were genotyped in a population-based cohort study of men from Southern Sweden (Malmö Preventive Project, n=3900). Genetic polymorphisms associated with plasma ceruloplasmin concentration were also tested for association with incident AF (n=520) during a mean follow-up of 29 years in the same cohort. Findings were replicated in an independent case-control sample (The Malmö atrial fibrillation cohort, n=2247 cases, 2208 controls).

A single nucleotide polymorphism (SNP) (rs11708215, minor allele frequency 0.12) located in the CP-gene promoter was strongly associated with increased levels of plasma ceruloplasmin (p=9x10^-10) and with atrial fibrillation in both the discovery cohort (hazard ratio=1.24 per risk allele, 95% confidence interval=1.06-1.44, p=0.006) and the replication cohort (odds ratio=1.13, 95% CI=1.02-1.26, p=0.02).

Conclusions
Our findings indicate a causal role of ceruloplasmin in AF pathophysiology, and suggest that ceruloplasmin might be a mediator in a specific inflammatory pathway that causally links inflammatory diseases and incidence of atrial fibrillation.
The non-hemodynamic component of arterial stiffness – epidemiology of vascular ageing

Objective
Arterial stiffness is a marker of vascular ageing and a predictor of cardiovascular events. Carotid-femoral Pulse Wave Velocity (c-f PWV) is the gold standard for measuring arterial stiffness. The aim of this observational study was to determine non-hemodynamic factors, with focus on glucose and lipid metabolism, associated with arterial stiffness in an elderly population.

Design and Method
In all, 3056 subjects (mean age 72 years, 39.5% men) were included and examined between 2007 and 2012 in a cross-sectional, population-based cohort study in Malmö, Sweden. c-f PWV was measured with Sphygmocor® and adjusted for heart rate (HR) and mean arterial pressure (MAP). Oral glucose tolerance test (OGTT; 75 g glucose) was performed with measurement of fasting and 2-h plasma glucose.

Results
Mean c-f PWV was 10.5 m/s. In univariate analysis correlations were found between c-f PWV and both fasting glucose (r=0.19, p<0.001) and 2-h glucose (r=0.24, p<0.001). c-f PWV was independently associated with age, male sex, triglycerides, waist to hip ratio and impaired glucose metabolism in multiple regression analysis (Table) after adjustment for HR and MAP. Low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol were not independently associated with PWV.
**Table**: Determinants of c-f PWV, stratified for sex. β-coefficients are standardized.

<table>
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<tr>
<th></th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>0.01</td>
<td>0.62</td>
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</table>

**Conclusion**

Arterial stiffness is independently associated with age and markers of disturbed glucose and lipid metabolism as well as abdominal obesity, adjusted for HR and MAP. This described the non-hemodynamic components of vascular ageing (increased c-f PWV). Gender differences were minor in this elderly cohort including postmenopausal women.
Reduced forced expiratory volume is associated with increased incidence of atrial fibrillation- the Malmö Preventive Project

Aims

Reduced forced expiratory volume in one second (FEV$_1$) and forced vital capacity (FVC) have been associated with increased incidence of cardiovascular diseases. However, whether reduced lung function is also a risk factor for atrial fibrillation (AF) is still unclear. We aimed to determine whether lung function predicted AF in the Malmö Preventive Project (MPP), a large population-based cohort with a long follow-up.

Methods and Results

The study population consisted of 7,674 women and 21,070 men, mean age 44.6 years. The cohort was followed on average for 24.8 years, during which time 2669 patients were hospitalized due to AF. The incidence of AF in relationship to quartiles of FEV$_1$ and FVC and per litre decrease at baseline was determined using a Cox proportional hazards model adjusted for age, height, weight, current smoking status, systolic blood pressure, erythrocyte sedimentation rate and fasting blood glucose. FEV$_1$ was inversely related to incidence of AF (per litre reduction in FEV$_1$) hazard ratio (HR): 1.39 (95% confidence interval (CI): 1.16-1.68; p=0.001) for women, and HR: 1.20 (95% CI: 1.13-1.29; p< 0.0001) for men. FVC was also inversely related to incidence of AF (per litre reduction in FVC) HR: 1.20 (95% CI: 1.03-1.41; p= 0.020) for women, and HR: 1.08 (95% CI: 1.02-1.14; p= 0.01) for men. This relationship was consistent in non-smokers as well as smokers, and among individuals younger than the median age of 45.8 years or normotensive subjects.

Conclusion

Impaired lung function is an independent predictor of AF. This may explain some risk of AF that is currently unaccounted for.
Addition of albumin/creatinine ratio to the continuous metabolic syndrome score improves discrimination of cardiovascular outcomes: the Czech post-MONICA Study

Objective – We evaluated the incremental yield of continuous metabolic syndrome score enriched with albumin/creatinine ratio (cMetSA score) compared with the validated continuous metabolic syndrome score (cMetS score) in cardiovascular outcomes (CVO) risk discrimination.

Research design and methods – We performed a cross-sectional survey of a representative Czech population random sample (n=3612). Urinary albumin excretion was determined using immunoturbidimetry in a morning spot urine and albumin/creatinine ratio (ACR) was calculated. cMetS score was derived using principal components analysis (PCA) applied to waist circumference, ln-triglycerides, HDL cholesterol, mean arterial pressure and ln-glucose. Similarly, cMetSA score was derived using PCA applied to ACR in addition to established variables of metabolic syndrome (MetS). A self-reported history of CVO was obtained by a physician-administered questionnaire.

Results – When PCA was applied only to established variables of MetS, one principal component was extracted (variance explained 49.1%), whereas two principal components had been identified after the addition of ACR (variance explained 41.2% and 17.0%, respectively). In logistic regression, cMetSA score yielded a stronger multivariate adjusted association with CVO, OR 2.52 [95% CI, 1.71-3.73], as compared to cMetS score, OR 1.51 [95% CI, 1.23-1.85]. In receiver operating characteristic curve analysis, cMetSA score showed a significantly better discriminating power of CVO as compared to cMetS score (AUC of 0.738±0.018 vs. 0.714±0.018; p<0.005). cMetSA score led to a 5.7% net reclassification improvement (NRI) of CVO from cMetS score (p<0.05).

Conclusion – The continuous metabolic syndrome score enriched with albumin/creatinine ratio outperforms the validated continuous metabolic syndrome score in cardiovascular outcome discrimination.
suPAR and presence of carotid plaques is associated with coronary event and ischemic strokes

Background
The low-grade inflammation biomarker soluble urokinase plasminogen activator receptor (suPAR) is associated with cardiovascular disease (CVD) and carotid plaques. The aim of this study was to explore the relationship between suPAR, carotid plaques and incidence of coronary event (CE) and ischemic stroke, respectively, in a prospective population-based cohort study.

Methods
Baseline levels of suPAR was assessed in 5378 subjects (59 % women), mean age of 57 years, participating in the Malmö Diet and Cancer Study cardiovascular program during 1991 to 1994. Sex-specific quartiles of suPAR were computed and Cox regression analyses were performed.

Results
During a mean follow-up time of 15.2 years, 385 CE and 328 ischemic strokes occurred. In a CVD risk factor (age, sex, smoking, systolic blood pressure, low density lipoprotein, diabetes mellitus, high sensitive C-reactive protein, leukocytes, lipoprotein-associated phospholipase A2) adjusted analyses, the top quartile of suPAR was associated with both CE and ischemic stroke, the hazard ratio (HR) was 1.66 (95% CI 1.13-2.43) and 1.53 (1.05-2.23), respectively, compared to the bottom quartile. Those subjects with both elevated levels of suPAR and presence of carotid plaques have the highest increased risk for ischemic stroke with HR 2.39 (1.63-3.45) compared to subjects with low levels of suPAR and without carotid plaques, adjusted for CVD risk factors.

Conclusion
Elevated levels of suPAR are associated to increased incidence of both CE and ischemic stroke. Subjects with presence of both high levels of suPAR and carotid plaque have the highest risk for ischemic stroke.
Incidence of ischemic stroke and CAD in categories of high or low suPAR and presence of carotid plaque

<table>
<thead>
<tr>
<th>Low suPAR/No plaque</th>
<th>High suPAR/No plaque</th>
<th>Low suPAR/Plaque</th>
<th>High suPAR/Plaque</th>
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<tr>
<td>HR 1.0 n=2060 Stroke n=58 (2.8%)</td>
<td>1.51 (0.99-2.30) n=805</td>
<td>1.56 (1.08-2.25) n=1385</td>
<td>2.39 (1.63-3.45) n=880</td>
</tr>
<tr>
<td>HR 1.0 n=2059 CAD n=78 (3.8%)</td>
<td>1.23 (0.83-1.84) n=804</td>
<td>1.54 (1.12-2.12) n=1374</td>
<td>1.80 (1.26-2.57) n=854</td>
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**Note:** The values in the table are hazard ratios with 95% confidence intervals.
The metabolic syndrome across europe – different clusters of risk factors

Metabolic syndrome (MetS) remains a controversial entity. Specific clusters of MetS components – rather than MetS per se’- were associated with accelerated arterial ageing and with CV events. To investigate whether the distribution of the “risky” clusters of MetS components differed cross-culturally, we studied 34,821 subjects from 12 cohorts from 10 European countries and 1 from US participants in the MARE (Metabolic syndrome and Arteries REsearch) Consortium. In accordance with the ATP III criteria, MetS was defined as an alteration ≥3 of the following 5 components: elevated glucose (G): fasting glucose ≥110 mg/dl; low HDL cholesterol (H): <40 mg/dl for M or < 50 mg/dl for W; high triglycerides (T) ≥150 mg/dl; elevated BP (B): ≥130/≥85 mmHg; abdominal obesity (W): waist circumference > 102 cm for M or >88 cm for W.

MetS had a 24.3% prevalence (8468 subjects) (23.9% in men vs 24.6% in women, p<0.001) with an age-associated increase in its prevalence in all the cohorts. The age-adjusted prevalence of the clusters of MetS components previously associated with greater arterial and CV burden differed across countries (p< 0.0001) and in men and women (gender effect p<0.0001). In details, the cluster T-B-W was observed in 12% of the subjects with MetS, but was far more common in the cohorts from UK (32.3%), Sardinia in Italy (19.6%), and Germany (18.5%) and less prevalent in the cohorts from Sweden (1.2%), Spain (2.6%), and USA (2.5%). The cluster G-B-W accounted for 12.7% of subjects with MetS with higher occurrence in Southern Europe (Italy, Spain, and Portugal - with 31.4%, 18.4%, and 17.1% respectively) and in Belgium (20.4%), than in Northern Europe (Germany, Sweden, and Lithuania – with 7.6%, 9.4%, and 9.6% respectively).

The preliminary analysis of the distribution of MetS suggested a gradient of “risky” clusters of MetS components across European countries.