Developments in epidemiology and biomarker research

A life-course perspective

21-22 March 2019

Skåne University Hospital (SUS), Malmö, Sweden

Organisers:

The Strategic Research Area (SRA) Epidemiology for Health (EpiHealth) at Lund and Uppsala Universities in collaboration with OLINK Proteomics AB, Uppsala, Sweden

Welcome address!

Dear colleagues,

You are all very welcome to this 2-day symposium on *"Developments in epidemiology and biomarker research - A life-course perspective"*, jointly arranged by the Strategic Research Area (SRA) Epidemiology for Health (EpiHealth) at the Lund and Uppsala universities, and OLINK Proteomics AB, Uppsala, Sweden.

Our intention is to bring together researchers from various fields interested in new developments in, for example, biomarker technologies and applications. We will review several projects based on the use of panels of biomarkers for a better understanding of risk of disease and prognosis. In addition we will address important areas of modern epidemiology such as the influence of early life factors on adult health and disease, but also the importance of the gene-diet-microbiota interaction for body function and health. Finally, we will invite speakers to talk about aspects of personalized or stratified medicine based on systems biology approaches for better care and future drug development.

We hope for two days of important lectures and discussion, but also networking and a good social atmosphere. In fact, the symposium also celebrates the *10-year anniversary* of the SRA EpiHealth and its activities as supported by the Swedish Government and the Research Council of Sweden. We are proud to work together with the leading biotech company in Sweden for biomarker panel analyses in epidemiology, OLINK Proteomics AB, Uppsala, and its representatives!

Welcome to Malmö!

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Programme **Thursday 21st March**

09:00 Registration and coffee

10:00 **Welcome and introduction** (Peter M Nilsson, SRA EpiHealth, Malmö, and Hanna Mann, OLINK Proteomics AB, Uppsala)

10:10 **Session 1. Technological developments, Big Data, and bioinformatics**
Chair: Peter M Nilsson, Malmö

A Systems biology/Multi-omics strategy to understand biology and drive precision medicine (AnnaLotta Schiller, Uppsala) – 10 min
Big Data and cohort collaboration (Johan Sundström, Uppsala) – 20 min
Mendelian Randomization analysis to assess causality in proteomics studies (Tove Fall, Uppsala) – 20 min
Opportunities and challenges with data analysis in biomarker research (Lina Hultin Rosenberg, Uppsala) – 20 min

State-of-the-Art 1: Biomarkers in cardiovascular research - An update 2019 (Lars Wallentin, Uppsala) – 30 min

12:00-13:00 LUNCH and posters

13:00 **Session 2. Biomarker panel applications in cohorts**
Chair: Isabel Goncalves, Malmö

CVD biomarker panels in Uppsala cohorts and EpiHealth (Lars Lind, Uppsala) – 20 min
Biomarker discoveries in MDC (Yan Borne, Malmö) – 20 min
Biomarkers and drug development (Olle Melander, Malmö) – 20 min
Biomarkers for the study of inflammation and repair after myocardial infarction - the LundHeartgene study (Alexandru Schiopu, Malmö) – 20 min
Biomarker discoveries in MPP (Martin Magnusson, Malmö) – 20 min
Biomarkers in a cohort from South Africa (Amra Jujic, Malmö) – 20 min

15:00 COFFEE and posters

15:30-17:00 **Session 3: Early life programming of adult health and disease**
Chair: Anna Rignell-Hybom, Lund

Update on the importance of early life programming (Peter M Nilsson, Malmö) – 20 min
The Medical Birth Register of Sweden (Karin Källén, Lund) – 20 min
Parent-of-origin effects and fetal programming for risk of diabetes (Rashmi Prasad) – 20 min
Stereotypic immune system development in newborn children (Petter Brodin, Stockholm) – 20 min
Prematurity and adult health (Kristina Sundquist, Malmö) – 20 min

17:00-17:25 Selected poster presentations (short oral communication)
Chair: Peter M Nilsson

17:25 Summary (Peter M Nilsson)

17:30 End of symposium

18:00-20:30 Buffet dinner at CRC, Malmö (pre-registration is mandatory)

Friday 22nd March

08:30-10:00 **Session 4: Gene-Nutrition and microbiota interaction**
Chair: Ulf Riserus, Uppsala

Microbiota in cardiometabolic disease (Marju Ohro-Melander, Malmö) – 20 min
Diet-microbiota patterns in a population-based study (Louise Brunkwall, Malmö) – 20 min
Novel Metabolome and Proteome derived biomarkers of type 2 diabetes and their association with food and lifestyle (Rikard Landberg, Göteborg) – 20 min
Probiotics in Prevention of Allergy among Children - Insights from the ProPACT study (Melanie Rae Simpson, Trondheim) – 20 min

10:00-10:30 COFFEE and posters

10:30-12:00 **Session 5. Precision medicine and biomarker use**
Chair: Karl Michaelsson, Uppsala

How can molecular pathological epidemiology contribute to improved prevention and treatment of cancer? (Karin Jirström, Lund) – 20 min
The Danish experience (Michael H Olsen, Odense) – 20 min
Systematic cross-platform comparison of genetic associations across the human plasma proteome (Eleanor Wheeler, Cambridge) – 20 min
State-of-the-Art 2: Drug discoveries built on precision medicine (Mark Caulfield, London) – 30 min

12:20 End of symposium. Poster Prize. Summary and Farewell (Peter Nilsson)
12:30 Lunch, grab and go

Biomarker discoveries in the Malmo Diet Cancer (MDC) Study

Yan Borné Ph.D., senior researcher

Cardiovascular Epidemiology, Department of Clinical Sciences in Malmö, Lund University, Sweden

Background

Incidence of cardiovascular disease and the prognosis of the patients have improved dramatically during the last decades. However, cardiovascular disease (CVD) is still the main cause of death in Sweden. Proteomics is a novel approach to discover biomarkers for CVD and to gain better mechanistic understanding. Our purpose was to explore whether circulating levels of proteins at baseline are associated with future development of CVD in Malmö diet and cancer (MDC) cohort.

Methods

MDC is a population-based cohort from Malmö, Sweden with baseline examination from 1991 to1996. Fasting plasma samples and information about carotid ultrasound is available in a subcohort of 6103 individuals, aged 45-68 years at baseline. Plasma proteins have been analysed using the Olink CVD I and Oncology I panels. Cardiovascular events have been followed up until 2016 using national and local hospital records and death certificates.

Results

The studies from this cohort have identified several circulating proteins associated with incident coronary events, atrial fibrillation, stroke, heart failure and diabetes, as well as associations with cardiovascular risk factors. An overview of these studies will be presented at the seminar.

Conclusions

Proteomics conducted in MDC showed that a number of circulating proteins were associated with increased incidence of CVD in subjects from the general population. Studies of the plasma proteome is a feasible method of identifying new risk factors with a potential role in development of cardiovascular disease.

Food patterns in relation to prediabetes and gut microbiota in the Malmö Offspring Study

Ulrika Ericson[1], **Louise Brunkwall[1]**, Sophie Hellstrand[1], Peter M Nilsson[2] and Marju Orho-Melander[1]

[1]*Department of Clinical Sciences, Malmö, Diabetes and Cardiovascular disease, Genetic Epidemiology, Lund University, Sweden*

[2]*Department of Internal Medicine, Skåne University Hospital, Lund University, Malmö, Sweden*

Background Diet is a determinant of gut microbiota composition, and both diet and gut microbiota have been linked to metabolic disease. Few observational studies have examined overall food patterns (FP) in relation to gut microbiota.

Objective To identify data driven FPs and examine them in relation to prediabetes and gut microbiota composition.

Methods Data driven FPs were extracted using principal component analysis in 1726 men and women with dietary data and without diagnosed diabetes from the Malmö Offspring Study.

Gut (fecal) microbiota was analyzed by 16S sequencing V1-V3) and OTUs were assessed by matching the sequence to the GreenGenes database (v.13.8) using QIIME. OTUs with low abundance (<0.01%) or OTUs only occurring in < 3 individuals were removed. Normalization by CSS (cumulative sum scaling) was applied in R using the *metagenomicSeq* package. Classification of prediabetes was based on fasting glucose ≥6.0 and/or HbA1C ≥ 42 at baseline among participants without diabetes diagnosis. Logistic regression was used to investigate association between FPs and prediabetes. The general linear model was used to examine association between FPs and 65 bacterial genera.

Results We identified two FPs: the *health conscious* and the *sugar and high-fat dairy* patterns. Adherence to the *health conscious* pattern associated with lower risk of prediabetes, as well as with abundance of several gut bacterial genera, and most robustly with lower abundance of *Eubacterium* and with higher abundance of *Lachnospira*, as well as *Roseburia*, which per se associated with lower risk of prediabetes. The association between the *health conscious* food pattern and prediabetes was attenuated after adjustment for *Roseburia* and BMI.

Conclusions

Our findings suggest that an observed inverse association between a "health conscious" food pattern and prediabetes, may partly be mediated by BMI and gut microbiota, specifically by abundance of *Roseburia.*

Genetic analysis of blood pressure in over one million people identifies novel loci associated with blood pressure and risk of cardiovascular disease

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[1] William Harvey Research Institute, Queen Mary University of London, London, UK, [2] NIHR Cardiovascular Biomedical Research Centre, William Harvey Research Institute, Queen Mary University of London, London, UK, [3]Genomics England, Queen Mary University of London.

Background

Hypertension is the leading cause of cardiovascular morbidity and mortality worldwide. Blood pressure (BP) is a highly heritable complex trait yet previously identified variants explain ~3% of the trait variance. Here we describe our latest work in BP and pulse pressure. More recently we have begun investigating syndromic Mendelian forms of hypertension in the 100,000 Genomes Project.

Methods

For BP as a complex trait our discovery meta-analyses combined the UK Biobank cohort (N=458,577) with the International Consortium for Blood Pressure data (N=299,024) and analysed ~7 million common genetic variants with systolic & diastolic BP and pulse pressure. Our analytical strategy incorporates both a one-stage design with internal replication and a two-stage design with independent replication from the Million Veteran Program (N=220,520) and the Estonian Biobank (N=28,742), totalling over 1 million individuals overall, of European ancestry. We use data on disease events available from UK Biobank, based on self-reported health data, record linkage to Hospital Episode Statistics and mortality follow-up data, to assess cardiovascular risk. In the 100,000 Genomes Project we assessed the results from analysis of all syndromic forms of blood pressure.

Results

We detected and validated 535 novel loci, replicated 92 loci for the first time, and confirmed all 274 published loci. Conditional analysis identifies 163 additional independent genetic signals across all 901 BP loci. By comparing the upper and lower deciles of the genetic risk score, the combination of all blood pressure variants is associated with ~13mmHg higher systolic BP and odds of 3.34 and 1.52 for increased risk of hypertension and cardiovascular outcomes, respectively. In rare syndromic BP phenotypes it has been possible to use whole genome sequencing to identify genes for BP.

Conclusions

In over 1 million individuals the combination of 901 novel and known loci for BP more than triples the number of previously known loci, with now over 1,000 independent genetic signals overall. The percentage trait variance explained is doubled, with 27% of the estimated BP heritability now explained. We have now extended these insights to include syndromic forms of BP. Collectively these findings offer new biological insights into BP regulation and suggest potential repurposing drug candidates and provide new genetic support for known anti-hypertensive targets.

Mendelian Randomization analysis to assess causality in proteomics studies

Tove Fall, PhD, Associate Professor, senior researcher

Department for Medical Sciences, Molecular Epidemiology, Uppsala University, Sweden

In epidemiology, analytical observational study designs are used to study the association of exposures of interest as they occur in the population on risk of disease, often with the goal to identify causal risk factors. As experimental studies are often not possible to pursue for economical, ethical or practical issues, such study designs have been shown valuable in medical research and are common. However, a major challenge in making causal inference from such observational studies is that conventional observational epidemiological studies are often troubled with bias from reverse causation and confounding, and there are several instances where associations found in epidemiological studies could not be replicated in randomized controlled trials. Recently, advances in causal inference testing in epidemiologic designs has fueled the emergence of a set of methods called 'Mendelian Randomization' - an adaptation of instrumental variable analysis that is heavily used in econometrics (**Figure 1**).

Figure 1 In Mendelian Randomization analysis, a genetic instrument (Gx) is identified and can be used to assess the causal effects of an exposure (X) on an outcome (Y), even in the presence of unmeasured confounders (U)

In Mendelian Randomization studies, germline genetic variation associated with a change of the risk factors assessed for association with an outcome to be able to draw unconfounded causal conclusions. Because of the random assignment of genotypes at conception, different subgroups in the population will, on average, be subject different degrees of the risk factor. This "quasi-randomization" creates a natural design analogous to different experimental groups in a randomized controlled trial. In order for a genetic variant to be a valid instrument for a risk factor, it must fulfill the basic instrument variable assumptions, for which we and others have explored methods to detect and account for violations:[1,2]

I. The genetic variant must be associated with the risk factor
II. The genetic variant must not be associated with any confounding factors of the risk factor-outcome relationship
III. The genetic variant can only affect the outcome through the risk factor

The most challenging part of a Mendelian Randomization study is to select appropriate instruments. We have applied this methodology in a series of articles and I will highlight some examples on how we have approached the instrument selection for proteins such as tPA[3], Cystatin C[4] and lipoprotein lipase.

1 Burgess, S., Bowden, J., Fall, T., Ingelsson, E. & Thompson, S. G. Sensitivity Analyses for Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic Variants. *Epidemiology* **28**, 30-42, doi:10.1097/EDE.0000000000000559 (2017).

2 Swerdlow, D. I. *et al.* Selecting instruments for Mendelian randomization in the wake of genome-wide association studies. *Int J Epidemiol* **45**, 1600-1616, doi:10.1093/ije/dyw088 (2016).

3 Nowak, C. *et al.* Protein Biomarkers for Insulin Resistance and Type 2 Diabetes Risk in Two Large Community Cohorts. *Diabetes* **65**, 276-284, doi:10.2337/db15-0881 (2016).

4 van der Laan, S. W. *et al.* Cystatin C and Cardiovascular Disease: A Mendelian Randomization Study. *J Am Coll Cardiol* **68**, 934-945, doi:10.1016/j.jacc.2016.05.092 (2016).

Opportunities and challenges with data analysis in biomarker research

Lina Hultin Rosenberg, PhD,

Head of Data Science Olink Proteomics, Uppsala, Sweden

The goal of the Olink Data Science team is to give customers as much value as possible from their Olink data and to accelerate the process from data to useful results. We offer statistical support from the initial study design to statistical analysis and to follow-up discussion of results. During this presentation I will talk about the services we offer and give examples of the type of analysis we do. One very important goal is to identify the best design and analysis approach leading to powerful and efficient studies, and the best use of the customer´s samples.

I will cover several things to consider in the design of a study, like power calculations, normalization between plates and projects as well as aspects related to controlling the different sources of variation. For longitudinal studies the design needs to allow for future projects to be merged to older data, by the use of overlapping or bridging samples for example. Batch effects in Olink studies are mainly explained by a baseline shift and can therefore to a large extent be handled by adjusting the medians between plates and projects.

Further, I will explain the main normalization methods we recommend and show examples of normalization in a customer project. As an alternative to adjusting the signal prior to statistical modeling by normalization, one can include the plate and project as variables in the statistical model. Another important aspect in the planning of a study, is trying to collect as much information as possible about the samples. With more parameters collected for our samples we have a better chance to design a study enabling us to detect the protein changes of interest, and correct for the sources of variation we are not interested in. Both pre-analytical variables related to sample handling and variables such as sex, age, medication are important to collect as well as other clinical variables we believe can have strong impact on the protein levels. I will also mention how different statistical models can account for the various parameters collected for our samples.

How can molecular pathological epidemiology contribute to improved prevention and treatment of cancer?

Karin Jirström, M.D, PhD, Professor of Pathology

Division of Oncology and Pathology, Department of Clinical Sciences Lund, Lund University

Molecular pathological epidemiology (MPE) is a scientific field that was first described by Ogino *et al*. in 2010[1]. For more than a decade, my research group has studied the influence of genetic, anthropometric and lifestyle-related factors on cancer development, progression, and prognosis utilizing a unique research platform with tumours from incident cancer cases in the prospective population-based cohort Malmö Diet and Cancer. Main focus is gastrointestinal and urothelial cancer. Some findings of potential clinical relevance have emerged, but we have a great challenge ahead in integrating these findings into prospective clinical trials and, ultimately, novel strategies for improved personalized prevention and treatment of cancer.

Reference

1. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. J Natl Cancer Inst 2010;102:365-7.

Exploration of biomarkers for subclinical atherosclerosis in an African population using a proteomics chip targeted at inflammation and cardiovascular disease

Anna Dieden[1], M.Sc, Leone Malan[2], Ph.D, Catharina Mels[2], MD. PhD, Peter Nilsson[3], MD, Ph.D, **Amra Jujic**[3,4*], Ph.D, Martin Magnusson[3-5*], M.D., PhD

*Denotes equal last author contribution

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[4] *Department of Cardiology, Skåne University Hospital, Malmö, Sweden*

[5] *Wallenberg Center for Molecular Medicine, Lund University, Sweden*

Background

The evolving use of multiplex proteomic platforms provides an excellent tool for investigating associations between multiple proteins and vascular health. In this study, we evaluated the impact of a multiplex protein panel, on carotid intima-media thickness (cIMT) as a marker of subclinical atherosclerosis. We used a multiplex proteomic platform to identify possible associations between proteins and subclinical carotid atherosclerosis as measured by carotid ultrasound in an African population.

Methods

In the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study, 92 proteins from the Proseek Multiplex CVD III 96×96 (Olink Bioscience, Sweden) were analyzed in 378 participants (mean age 44.7±9.6 years, 50.6% women, 10.8% with known cardiovascular disease). Carotid ultrasound was performed for measurements of the carotid intima-media thickness (cIMT, mean 0.663±0.127 mm) and calculation of cross-sectional wall area (CSWA, mean 13.5±4.4mm2), a measure of target organ damage. Possible associations between the proteins, and cIMT and CSWA, respectively, were explored using linear regression models. A two-sided Bonferroni corrected P-value of 0.05/92=5.4x10-4 was considered statistically significant in the crude analysis.

Results

Of 18 proteins (1 standard deviation of change of ln-transformed values) that were Bonferroni-corrected ($p \leq 5.4x10-4$) significantly associated with cIMT and/or CWAS in crude analyses, the following remained significant after further adjustment for age, sex, waist circumference, systolic blood pressure, smoking and total cholesterol: growth-differentiation factor-15 (GDF15; β 0.017, p=0.050), E-selectin (SELE; β 0.019, p=0.017), carboxypeptidase A1 (CPA1; β 0.019, p=0.019), C-C motif chemokine 15 (CCL15; β 0.031, $p<0.001$), chitinase-3-like protein 1 (CHI3L1; β 0.021,

p=0.007), the hemoglobin scavenger receptor (CD163; β 0.021, p=0.008) and osteoprotegerin (OPG; β 0.022, p=0.004). As for target-organ damage defined by CSWA, SELE (β 0.459, p=0.018), CCL15 (β 0.398; p=0.032) and CD163 (β 0.541, p=0.005) showed multivariate adjusted significant associations. Further analyses revealed that several proteins also were associated with prevalence of hypertension in fully adjusted model (age, sex, waist circumference, smoking, estimated glomerular filtration rate and alcohol use): GDF15 (OR 1.94; CI95% 1.39-2.71, p<0.001); CPA1 (OR 1.31; CI95% 1.01-1.70, p=0.046); CHI3L1 (OR 1.58; CI95% 1.21-2.07, p=0.001); CD163 (OR 1.34; CI95% 1.04-1.73, p=0.024); and OPG (OR 1.45; CI95% 1.12-1.88, p=0.005). Each 1 SD elevation of GDF15 (β 0.49; p<0.001), SELE (β 0.28; p=0.003) CPA1 (β 0.20; p=0.034), and CCL15 (β 0.20; p=0.025) were also associated with arterial stiffness measured by pulse wave velocity in a fully adjusted model (age, sex, waist circumference, smoking, diabetes, systolic ambulatory blood pressure, and total cholesterol).

Conclusions

In an African population, we could confirm five proteins (GDF15, SELE, CHI3L1, CD163 and OPG) associated with vascular cIMT, but in addition identified two proteins (CPA1 and CCL15) with novel associations with cIMT and/or CSWA, and augmented pulse wave velocity.

The Swedish Medical Birth Register

Karin Källén, PhD, Professor

Clinical Sciences, Lund, Dep of Obstetrics and Gynecology, Lund University, Sweden

Background

The Swedish Medical Birth Register (MBR) is, especially in combination with the other health registers kept at the National Board of Health and Welfare (Sw. "Socialstyrelsen"; SoS), a goldmine for epidemiologic research in Sweden. The MBR was revised in 1998, and a new revision is heavily due. However, despite the fact that a revision is needed, the register is frequently used by researchers all over Sweden, and is also used to produce numerous reports from SoS - reports that are often commissioned by the Swedish government. The current speech will give a short overview of the planned revision of the MBR, and will also present highlights from four recent reports published by SoS (Socio-economic factors and maternal and child health during pregnancy and delivery, Congenital malformations and chromosomal abnormalities, 2018, Maternal complications after child birth, and Stillbirth in Sweden-an inventory and suggested interventions).

Methods

The highlights of four reports published between December 2016 and December 2018 will be presented. In all four publications, the MBR was linked with the patient register (PAR) and the cause of death register (DOR). For one report, data from Statistics Sweden were also used, and for another, data from the certain data collection on congenital malformations was used.

Results

1. Socio-economic factors have a substantial impact on maternal- and child perinatal health. Women born in Africa south of Sahara, and their children, have substantial increased risks for poor perinatal outcome when giving birth in Sweden. Women born in Middle East (and their children) were also at higher risk than Swedish women and their offspring, but far less pronounced than African women and their children. Maternal and paternal educational level also influenced the maternal- and child health, but the income level did not have any major impact on the selected outcomes.
2. Because of the expanding prenatal screening for chromosomal abnormalities, the rate of children born with Down syndrome has not increased in spite of the increased maternal age at child birth. In 2018, (data from 2016), the rate of children born with Down syndrome rapidly decreased.
3. Vaginal birth is associated with perinatal ruptures immediately at birth, and risk of anal- and urine incontinence, and prolapse, respectively, in the long run. Cesarean section, on the other hand, is associated with bleedings and infections immediately after birth, life-threatening conditions like placenta percreta or uterine rupture in subsequent deliveries, and abdominal hernias and adhesions in the long run. The complications after caesarean section are more, and are more severe than the complications after vaginal births. Thus, if no certain risk situation is present, jeopardizing maternal or fetal health, vaginal birth is to prefer.

4. Until some years ago, Sweden was one of the countries with the lowest stillbirth rate. Since then, the other Nordic countries have managed to lower their stillbirth rate, while the Swedish stillbirth rate has been stable. Now, Sweden has the highest stillbirth rate among the Nordic countries. Large regional differences indicate that more could be done to lower the stillbirth rate in Sweden.

Conclusions

The health registers kept by SoS are important tools for reproduction epidemiology in Sweden. The SoS publish numerous reports of public interest in the field of reproduction epidemiology.

Novel Metabolome and Proteome derived biomarkers of type 2 diabetes and their association with food and lifestyle

Lin Shi[1] Ph.D, Carl Brunius[1] Ph.D, Ingegerd Johansson[2] Ph.D, Ingvar Bergdahl[3,4], Olov Rolandsson[4] M.D., Kati Hanineva[5,6] Ph.D, Seppo Auriola[5] Ph.D, Carolina Donat Vargas[7], Hannu Kiviranta[8], Agneta Åkesson[7], Alicja Wolk[7] Ph.D, Karl Michäelsson[9] MD, **Rikard Landberg[1,3] Ph.D.**

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Background

Identification of individuals at risk of developing type 2 diabetes (T2D) is of great importance for prevention and early interventions. Metabolomics and proteomics are emerging tools to discover metabolic alterations before onset of disease, thereby potentially providing novel insights into disease pathophysiology and/or improving disease prediction. Our aim was to discover metabolite- and protein biomarkers that predict T2D risk in Swedish cohorts and investigate their association with food intakes.

Methods

For metabolomics, we established a nested case-control study within the Swedish prospective population-based VIP- cohort. We used untargeted LC-MS metabolomics and analyzed plasma samples from 421 case-control pairs. Moreover, 16 plasma persistent organic pollutants (POPs) were analyzed for a sub-population for which repeated samples were available. *For proteomics*, 276 proteins were analyzed by three OLINK panels in plasma samples from 4103 women, including 173 incident T2D and participants who were free of T2D after a median time of 10 follow-up years, from the Swedish Mammography Cohort Clinical study.

Results

We identified 46 predictive plasma metabolites of type 2 diabetes. We also identified 31 metabolites associated with fish intake and 46 metabolites associated with coffee intake. Integration of fish-related metabolites and POPs led to a unique metabolite component independent of plasma POPs, which showed inverse association with T2D risk at borderline significance (P=0.07). Biomarkers reflecting filtered coffee were inversely associated with T2D risk (P=0.04). We identified 24 proteins associated with T2D risk, independent of established T2D risk factors (including 15 novel associations). Optimal selection of protein biomarkers using machine learning significantly improved early risk prediction of T2D beyond the optimal use of established traditional risk factors. Furthermore, we identified food items associated with several of the proteins associated with T2D, after adjusting for other lifestyle factors.

Conclusions

Predictive metabolites may improve understanding of the pathophysiology of type 2 diabetes, but they provide limited incremental value in risk prediction beyond optimal use of traditional risk factors. Filtered coffee as well as fatty fish intake may be beneficial for T2D prevention. Our data suggest that protein biomarkers are useful for T2D risk prediction and identified diet-protein associations linked with T2D risk may provide targets for specific dietary prevention strategies.

CVD biomarker panels in the Uppsala cohorts and EpiHealth cohort

Lars Lind, Professor

Department of Medical Sciences, Clinical Epidemiology, Uppsala University, Sweden

We have in Uppsala applied OLINK proteomic analyses to study biomarkers and biological pathways for cardiovascular diseases (CVD) and CVD risk factors in several Uppsala-based cohort studies, like PIVUS, ULSAM, POEM and the EpiHealth cohort [1].

Using the first launched CVD-chip, we could link several new proteins to atherosclerosis in the carotid arteries in PIVUS. We thereafter published data on protein biomarkers and ischemic stroke and atrial fibrillation using both PIVUS and ULSAM in a discovery/validation approach. We have also recently found novel proteins being linked to endothelial function in PIVUS and POEM.
Using different combinations of samples, we have published and unpublished data on associations between protein biomarkers and CVD risk factors, such as diabetes, dyslipidemia, smoking, blood pressure, and obesity.
We are currently performing a meta-analysis of 7 studies on protein biomarkers and atherosclerosis involving >11,000 individuals.

During the last year, we have analyzed the currently manufactured CVD 2 and CVD 3-chips in the EpiHealth cohort (n=2,500) and the national SCAPIS studies (n=5,000). A number of novel proteins biomarkers for diabetes have been discovered in the EpiHealth cohort.

In conclusion, multiplex proteomics analysis is a useful tool to discover novel biomarkers and biological pathways for cardiovascular diseases.

Reference

1. Lind L, Elmståhl S, Bergman E, Englund M, Lindberg E, Michaelsson K, Nilsson PM, Sundström J. EpiHealth: a large population-based cohort study for investigation of gene-lifestyle interactions in the pathogenesis of common diseases. *Eur J Epidemiol*. 2013; 28(2):189-97.

Galectin-4 bridging the gap in cardiometabolic disease predicting diabetes, coronary events and mortality in a Swedish population cohort

Martin Magnusson. MD, PhD, Associate Professor, Senior Consultant Cardiologist

Department of Clinical Sciences, Lund University, Clinical Research Center, Department of Cardiology, Skåne University Hospital Malmö, Sweden, and *Wallenberg Center for Molecular Medicine, Lund University*

Multiplex proteomic platforms provide excellent tools for investigating associations between multiple proteins and disease (e.g., diabetes) with possible prognostic, diagnostic, and therapeutic implications. In this study our aim was to explore novel pathophysiological pathways by examining 92 proteins and their association with incident diabetes in a population-based cohort (146 cases of diabetes versus 880 controls) followed over 8 years. After adjusting for traditional risk factors, we identified seven proteins associated with incident diabetes. Four proteins (*Scavenger receptor cysteine rich type 1 protein M130, Fatty acid binding protein 4, Plasminogen activator inhibitor 1* and *Insulin-like growth factor-binding protein 2*) had a previously established association with incident diabetes and 3 proteins (*Cathepsin D, Galectin-4, Paraoxonase type 3*) with a novel association with incident diabetes. Of the proteins with a novel association, *Galectin-4,* with an increased risk of diabetes, and *Paraoxonase type 3,* with a decreased risk of diabetes, remained significantly associated with incident diabetes after adjusting for plasma glucose, implying a glucose independent association with diabetes (1).

When further investigating associations between the seven diabetes associated proteins and risk of cardiac disease and mortality we found *Galectin-4* to be significantly associated with increased risk of incident major adverse coronary events, incident heart failure as well as cardiovascular- and all-cause mortality suggesting *Galectin-4* as a novel link between diabetes and cardiac disease.

Reference

1. John Molvin, Manan Pareek, Olle Melander, Lennart Råstam, Ulf Lindblad, Margrét Leósdóttir, Peter M. Nilsson, Michael H. Olsen, Martin Magnusson**.** Using a Targeted Proteomics Chip to Explore Pathophysiological Pathways for Incident Diabetes– The Malmö Preventive Project. *Scientific Reports*. 2019; 9: 272.

Gut microbiota in cardiometabolic disease

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In 2018, more than 4,000 gut microbiota studies were published, and over the past 10 years, several studies have associated obesity, type 2 diabetes (T2D) and cardiovascular disease (CVD) with specific changes in the gut microbiota composition and functional capacity. Further, many investigations have focused on the influence of nutrients and demonstrated how specific microbial-derived metabolites or bacteria might be linked to nutrients such as amino acids, offering future perspectives for possibilities to develop novel preventive and even therapeutic applications. Novel mechanisms of importance for metabolic diseases and CVD have emerged, some of which have focused on specific bacteria while others on bacterially produced metabolites. These studies, often in animal- and *in vitro* models, have provided important contributions to the field, and studies in germ-free mice have provided support for causal connections. However, human studies have so far mostly been small cross-sectional case-control studies or intervention studies, gut microbiota variation in the overall healthy population has remained under-investigated, human data favouring causality is clearly insufficient, and prospective studies are lacking.

In two large prospective population cohorts, the Malmö Offspring Study (N=3300) and SCAPIS-Malmö (N=4526), we investigate how gut microbiota composition and functional capacity associate with cardiometabolic risk traits and incidence of T2D and CVD. By genome-wide association analyses we identify host genetic variants that associate with gut microbiota composition and function, circulating metabolite levels including short chain fatty acids (SCFA), and will utilize this knowledge in Mendelian Randomization (MR) and network-MR to assess causal connections and directions.

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Biomarkers and drug development

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Background

Biomarkers have huge potential to predict disease risk. However, an ultimate goal is to improve health by therapeutic manipulation of the pathway indicated to be in disequilibrium by the biomarker/s in question. Examples of possible approaches will be reviewed in this lecture.

Methods

So far, the most successful strategy in cardiometabolic drug-target identification comes from studies combining genetics and circulating biomarkers. Moreover, top-down approaches starting with large epidemiological discovery studies in humans followed up by mechanistic studies in animal models and cell systems for validation have turned out to be attractive.

Results

A number of studies have been published over the last years showing that both small biological effects conferred by common genetic variants and larger biological effects conferred by rare genetic variants, result in distinct blood biomarker profiles, and can be blocked by drugs interfering with the pathway in question. Within the cardiometabolic field, lipid genetics stands out as perhaps the most successful example including drug targets involved in triglyceride- and lipoprotein metabolism as well as in endocrine regulation of lipid metabolism.

Conclusions

Using a top-down approach starting with large human cohorts and the combination of genetics and circulating biomarkers has proven very useful for drug target identification. With increasingly powerful omics techniques, this methodological principle is likely to become central for drug development during the upcoming decades.

pQTL studies to maximize understanding of biological mechanisms and create a foundation for Precision Medicine

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Background

Circulating protein biomarkers play vital roles in human physiology and are frequently used as biomarkers to diagnose and predict disease. The arrival of immuno-proteomic methods such as Olink PEA has enabled simultaneous measurement of 100s of circulating proteins at scale, which should ultimately lead to improved ability to diagnose and predict disease as well as identifying subtypes of disease. However, the characterization of circulating proteins at scale, including inter-individual differences due to DNA variation, regulatory pathways, and potential causal roles in disease is so far incomplete. Therefore, we created the SCALLOP consortium, which is a collaborative framework that brings together researchers seeking to identify protein quantitative trait loci (pQTL) and disease biomarkers (www.scallop-consortium.com).

Methods

PIs of study collections with genome-wide genotyping and Olink PEA proteomics data were brought together and the genetic and Olink protein data were harmonised within each study collection. Genome-wide association studies of Olink protein levels were conducted according to a common protocol, and the data were stored and analysed at the TRYGGVE server infrastructure, which is part of the EU ELIXIR programme. Summary statistics from each cohort were meta-analysed by Olink panel using METAL after deep quality control.

Results

A meta-analysis conducted across 13 studies and 22,000 participants, based on the Olink CVD-I panel proteins, has been completed. A total of 401 pQTLs for 90 Olink CVD-I proteins were identified, of which 75 were cis-pQTLs. An additional 144 signals were conditionally significant after adjustment for the primary signal. By combining trans-pQTLs with protein-protein interaction networks we identified the most likely mechanisms by which the pQTLs regulate protein levels, which confirmed known pathways and suggested novel pathways. A Mendelian Randomization framework for combining cis- and trans-acting pQTLs to identify causal associations with coronary artery disease (CAD), ischemic stroke (IS), type-2 diabetes (T2D) and rheumatoid arthritis (RA) was developed, which confirmed a causal role of the interleukin-6 receptor in CAD and RA and matrix-metalloproteinase 12 in IS.

Conclusions

GWAS meta-analyses of Olink proteins is feasible but requires carefully standardised protocols. Almost 90 % of the Olink CVD-I proteins were influenced by cis-pQTLs and the large sample-size greatly increased detection of trans-pQTLs. Cis- and trans-pQTLs contributed to identification of novel causal pathways and highlighted opportunities for disease-modifying pharmacological interventions.

Update on the importance of early life programming

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The importance of early life programming for adult health and disease has been a research focus of interest ever since the 1970´ies (Forsdahl in Norway) and 1980´ies (Barker, UK, and Gennser, Sweden) when the first observational studies were published linking perinatal health and birth weight with adult risk of hypertension, cardiovascular disease and type 2 diabetes. This scientific thinking was later on further developed by Nick Hales, Peter Gluckman and Mark Hanson [1], but also in Sweden [2], with several reports from large cohort studies from Uppsala and the Swedish Twin Register. More recently, the International Society for Developmental Origins of Health and Disease, DoHAD (https://dohadsoc.org/) was set up to promote research into the fetal and developmental origins of adult disease and this society involves scientists from many backgrounds. Adult lung function and lung ageing have, for example, been associated with early life factors and birth weight [3]. A special focus of interest could be the long-term prognosis of children born pre-term, now surviving to a higher degree as compared to historical cohorts, and at risk for disease.

Ongoing cohort studies on early life influences on adult health in southern Sweden include the Malmo Birth Data Cohort (MBDC), the Malmö Offspring Study (MOS), the Helsingborg Birth Cohort (HbgBC), and the Halmstad Birth cohort. In the MBDC we are currently analyzing the influence of early life factors on the incidence and prognosis of cancer (S. Sharma) and fractures (B. Rosengren, L. Moberg), and in MOS the association between low APGAR scoring and adult health problems (A. Sharma) as well as birth weight and cognition, as modified by weight trajectories (E. Mimarian). In the HbgBC, preliminary analyses have shown an association between early life factors and lung disease in adulthood (C. Johansson), and finally in the Halmstad Cohort (A. Pontén) we try to explore the association between higher mean birth weights in general with longevity, where Halland has been known for increased longevity compared to national means since more than a century.

Conclusions

The importance of early life factors for adult health and disease have been documented in several national and international studies. We now aim to explore several cohorts in southern Sweden for various outcomes based on national register linkages. In addition to that we also have phenotypic data from health examinations in MOS and LifeGene to be further explored in this perspective.

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Biomarkers and risk stratification in cardiovascular prevention

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Traditional cardiovascular (CV) risk factors are inadequate to predict CV events making primary prevention difficult. To improve the predictive value of traditional risk factors, SCORE is calibrated to country specific CV death rates in many European countries. However, as age specific CV death rates and the ratio between fatal and non-fatal CV events are decreasing in Europe especially in subjects younger than 65 years and in women, we tend to under-treat these groups.

Traditional CV risk factors have two problems: The need for many years of exposure and a huge variation in individual susceptibility to the harmful effect of the risk factors. In general, the hope is that tissue or circulating markers of subclinical target organ damage (TOD) will enable us to offer individualized CV prevention and treatment:

1) Markers of TOD may identify more people at high CV risk in whom we should reduce traditional risk factors if needed and even try specifically to prevent progression of a specific subclinical organ damage
2) Markers of TOD may identify patients with hemodynamic imbalance or progression of subclinical cardiovascular damage in whom we should adjust or intensify preventive treatment

In many population studies, several tissue or circulating markers of subclinical TOD have predicted CV events independently of traditional CV risk factors, but it has been difficult to demonstrate a clear clinical benefit of using new biomarkers in the general population [1]. However, in a recent large study, the Natriuretic Peptides Studies Collaboration have demonstrated that Nt-proBNP added predictive value to a model including all SCORE risk factors, history of diabetes and HDL-cholesterol [2]. In an elderly Swedish population without previous CV disease, the combination of Nt-proBNP and GDF-15 improved risk prediction significantly even after adjusting for SCORE risk factors and CV and metabolic treatment [3]. Furthermore, in a Danish population study of 2059 apparently healthy subjects, 1040 subjects had moderate CV risk according to SCORE. However, 242 of these subjects with moderate risk had either slightly elevated urine albumin/creatinine ratio or presence of ultrasonic atherosclerotic plaques in the carotid arteries which was associated with high CV risk during 12-13 years of follow-up (40 CV events in 242 subjects). Among these 242 reclassified subjects 53% had untreated hypercholesterolemia (>5.5 mmol/L), 13% had untreated hypertension (≥140/90 mmHg), 18% had both and only 16% had neither hypercholesterolemia nor hypertension. Based on known treatment effects, primary prevention would be expected to reduce the relative CV risk by 24% if implemented and followed. As 40 subjects were correct reclassified (i.e. had a major CV event) a 24% benefit equals 10 events that would be prevented out of 242 treated out of 1040 screened. In other words, 1 major CV event may be prevented by treatment of 24 reclassified found by screening of 104 subjects with moderate SCORE risk [4]. This clinically very relevant result should be tested in a randomized prospective study.

In theory, documenting subclinical TOD in patients on antihypertensive treatment may improve future patient compliance/adherence to antihypertensive treatment, may support more intensive treatment of traditional risk factors (lower targets) and may lead to adjustment of treatment targeting specific subclinical TOD. None of these hypotheses have been tested prospectively yet, but data are on the way (SPARTE).

Conclusion

In conclusion, tissue or circulating markers of subclinical TOD can identify moderate risk subjects with high risk that might benefit from preventive treatment. Although changes in markers of subclinical TOD have independent prognostic importance, we lack data supporting the use of these as treatment targets.

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Parent-of-origin effects and fetal programming for risk of diabetes

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Background and aims

T2D encompasses a set of heterogeneous disorders characterized by *impaired insulin secretion* and *increased insulin resistance*. While T2D has a strong heritable component, >400 T2D risk SNPs discovered through genome-wide association studies (GWAS) explain <15% of the heritability. It has been observed that T2D is seen more often in offspring of T2D mothers rather than of T2D fathers. Parent-of-origin effects (POE), wherein the phenotypic effect of an allele depends on whether it is inherited from the mother or the father could explain part of this missing heritability, maternal transmission of T2D and relate to fetal programming.

Materials and methods

We leveraged RNA sequencing, GWAS and global DNA methylation data from 80 independent trios enriched for type 2 diabetic offspring (77 offspring with T2D) to identify parental biases in expression. Expression patterns of genes showing POE in trios were studied in adult and fetal pancreas. Knockdown studies are ongoing to assess their influence on beta-cell mass (proliferation) and function (insulin secretion).

Results

Multiple protein-coding genes showed parental biases in gene expression in trios. Variants in the *KCNQ1* gene have been associated with increased T2D risk especially when transmitted from mothers. We replicated the association of the maternal allele increasing risk of T2D in our family trios; distinct methylation and POE-specific expression patterns were observed. The Bone Morphogenic Protein 8A coding *BMP8A* showed significant parental bias in expression and was consistent even when only sons or only daughters were considered. Differential expression analysis between genders was not significant either in blood or in pancreas ($p>0.05$), showing clearly that these differences in correlations were not driven by gender. *BMP8A* showed significantly high expression in the fetal pancreas whereas almost no expression was observed in the adult pancreatic islets. siRNA knockdown (KD) of *BMP8A* in INS-1 832/13 and EndoC-BH1 cells showed increased viability, decreased cytotoxicity and apoptosis but no effect on glucose stimulated insulin secretion. BMP8A belongs to a family of proteins involved in orchestrating tissue architecture. These preliminary results suggest that *BMP8A* might play a role in beta-cell development. Similar studies in iPS cell lines are underway to further elucidate the role of *BMP8A*.

Conclusion

This study demonstrates that parental biases in gene expression exist beyond imprinted genes and can have strong spatial and temporal effects independent of gender. Some of these genes could have significant roles in fetal development.

Probiotics in Prevention of Allergy among Children - Insights from the ProPACT study

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Background

Since the 1950's, we have been witness to a dramatic increase in the prevalence of allergy related diseases, such as atopic dermatitis (eczema), asthma and allergy rhinoconjunctivitis (hay fever). Modern interpretations of the so-called "hygiene hypothesis" suggest that recent changes in the patterns and diversity of microbial exposures in early infancy may be partially responsible for this increased prevalence. As such, probiotics – "Live organisms which, when administered in adequate amounts, confer health benefit on the hosts" – have been trialed in both the prevention and treatment of allergy related diseases with partially conflicting results. The most promising results have come from trials aimed at preventing atopic dermatitis.

Although individual randomised controlled trials have employed a range of bacterial strains and administration regimes, meta-analyses indicate that supplementation during the later stages of pregnancy and during the first months of life can prevent the development of atopic dermatitis in infancy. Our own randomised controlled trial, the Probiotics in the Prevention of Allergy among Children in Trondheim (ProPACT) study, indicated that maternal probiotic supplementation alone resulted in a nearly 40 % reduction in the cumulative incidence of atopic dermatitis at 2 years of age. The biological mechanisms behind this effect are incompletely understood. We have been investigating potential microbiological and immunological mechanisms using samples collected from mothers and children during the ProPACT trial, including plasma, peripheral blood mononuclear cells, stool, vaginal and oral swabs, and breast milk samples.

This presentation will review the current understanding of how probiotics prevent atopic dermatitis, with a particular focus on insights from the ProPACT trial.

A systems biology/multi-omics strategy to better understand biology and drive precision medicine

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Identifying relevant protein biomarkers helps to bring new insights into disease processes, improve disease detection, and contribute to a better understanding of pathophysiology. Using multiple proteins that form a signature is more powerful and reliable than looking at a single protein, but this requires studies that examine many different proteins simultaneously, in large numbers of human samples.

Olink Proteomics has an enabling solution for such studies, based on the Proximity Extension Assay, an innovative technology providing a sensitive, high-multiplex approach that overcomes the cross-reactivity problems normally associated with multiplexed immunoassays, using a dual-recognition, DNA-coupled methodology that provides exceptional readout specificity.

Details of the thorough validation procedures applied to these assays will be presented, along with the design strategy behind the disease or biological process-focused 92-plex panels that can be used for targeted protein biomarker discovery.

Bridging over several omics strategies to enable a systems biology approach is imperative for a deeper understanding of biology and to hopefully drive the development of precision medicine and take us closer towards personalized health.

Biomarkers for the study of inflammation and repair after myocardial infarction - The LundHeartGene study

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A good prognosis in myocardial infarction (MI) patients depends on a finely-tuned balance between inflammation and repair, on the interplay between pro- and anti-thrombotic mechanisms, efficient neovascularization and adequate fibrotic repair of the damaged myocardium. Excessive inflammation promotes extensive injury and cardiac remodeling, leading to heart failure and cardiovascular (CV) death. Conversely, rapid resolution of inflammation and efficient repair are associated with a favorable prognosis. The recent CANTOS trial has demonstrated that targeting inflammation in stable MI patients improves prognosis. Development of clinically-relevant biomarkers able to monitor the pathogenic processes involved in MI and to identify novel candidates for potential treatments is an important step towards personalized medicine in MI patients.

The LundHeartGene study has been initiated to find biomarkers able to monitor the response to treatment and to identify patients with high residual risk. The study is designed to prospectively investigate post-MI cardiac function and future events in relation to plasma biomarkers and genetic background. MI patients are recruited at the Department of Cardiology SUS Lund and Malmö. Blood samples are collected within 24h after MI, during the acute phase and at 6 weeks post-MI, during the recovery phase. Serum, plasma and genetic material are saved in the Region Skåne biobank. Echocardiography is performed at baseline and at 6 months, to assess the degree of post-ischemic cardiac recovery. Cardiac MR will be performed at the same time points in 100 participants, to obtain detailed information on myocardial structure and function. Clinical and follow-up data will be acquired from national registries. The method of sampling by clinical routines coupled to the SWEDEHEART registry has now spread to Uppsala and Stockholm and a network called SWEDEHEART-BIOBANK has been developed.

The biomarker discovery platforms provided by OLINK Proteomics will be used for mediator measurement in patient plasma. Plasma concentration of the biomarkers during the acute and post-acute phase, as well as the dynamic changes between the two phases will be examined. We will prospectively evaluate the incidence of recurrent MI, heart failure and CV death in the cohort, and their relationship with the biomarkers and the genetic background.

The main aim of the study is to identify strong biomarker candidates to identify patients with an unfavorable post-MI evolution. Additionally, we aim to identify novel treatment targets that can be used to limit the post-ischemic damage to the myocardium and to promote efficient repair. Neither of these important goals is currently met in clinical practice.

Preterm birth and SGA are associated with multiple short- and long-term health consequences

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Background

As premature infants survive in greater numbers and with fewer short-term comorbidities, there has been increasing interest in also examining the long-term outcomes for these infants.

Methods

The National Board of Health and Welfare and Statistics Sweden collect and create nationwide registers that includes (examples): health care data, mortality data and sociodemographic population data that are very useful for studying short- and long-term health consequences in prematurely born and SGA infants who survive into adulthood.

Results

Prematurely born and SGA infants who survived into adulthood had increased risks for multiple short- and long-term health consequences.

Conclusions

Persons born prematurely or SGA need long-term follow-up for monitoring and preventive actions to achieve good health across the life course.

Big Data and cohort collaboration

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The scientific literature is continuously flooded with non-replicable results from underpowered studies. The insight that very large sample sizes are needed in order to generate robust results has led to the establishment of very large cohorts and cohort consortia in the last decades. This presentation will outline the rationale for a Swedish cohort consortium, aiming to facilitate greater use of Swedish cohorts for better-powered research [1]. Coordination of all Swedish prospective population-based cohorts in a common infrastructure would enable more precise research findings and facilitate research on less common exposures and outcomes, leading to better utilization of study participants' data, better return of funders' investments, and higher benefit to patients and populations. The proposed infrastructure will be motivated by lessons learned from two pilot studies.

A standing Swedish cohort consortium may drive development of epidemiological research methods and strengthen the Swedish epidemiological competence, community, and competitiveness.

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Biomarkers in cardiovascular research - An update 2019

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Background

Cardiovascular disease remains the most common cause of death world-wide despite a substantial decline in age-related cardiovascular mortality and morbidity. [1,2] The most common cardiovascular diseases are a) coronary atherosclerosis, associated with angina pectoris, myocardial infarction, heart failure and sudden death; b) peripheral atherosclerosis with claudication and amputation; c) carotid atherosclerosis with ischemic stroke and death and d) myocardial fibrosis with atrial fibrillation, heart failure and sudden death. Recently several new treatment options have been introduced in all these areas, which though provide challenges because of cost issues and risks of side effects. Thus, there is a need for better precision in the selection of the best balance between efficacy, safety and cost for the individual patient. There is also a need for new tools for monitoring treatment effects over time, allowing timely adjustment for maximal effectiveness. Despite the new interventions there remains a substantially increased risk of new events in certain patients in whom there is a need to identify additional pathophysiological mechanisms and new treatment targets.

Biomarkers

Currently there is a rapid expansion in the opportunities of measuring circulating biomarkers reflecting many different processes of importance for disease development, organ dysfunction, treatment response and clinical outcomes. The availability to methods for precise determinations of key proteins with high sensitivity assays and to simultaneous screening of hundreds or thousands of proteins with multiplex assays allow the development of new tools for decision support in several cardiovascular diseases. If the biomarker levels are genetically regulated, there are also opportunities to perform Mendelian randomization analyses to learn if the genetically regulated protein expression might be causally associated with disease development and thereby might identify potential new treatment targets.

Recent developments in our area of research

During the last years we have documented the prognostic importance of a series of biomarkers (NT-proBNP, troponin-T or -I high sensitivity (TnT, TnI), interleukin-6 (IL-6), D-dimer and cystatin-C concerning ischemic events both in acute and chronic coronary artery disease and atrial fibrillation.[3-11]. We have also presented the prognostic importance of growth differentiation factor 15 (GDF-15) and D-dimer for bleeding during antithrombotic treatment in the same conditions.[12] Finally we have shown that these biomarkers are more strongly related to cardiovascular mortality than any clinical information including age.[13] Based on these biomarkers we have, in patients with atrial fibrillation (AF) developed a validated and well-calibrated biomarker-based risk scores for stroke (ABC-AF-Stroke), major bleeding (ABC-AF-bleeding) and death (ABC-AF-death) with better predictive performance than any score based on clinical information.[14-16] Similarly, we have for patients with CAD presented the validated and well-calibrated ABC-CHD risk score based on the biomarkers NT-proBNP and TnT with better performance than any clinically based prognostic tool.[13] Currently we are working on the finalization of the biomarker-based risk scores on ischemic events (ABC-ACS-ischemia) and bleeding (ABC-ACS-bleeding) for risk stratification and decision support after the revascularization in patients with acute coronary syndrome. During the last year we have also

documented and validated the potential usefulness of monitoring the risk scores over time in order to modify treatments in relation to alterations in risk[27]. Although the biomarker-based risk scores perform better than scores based only on clinical data, the c-statistics are only excellent concerning mortality (C-index above 0.80) but modest concerning ischemic events and major bleeding (C-indices around 0.70). Thus, there is a large need for better precision in the prediction of ischemic and bleeding events, which might be accomplished by the utilization of multiple biomarkers, genomics and imaging information. The ongoing progress in research using multiplex omics will be reviewed at the meeting. 17-19

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Systematic cross-platform comparison of genetic associations across the human plasma proteome

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Background

'Omic' blood biomarkers can now be measured at population-scale using different techniques that have broad ranging coverage, test characteristics and costs. Genetic association studies require large sample sizes to provide sufficient power for discovery, yet platform-specific analysis is limited in scope by focusing on measures assessed using a single method. The aim of this study is to test the feasibility of integrating different proteomic platforms for genetic discovery, and thereby enable inclusion of publicly available data from different sources.

Methods

We conducted genome-wide association studies (GWAS) of 1104 protein measurements across all currently available Olink panels (12 panels, 35 proteins measured >= 1 panel) in fasting EDTA plasma samples from 500 of a total of 12,435 Fenland study participants, randomly selected from those attending the Cambridge recruitment centre (36% of all Fenland participants) and successfully genotyped using the Affymetrix UKBB genotyping array imputed to the the HRC and UK10K imputation panels. We compared genetic effects for proteins also measured using the Somalogic V3 assay and included in the genetic discovery of Sun et al., Nature 2018.

Results

A total of 93% samples were successfully measured across all 12 panels and only 15 samples failed for more than one panel and were excluded. Of the 1104 target proteins, 78% were above the lower limit of detection across all samples. Correlations of the same protein measured on different panels were high (35 comparisons, mean r=0.90 [range 0.68-0.99]), even when measured using different dilutions across panels (4 comparisons, mean r=0.84). Genetic effects in Fenland were correlated with those published in the literature.

Conclusions

Measurement of 12 O-link panels has provided in-depth, high quality proteomic characterisation of Fenland participants. Comparison to published results showed that integration of genetic associations for proteins measured using different techniques and platforms is feasible to boost power for discovery.

POSTER abstract

Potential intake biomarker of added sugar in spot urine in comparison to intake from 4-day food records for examining cardiometabolic risk

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Introduction

The research on added sugar intake and its link with cardiometabolic disease tend to show inconsistent results, plausibly partly because of dietary misreporting. Therefore, cheap and easily-measured nutritional markers are highly necessary and warranted to support the dietary data. 24-hour urinary sugars are established as a biomarker for sugar intake; however, there is a huge lack of knowledge regarding how this holds up in spot urine.

Objective

To compare the spot urinary sugars biomarker with reported sugar intake and to compare the associations with cardiometabolic risk markers between the two different methods for assessing sugar exposure as well as when combined to one unite measure.

Method

Among 992 subjects in the Malmö Offspring Study, urinary sucrose and fructose concentrations were measured using liquid chromatography and mass spectrometry. Sugar intakes were assessed using a web-based 4-day diet record.

Results

The correlation coefficients between sugar intakes and the urinary sugars varied between 0.20-0.30. Important differences between men and women were seen for the associations with several cardiometabolic risk markers. In women only, urinary sugars, but not added sugar intake, were positively associated with blood pressure and fasting plasma glucose. For BMI and waist circumferences, both added sugar intake and urinary sugars were positively associated in women, while urinary sugars were negatively associated in men. Added sugar intake was negatively associated with HDL in both sexes. A combined measure of dietary and urinary sugars was positively associated with BMI, waist circumference and blood pressure in women, and negatively with HDL for both sexes.

Conclusion

We here demonstrate correlations at levels that indicate good potential for the spot urinary sugars biomarker to support the self-reported dietary data. A combination with urinary and dietary sugars can strengthen the confidence of the associations between sugar exposure and health outcomes. However, we do not yet know all the determinants of the spot urine sugars and it is crucial for future studies to validate the spot urine sugar biomarker against controlled food intake.

POSTER Abstract

Circulating ErbB2 levels are associated with increased incidence of diabetes: a population based cohort study

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Background

ErbB2 is a member of the epidermal growth factor receptor family. It is widely used as a marker of tumors but it has recently also been associated with insulin resistance. Both ErbB2 and diabetes have been shown to be associated with cancer. However, the relationship between ErbB2 and diabetes is not well explored. The aim of this population-based cohort study is to assess the association between plasma ErbB2 and incidence of diabetes.

Methods

The study population included participants from the Malmö Diet and Cancer-cardiovascular cohort (age range: 46-68 years). After excluding participants with history of diabetes, missing data on ErbB2 and other co-variates, the final study population consisted of 4220 individuals. Incidence of diabetes was followed by linkages to local and national registers. Cox proportional hazard regression was used to assess the incidence of diabetes in relation to the quartiles of ErbB2, adjusted for potential confounders.

Results

ErbB2 in plasma was significantly and positively associated with glucose, insulin and HbA1c, after adjustment for potential confounding factors. During a mean follow-up period of 20.20 ± 5.90 years, 615 (14.6%) participants were diagnosed with new onset diabetes. Individuals with high levels of ErbB2 had a significantly increased risk of diabetes. The multivariable adjusted hazard ratio for the highest vs the lowest quartile of ErbB2 was 1.31 (95% confidence interval [CI]: 1.03-1.66) (p<0.05) and was 1.15 (95% CI: 1.05-1.25) (p<0.05) per 1 standard deviation increase of ErbB2.

Conclusions

Elevated levels of ErbB2 are associated with increased incidence of diabetes.