Redressing the balance

A critical lack of understanding of the links between pre-eclampsia and cardiovascular disease in women still remains. **Dr Ann-Charlotte Iversen** illustrates how her team is tackling this topic

Could you begin by offering an overview of your research objectives and what prompted this study?

Pre-eclampsia is a major cause of morbidity and mortality in pregnant women and no curative therapy exists, other than delivering the baby. Women with pre-eclamptic pregnancies have up to an eight-fold increased risk of cardiovascular disease (CVD) later in life. Similar inflammatory manifestations have been observed in pre-eclampsia and CVD – thus, pathogenic mechanisms are assumed to be shared. We believe pregnancy represents a stress-test to the vascular system and that pre-eclampsia may be one of the first clinically identifiable markers of a woman's increased risk of CVD.

This approach could be helpful in understanding the gender-associated mechanisms underlying CVD in women. The newly defined and central role of pattern recognition receptors in inflammation helps us understand the underlying inflammatory mechanisms in these diseases, and we aim to investigate the involvement of pattern recognition in pre-eclampsia and CVD, using several large and unique Norwegian biobanks.

What evidence led to the hypothesis that pre-eclampsia is one of the first clinically identifiable markers of a woman's increased risk of CVD?

The obvious link between pre-eclampsia and CVD is from epidemiologic studies showing a significantly increased risk for CVD later in life following a pre-eclamptic pregnancy. However, it is debated whether experiencing pre-eclampsia itself induces this enhanced CVD risk, or if the woman is constitutionally predisposed for both pre-eclampsia and CVD before her pregnancy. Pre-eclampsia and CVD also share risk factors, such as obesity, diabetes and metabolic syndrome, and knowledge from genetic and molecular studies of these inflammatory diseases clearly substantiates that at least some of the harmful inflammatory processes must be shared.

Molecular pattern recognition is a fairly new field of study. Could you tell us more about it?

New findings have revealed a common set of receptors, which recognise microbial components upon infection and initiate the immune response to defeat the microbes. These pattern recognition receptors are expressed by many cell types and their activation is crucial for initiation of immune activation, such as release of potent pro-inflammatory cytokines. In addition to recognising microbes, more recent findings have shown that the same receptors are activated by endogenous cell damage signals, such as dying cells, saturated fatty acids, stress-induced factors, and free DNA. This implies that pattern recognition triggers inflammation both in the presence and absence of infection, and thus is presumed to be involved in infectious and inflammatory diseases. Since the same set of receptors responds to microbial intruders and endogenous danger, pattern recognition could also explain why some infections have an enhancing effect on some inflammatory conditions.

Given that both pre-eclampsia and atherosclerosis/CVD are linked to genetics, has the search for early pre-eclampsia risk biomarkers yielded any promising results?

The ongoing search for early biomarkers for pre-eclampsia is complicated by the fact that it is a complex disease, with several subgroups which – due to the lack of good phenotype characterisation – are not easily separated. Several biomarkers have been suggested, among these angiogenic factors, but no single one with high impact.

Some of the inflammatory mediators common for both pre-eclampsia and CVD are pointed out as potential early biomarkers for CVD, but


© GEIR MOGEN/NTNU

are not yet properly assessed in pregnancy. The search for other biomarkers and better subgrouping of pre-eclampsia is ongoing: we believe our approach, based on a combined look at pre-eclampsia and CVD, is particularly promising.

Why is it important to focus on women in particular with respect to cardiovascular disease?

CVD has been extensively studied for many years, but focus has traditionally been set on men or the two sexes combined. We now see an alarming gender difference in CVD: the number of cardiovascular deaths has steadily declined in men, but the same rate of decline is not observed in women. Gender-associated differences are observed in both CVD risk profile and clinical course, but female-specific factors are yet to be elucidated. Importantly, inflammation appears to have a more fundamental role in female CVD pathogenesis. Pattern recognition is crucial for inflammation and our approach looking at underlying inflammatory mechanisms with a combined look at CVD and the female specific disease pre-eclampsia, provides an excellent setting in which to specifically address female CVD.

Pre-eclampsia and female heart disease

FP7-funded work at the **Norwegian University of Science and Technology**'s Faculty of Medicine could reveal the genetics and molecular mechanisms connecting hypertension and proteinuria in pregnancy to the increased likelihood of heart disease later in life

AROUND FIVE IN every 100 pregnant women will suffer from pre-eclampsia: the onset of hypertension – high blood pressure – and proteinuria during pregnancy. This is a dangerous condition, not only for the woman, but can also lead to health issues in the developing foetus, such as restricted growth and premature delivery. The up to eight-fold increase in likelihood that women who suffer from pre-eclampsia will also develop cardiovascular disease (CVD) later in life is poorly understood. It has been hypothesised that pre-eclampsia might, in fact, be a pre-cursor to CVD, indicating during the 'metabolic stress test' of pregnancy, the early warning signs of abnormal metabolic or inflammatory responses in women.

Dr Ann-Charlotte Iversen, of the Norwegian University of Science and Technology's Faculty of Medicine, whose work over the last 12 years has focused on the role of pattern recognition in immune activation and viral immune evasion, is ideally placed to take on this important issue. With her strong combined molecular and clinical research backgrounds, she believes work in this area is vital and carries with it specific challenges: "Pregnancy is a delicate setting which can complicate treatment, but all knowledge that helps us better understand the disease will help us pinpoint women at risk at an earlier stage, both in pregnancy and for their risk of CVD later in life".

NEW THERAPEUTIC APPROACHES

Presently, there is no curative treatment for pre-eclampsia, while CVD is also a health issue of global impact. But while the rate of CVD in men has been falling, the gender-specific elements of the condition in women are understudied. Also, there is not sufficient knowledge of pre-eclamptic pregnancies to properly subgroup the disease which may have multiple causes. Through her work, Iversen hopes to give a more comprehensive range of sub-categories of pre-eclampsia, with a more complex understanding of the condition's pathogenesis, to help facilitate treatments in the future. To arrive at such sub-categories, Iversen's functional studies are aiming to reveal how specific genes and molecules contribute in this process.

The potential of this combined genetic-function approach is one Iversen is clearly excited about: "We strongly believe our translational approach, using novel molecular knowledge to study the underlying harmful inflammation of pre-eclampsia and CVD in women, will provide a more solid understanding of what causes these diseases and how they are related". The datasets they will garner are hoped to bring promising insights into the mechanisms involved in pre-eclampsia and CVD pathogenesis, offering the possibility of new therapeutic approaches.

STUDY AND COMPARE

Towards that end, the project's primary aim is to study and compare inflammatory mechanisms associated with pre-eclampsia and CVD, in women specifically. One of the objectives with this, the evaluation of the contribution of pattern recognition receptors (PRRs), will be achieved through evaluating large cohorts of women with and without pre-eclampsia and/or CVD. It represents a novel approach to these conditions, as she explains: "Pattern recognition has provided an understanding of the similarities in the underlying mechanisms of pre-eclampsia and CVD, and addressing pre-eclampsia in a bigger picture – including the knowledge from CVD pathogenesis – and is an exciting approach". In both pre-eclampsia and CVD, endogenous danger signals from stressed and dying cells – those emanating from within the body – are known factors in the ongoing harmful inflammation.

Such danger signals might range from heat shock proteins, unsaturated fatty acids and oxidised lipids and proteins. Presently, though, their part in how these diseases play out is unknown. But with the advent of pattern recognition, an explanation is emerging; these danger signals may, in fact, initiate and enhance inflammation – systemic and local –

The datasets are hoped to bring promising insights into the mechanisms involved in PE and CVD pathogenesis, offering the possibility of new therapeutic approaches

in pre-eclampsia and CVD. This will take place through central processes like endothelial activation, release of pro-inflammatory cytokines, and recruitment and activation of immune cells. Iversen's team will compare these initiating signals and receptivity, as well as genotyping to assess genetic influence, in women with and without pre-eclampsia/CVD, with the level of inflammatory signals and activation. Local inflammatory responses at the maternal-foetal site will be studied, through placental/decidual tissues and *in vitro* studies of foetal trophoblasts.

These trophoblasts – the foetal cells of the placenta – have a key role to play. Molecular analyses have confirmed that they are much more immunogenic than previously thought, and could amplify harmful inflammation in pregnancy. "This is of particular interest," Iversen outlines, "since our study of molecular inflammatory mechanisms clearly identifies specific receptors as crucial for immune activation and inflammation." This knowledge could prove vital in their work establishing how the same mechanisms are at play in the placental tissue of pre-eclampsia and non-pre-eclampsia pregnancies.

UNIQUE BIOBANKS

Such multidisciplinary work requires the very latest techniques, and to that end leading-edge methodologies. Genome-wide association analysis has been used for assessing genetic predisposition, as well as targeted SNPlex analysis. Pro-inflammatory cytokine profiles are assessed by Multi-Plex, metabolic serum profiles through NMR analysis, and inflammatory mediators analysed with different ELISA-techniques. Molecular *in vitro* studies of placental cells and tissue involve various immunologic techniques, gene expression analysis and advanced molecular imaging, to define inflammatory receptors and activators involved pre-eclampsia development.

But one of the key aspects in her research has been the unique and extensive biobanks and health registries at her disposal. Managed by Professors Rigmor Austgulen and Line Bjørge, biologic materials and clinical information have been collected from both pre-eclamptic and non-pre-eclamptic pregnancies – The pre-eclampsia Biobank – as well as families with high occurrence of pre-eclampsia and CVD – The pre-eclampsia Family Biobank. Other contributory biobanks have been an early pregnancy biobank, and a large Norwegian national population-based health study – The HUNT Study. Use of valuable CVD-based biobanks in Western Norway is also planned through collaborations.

These biobanks aid the comparison of pre-eclamptic and non-pre-eclamptic pregnancies, using serum analysis of systemic inflammatory mediators and genetic predisposition analyses of DNA. Pregnancy biobanks have enhanced the knowledge of underlying molecular inflammatory pathways in placental and decidual tissues. "These invaluable collaborations and use of unique Norwegian biobanks is fundamental for our research," Iversen enthuses.

CRUCIAL COLLABORATION

The collaborators in this project come from a range of partners across Norway and internationally from the UK, U.S. and Australia. Professor Eric Moses from the University of Western Australia served as the basis for both completed and ongoing collaborative genetic population studies. At the La Jolla Institute for Allergy and Immunology in San Diego, Associate Professor Chris Benedict has been invaluable in researching molecular immunity as well as current studies of molecular mechanisms of inflammation. Furthermore, Iversen is part of the EU InterPregGen project, coordinated by Professor Linda Morgan at the University of Nottingham, which addresses genetic predisposition for pre-eclampsia and CVD in large population studies in Europe and Central Asia. Close collaboration with Professor Rigmor Austgulen at NTNU, Professor Line Bjørge at Haukeland University Hospital/University of Bergen, and Professor Terje Espevik at NTNU – as well as others in hospital departments and universities in Bergen and Trondheim – is at the core of her work. "The well-established collaboration between highly competent molecular and clinical research groups and hospital departments ensures solid knowledge – ranging from basic molecular research to clinical research on patient materials – underlies the entire project," she concludes.

INTELLIGENCE

MECHANISMS OF VASCULAR DISEASE IN WOMEN - INFLAMMATION IN PRE-ECLAMPSIA AND ATHEROSCLEROSIS

OBJECTIVES

The main aim is to study and compare inflammatory mechanisms associated with both pre-eclampsia and atherosclerosis in women.

KEY COLLABORATORS

Professor Rigmor Austgulen, Professor Terje Espevik, Professor Jan Kristian Damås, **Research Scientist Tone Frost Bathen**, **Professor Frank Skorpen, Professor Torstein Vik**, **Professor Siri Forsmo, Professor Pål Romundstad**, NTNU, Norway • **Professor Kjell Salvesen**, **Senior Consultant Svein-Arne Nordbø**, **Gynecology Consultant Merete Myklebost**, **Consultant Dr Med Eszter Vanky**, St Olavs Hospital, Trondheim University Hospital • **Professor Line Bjørge**, **Professor Ottar Nygård**, Haukeland University Hospital, Bergen • **Professor Grethe Tell**, University of Bergen • **Professor John-Anker Zwart**, Oslo University Hospital • **Professor Eric Moses**, University of Western Australia • **Associate Professor Chris Benedict**, La Jolla Institute for Allergy and Immunology, USA • **Professor Linda Morgan**, University of Nottingham, UK

FUNDING

The Research Council of Norway • The Central Norway Regional Health Authority • The Faculty of Medicine, NTNU • St Olavs Hospital, Trondheim University Hospital • European Commission Seventh Framework Programme (FP7)

CONTACT

Dr Ann-Charlotte Iversen
Project Leader

Department of Cancer Research and Molecular Medicine.
NTNU
Gastro Centre
Prinsesse Kristinas gate 1, N-7491 Trondheim
Norway

T +47 725 73305
E ann-charlotte.iversen@ntnu.no

ANN-CHARLOTTE IVERSEN has worked in the field of host-pathogen interactions, molecular immunology and inflammation research for more than 19 years. She received her PhD in Molecular Immunology at NTNU and worked as a postdoc at LIAI in San Diego. Currently, she serves as Research Scientist at NTNU and leads projects funded by the Research Council of Norway, the Central Norway Regional Health Authority and FP7.

NTNU – Trondheim
Norwegian University of Science and Technology