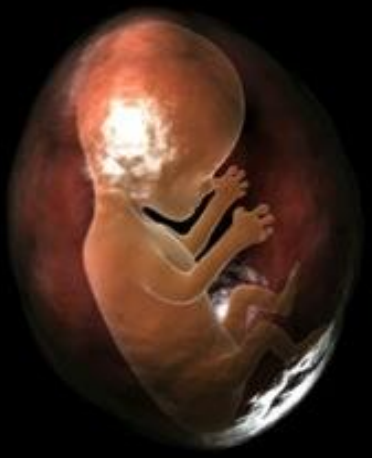
in-FLAME

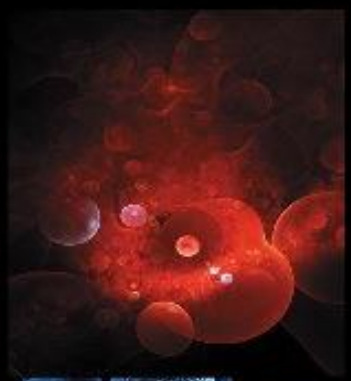
The International inflammation Network

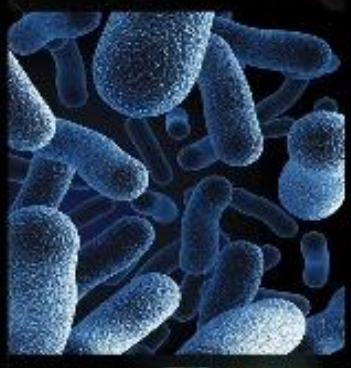


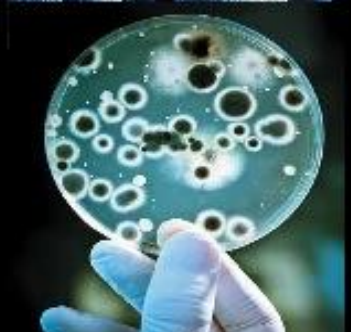
# 4th Annual Workshop

Marburg, Germany, 2015

# Meeting Report







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# Introduction from our *in-FLAME* Network Chair:

Prof Susan Prescott,
University of Western Australia
Telethon Kids Institute

This has been our best year yet! Our 2015 annual workshop in Marburg Germany was a resounding success, not just because of the many new collaborative projects that were developed, but because of all of the many new friendships and collaborations that were established as a result. The positive feedback has been overwhelming, and I believe that this is largely because of the strong spirit of collaboration, commitment and active participation by our many network members. As always, the informal discussions over lunch and dinner were just as fruitful and important as the main working sessions. Thanks to our local host Professor Harald Renz and his team, we had the perfect location for our retreat at the Schloss Rauischholzhausen nestled in the beautiful German countryside. I would also like to thank Professor Diane Campbell (University of Sydney) for her pivotal role and assistance in organising the meeting.

Now entering our 4th year, the *in-FLAME* has quickly matured towards a critical mass, now generating a broad range of collaborations examining key early *exposures* that modify early immune development to impact multisystem health *outcomes. In many ways in-FLAME* has become a showcase for collaborative success within the WUN global challenge program.

As Chair of the in-FLAME network, I am truly proud of what we have achieved so far. In addition to our international research collaborations, fostering the next generation of researchers is a high priority. Around one third of attendees this year were Early-Career Researchers (ECR), and our network provides an important opportunity for networking and mentoring. In the coming year *in-FLAME* will establish an Early-Career Researchers (ECR) Network and a Mentoring Program. This will be led by Dr Daniel Munblit (Imperial College London) with help from Dr Chrysanthi Skevaki (Marburg), with support from Prof Anita Kozyrsky (University of Alberta). This will build general research capacity, sustainability and contribute to specific projects through exchange programs and/or specific training.

Going forward, we will further consolidate our activities and collaborations through an expanding number of projects, funding initiatives and publications, sharing resources through our new website http://www.wuninflame.org. We welcome new members from every region.

I am looking forward to our ongoing collaborations in the coming year, and to welcoming you to our next workshop in Maastricht Netherlands, April 1-3, 2016.

Prof Susan Prescott
Founding Chair, *in-FLAME* Network

Meeting Co-Chairs
Prof Dianne Campbell
and Prof Harald Renz

# About the *in-FLAME* Network

Launched in 2012, the *in-FLAME* Network addresses the risk factors, pathways and strategies to overcome the rising propensity for chronic inflammatory disorders, with a focus on early effects on the developing immune system. Led from UWA by Professor Susan Prescott, it involves 9 WUN universities and WUN+ partners from 47 institutions, and 20 countries around the world. Together our 175 current members are working on an integrated program of population studies, biological studies and intervention studies aimed at preventing inflammation and the burden of subsequent disease.

'*...There has been an unprecedented rise in non-communicable diseases (NCDs) such as allergies, asthma, cancer, diabetes, mental ill health and obesity. Inflammation and immune dysregulation are common features, often associated with similar environmental and lifestyle risk factors such as dietary patterns, environmental pollutants, microbial patterns and stress. Given the central role of the immune system in health and development, inflammation must be examined as both a common element and target for the prevention of NCDs...*'

For further information please visit our website or the WUN general links:
http://www.wuninflame.org.
http://wun.ac.uk/wun/research/view/in-flame-international-inflammation-network
http://wun.ac.uk/article/early-life-solutions-to-the-modern-health-crisis

in-FLAME

# Our alignment with WUN goals and values

WUN's values underpin our approach to maximising the network's core strengths.

| DEVELOP | NURTURE | INNOVATE | COLLABORATE | IMPACT |
|---|---|---|---|---|
| WUN actively develops our global research alliance and education programs to meet our objectives. | WUN nurtures and invests resources in the intellectual strengths and priorities of our members. | WUN seeks and instigates innovation in knowledge management and educational exchange. | WUN promotes opportunities that accelerate internationalisation for global collaborations between universities and with government, international organisations and industry on issues of global significance. | WUN exists to make a difference. Our research produces new knowledge that influences policy and impacts society to improve lives. |

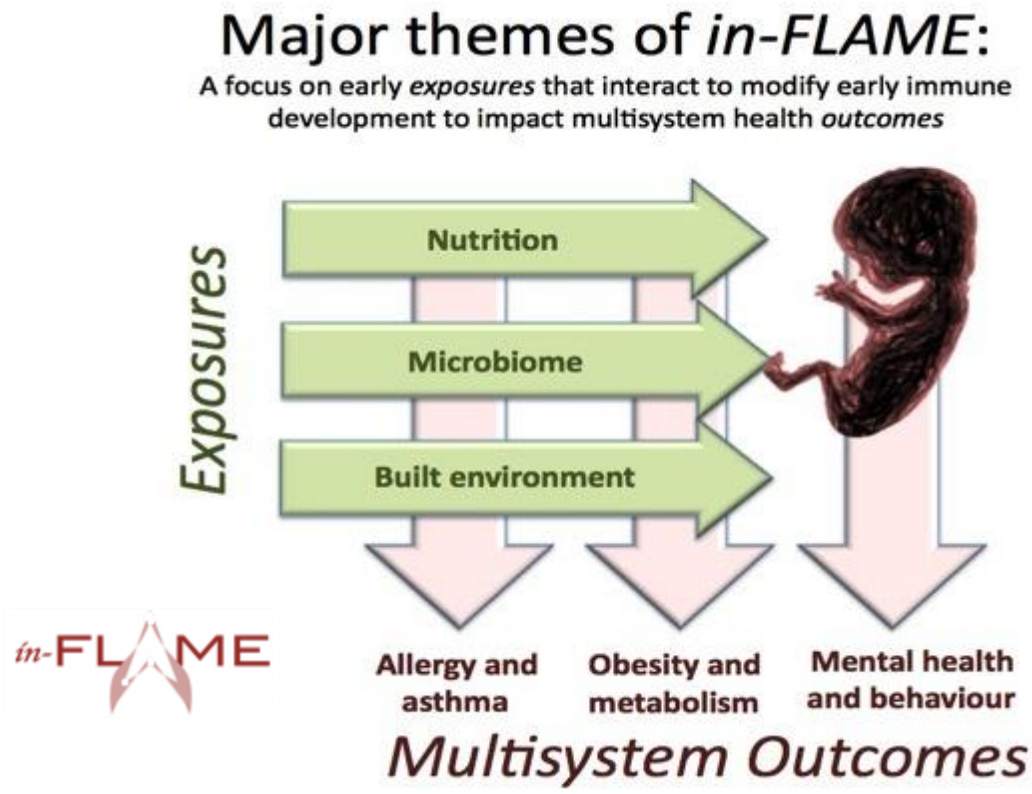
## OBJECTIVES & STRATEGIES

| | | |
|---|---|---|
| 1 | Strengthen and grow our university network | • Develop and maintain a dynamic portfolio of research and education programs in alignment with WUN members' priorities.<br>• Strategically grow the membership as a network of peer universities with mutual strengths and regional diversity.<br>• Build ownership and leadership within WUN to increase collaboration, commitment and sense of community. |
| 2 | Foster influential research communities | • Focus our efforts on four *Global Challenges*:<br>– Responding to Climate Change (food security, urbanisation, oceanography);<br>– Public Health (lifecourse approaches to obesity, heart disease, diabetes);<br>– Global Higher Education and Research (access and equity, new technologies);<br>– Understanding Cultures (migration, digital futures, ageing).<br>• Incorporate cross-cutting themes in big data, macroeconomics, and regional programs on China and Africa into our *Global Challenge* programs.<br>• Strengthen leadership and accountability to ensure quality and delivery. |
| 3 | Nurture research talent | • Create opportunities for the engagement and career development of talented researchers at the postdoctoral, postgraduate and undergraduate level in international research collaborations.<br>• Facilitate the mobility of students and academic staff.<br>• Promote equity for researchers in our programs. |
| 4 | Enhance the WUN profile | • Position WUN as a recognised thought leader in our areas of expertise, engaging the WUN Presidents and experts as an international think-tank and as policy advisors.<br>• Increase the power of the "WUN voice" in an ambassadorial and lobbying role.<br>• Strengthen the WUN brand and profile with internal and external audiences, ensuring WUN is recognised for the vast potential of its intellectual resources. |

# Goals of the *in-FLAME* 2015 workshop

Our core goal of our workshop was to develop and consolidate our research platform through new and existing projects. Continuing the themes of our previous meetings, we have a core focus on key early exposures (namely a) nutrition, b) the microbiome and early microbial diversity, and c) pollutants and the built environment), and how these interact to modify early immune development, to impact many aspects of development. Our multisystem focus includes a range of early outcomes including 1) allergy and asthma 2) obesity and metabolism 3) mental health of behaviour.

Discussions also focused on building capacity and productivity through new collaborations, technologies, funding streams, and publications. There was also a key emphasis on opportunities for our student and ECR, who all played an active role in the program.



# Travel Grant recipients:

Thanks to generous support from Danone Nutricia we were very pleased to provide travel grants for early and mid career researchers, to attend the meeting and present their work. Congratulations to:

Susanne Brix
Silvia Carraro
Shantelle Claassen
Elloise du Toit
Aveni Haynes
Petya T Koleva
Jennifer Koplin
David Martino
Sofia Nordlander
Debra J. Palmer

# Meeting sponsors:

The *in-FLAME* meeting was generously supported by a DFG (Deutsch Forschungsgemeinschaft) grant with matching funds from a University of Western Australia Research Collaborative Award (RCA). We are also grateful for support from our WUN collaborators from the University of Sydney and University of Alberta. Importantly, we recognise the in-kind contributions of all of the participating members.

# Meeting Photographer:

We thank Dr Peter Hsu for capturing our meeting so beautifully.




# Key Achievements in 2014-2015

| | |
|---|---|
| Scientific Publications | We now have **46 publications** accepted or under review (see full list on page 45), with the majority in the last 12 months. The number of original research papers is anticipated to increase as the number of new projects and new collaborations come to fruition. |
| Major general communications (public audience) | The book *"Origins: Early-life solutions to the modern health crisis"* by Susan Prescott was published, April 2015. This has a major focus on the impact of early environments on the developing immune system, as well as the wider so social, cultural and economic determinants of health. The themes of the book resonate with the objectives of both in-FLAME and the wider WUN philosophies, and it is aimed at engaging the general community in these discussions. |
| Major Grant Applications | **EU 2020 Horizon:** €7.1 million (invited to submit stage 2). This considerable application (269 pages) was led by Imperial College London (Prof John Warner, and early career researcher Dr Daniel Munblit) and includes a large number of collaborating institutions across the *in-FLAME* network. Although the results are still pending, the enormous amount of work that has gone into planning this collaboration will be put to good use regardless of the outcome.<br>**NIH application** (Alberta, Detroit and multiple collaborators). This application is led by Prof Anita Kozyrskyj, together with Profs Christine Johnson and Ganesa Wegienka. Preliminary studies have been initiated. |
| New Grants and Awards | To support our activities this year we received the following grants:<br>• Danone Travel Awards: **€5000**<br>• Deutsche Forschungsgemeinschaft **€15000**<br>• Research Collaborative Award, University Western Australia **$20,000**<br>• Research Support, University of Sydney **$20,000**<br>• Research matching funds, University of Alberta **$5,000**<br>• Nutricia foundation grant **€25000 (**University of Nice and Utrecht) |


**Prof Peter Gluckman** and **Prof Keith Godfrey** launch Susan Prescott's *book* '*Origins'* in April At Telethon Kids Institute

# Other Achievements in 2014-2015

| | |
|---|---|
| Launch of the DOHaD Society of ANZ | The Australian members of *in-FLAME* (Adelaide, Melbourne, Sydney and Perth) were the foundation members of the Developmental Origins of Health And Disease (DOHaD) Society of Australia and New Zealand – and successfully launched this society in Perth, 2014, with the support of UWA and Telethon Kids Institute. We have just successfully held Second annual conference (Melbourne 2015). Inflammation has been a strong theme at the DOHaD ANZ conferences, because of the influence and interests of our Australian *in-FLAME members.* |
| Exchanges and Travel plans | Dr Anita Kozyrskyj (Alberta, Canada) received a travel award from the University of Alberta WUN Research Mobility Fund to visit the Telethon Kids Institute and the University of Sydney in order to prepare a WUN Research Development Fund application with Profs Prescott and Campbell. |
| | Dr Marie Bodiner (Nantes, France) is applying for a Marie Curie Fellowship to travel to Australia to work on the prebiotic clinical trial in Perth (at Telethon Kids Institute) with Dr Debbie Palmer and Prof Susan Prescott. |

Inflammation remains
a central theme of DOHaD ANZ
(the founding members are also all
members of WUN *in-FLAME*)

# Report: pre-Workshop 'LactoActive' meeting

Prof Anita Kozyrskyj
University of Alberta

*Our breast milk interest group head, Lactation Biome-Active Partners Against NCDs (LactoActive), held a pre-workshop meeting to discuss our new collaborative project – bringing new collaborations with the 'Symbiota' group.*

## Goals and results of our preliminary studies:

Background: Combined, breast milk and the gastrointestinal tract (gut) harbour trillions of microbes that are essential to metabolic function (energy production) and the immune system of the infant. Early life exposures such as caesarean delivery and antibiotic treatment, can affect the composition of breast milk and gut microbiota in infants. Alterations in which microbes are present and what metabolites they produce have been implicated in the development of many metabolic (eg. obesity) and inflammatory diseases (eg. asthma). Microbial metabolites such as short chain fatty acids (SCFAs), are used as an energy source by human cells in the intestinal lining. SCFAs can also dampen inflammatory responses to ingested dietary substrates which are not pathogens. Changes in gut microbiota and metabolite composition, even transient ones, can induce long term changes in human cellular metabolic processes. This last point is important since early life changes to metabolic processes predict further overweight, insulin resistance and subsequently NCD in adulthood. Adolescents who develop insulin resistance are more likely to be overweight when they are younger.

Rationale: We therefore proposed to analyse concurrent mother-infant samples of breast milk, and infant stool to assess the energy substrates (e.g., fats and sugars from breast milk) available to the infant and the gut microbial machinery possessed by the infant which will metabolize these substrates to produce key metabolites. *Appreciating that pre and postnatal nutrition and environmental exposures are socially patterned, we require representation from different socioeconomic, ethnic/immigrant and social policy environments*. This will be achieved through a comparative study of breast milk and infant fecal samples from birth cohorts in Canada, Australia, Europe, USA, Africa and Asia, and by the subsequent testing of associations with child overweight. Since environmental context is so important to shaping breast milk and infant gut microbiota composition and what metabolites are produced, a country comparison of this magnitude will yield substantial insight into the early life exposures that contribute to the obesity epidemic in children and adolescents, and into those exposures which are protective.

The study plan: With initial funding from the University of Alberta WUN Research Development Fund, and the Universities of Western Australia and Sydney, and coverage of costs by individual cohorts to transport samples to the Dr. Wishart Metabolomics Innovation Centre at the University of Alberta in Edmonton, Canada, a preliminary comparison of milk metabolites

Petya Koleva (ECR) presents preliminary results

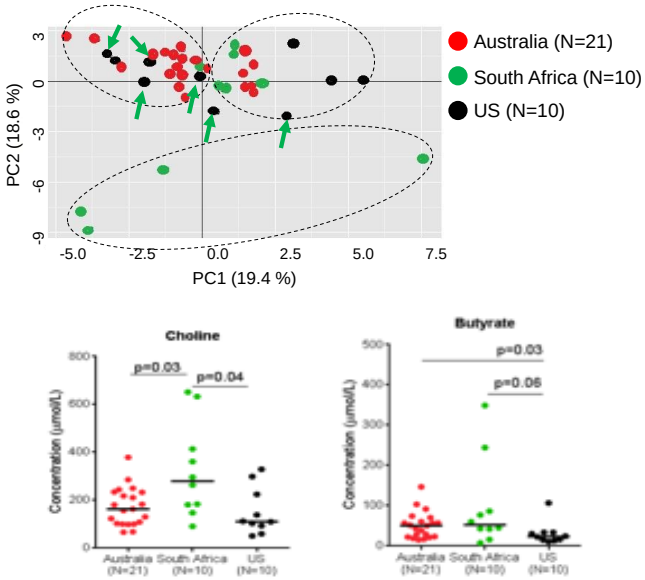
was completed with samples from the Drakenstein Child Health vanguard cohort (South Africa: Cape Town), PRB high-risk trial (Australia: Perth), WHEALS birth cohort (Detroit, USA), CHIBA birth cohort (Chiba region, Japan) and NOMIC birth cohort (Oslo, Norway). Based on an inventory of breast milk samples among existing cohorts worldwide conducted in 2014, selected breast milk samples were those obtained 1 month after vaginal delivery from mothers or infants who had not been treated with antibiotics. Samples were frozen and thawed a maximum of one time, and represented atopic and non-atopic mothers of Caucasian and non-Caucasian ethnicity, who had delivered female and male infants. They were processed by NMR to detect breast milk sugars, amino acids, polyols, vitamin compounds, short-chain fatty acids, energy metabolites and their derivatives.

**The goal of our meeting:** The planned outcome of the satellite symposium was to present our preliminary data and to further devise comparative studies of samples in established birth cohorts across the globe. The ability to access existing samples will represent a major inkind contribution since millions of dollars and many human resources are required to collect data from just one birth cohort. The proposed program will capitalize on the expertise of inFLAME network researchers, yet introduce a new element to network-led research by studying microbiota metabolomics (what metabolites the bacteria are producing).

**Preliminary Findings:**
Petya Koleva, Dr. Kozyrskyj's postdoctoral fellow, who helped process the breast milk samples, then analyzed the NMR output of metabolite data, presented preliminary findings. Regional and ethnic differences in metabolic composition of breast milk were evident, and noteworthy, samples from atopic women were clustered together. Specifically, maternal atopy was also associated with differences in sugars, choline, energy metabolites and amino acids. Comparisons of the NMR spectra revealed that 2-oxoglutarate and pyruvate differed by infant sex. These results are preliminary and need to be explored further in a larger sample set. We also now aim to:

- Identify fucosylated HMOs in the NMR spectra.
- To perform lipidomic and proteomic assays.
- To investigate human milk microbiome.

**Breast milk metabolites by geographical location**




**Preliminary data using samples from UWA, UCT and Detroit (Prof Kozyrskyj) – suggests regional differences according to diet.**

## EU 2020 Horizon Application (2015) – 'BIRTH'

As part of the breast milk discussions, Dr Daniel Munblit (Imperial College London) presented a summary of the EU application that has been lead from Imperial College London with multiple in-FLAME collaborators:

**Overview:** Breast milk (BM) is the principal source of nutrition during early infancy - a critical period of immune and metabolic programming for lifelong health and development. Numerous bioactive components have been identified in BM, and it is clear that maternal exposures can change both BM composition and infant health outcomes. There is conflicting evidence on the protective role of breastfeeding in relation to several biological outcomes, including metabolic (eg. obesity), intellectual development, and immune (allergic and autoimmune) outcomes.

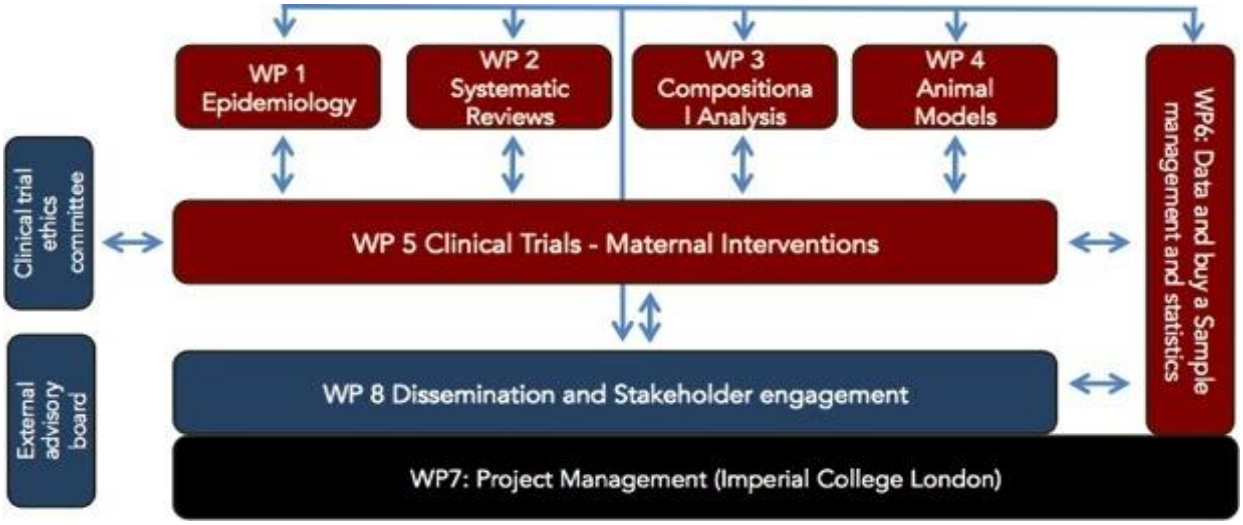
The use of maternal interventions during pregnancy/ lactation to influence infant development is a new and attractive approach for promoting long term health in the infant. However systematic methodologies have not been applied to understand variations in BM composition and their determinants. This defect in our understanding places limits on the potential for maternal dietary and other environmental interventions to be studied for their

Dr Daniel Munblit (ECR)

potential to promote infant health. BM composition is highly variable both within and between women. Maternal impact on infant health, and in some cases this may be mediated through altered BM composition. BM composition modification through maternal diet therefore offers potential to drive a new and acceptable approach to promoting long term health. The main purpose of this collaboration is to develop standards for BM analysis, identify maternal genetic and environmental factors that modify milk composition, systematically review existing evidence on BM research and conduct intervention trials in mothers aiming to modify BM composition and thereby promote improved health outcomes in the child. Our collaborations will allow us to rapidly commercialise and market the successful intervention(s) to ensure a rapid impact on future EU and non-EU populations who will then benefit from improved health, economic and societal outcomes

phenotype and diet can have a profound

Proposed work packages (WP) will provide ongoing framework for collaboration regardless of the outcome o the application. The project development so far will also provide opportunities to apply for other funding sources.




## Pollutants and breastfeeding

(*also see Pollutants report page 31*):

As part of this session Prof Merete Eggesbø (Oslo, Norway) discussed exposure to pollutants in breast milk level. Although the amount of environmental pollutants in breast milk has decreased significantly in recent years (citing data from Norway), persistent organic pollutants (POPs) PCB and DDE may still have harmful effects. Depending on maternal levels, breastfed infants can be exposed to much higher levels than formula fed babies, and this is proportional to the duration of feeding. Eggesbø led a new EU study that EU pooled data from seven European birth cohorts (*n* = 2,487). This reveals that if women had higher prenatal levels of DDE (a metabolite of DDT) this was associated with a significant increase in postnatal infant growth (a predictor of obesity). They also looked at the effects PCB on breast milk, and found that breast fed babies had higher levels of PCBs, and had reduced growth trajectory. This indicates that more reductions in pollutants needed. Her presentation also highlighted the need more such studies so that we can issue optimal breast feeding recommendations.

Prof Merete Eggesbø is senior author in newly published paper: *Iszatt et al Prenatal and Postnatal Exposure to Persistent Organic Pollutants and Infant Growth: A Pooled Analysis of Seven European Birth Cohorts. Environ Health Perspect. 2015 Jul;123(7):730-6*

# Summary of next steps

A manuscript based on our current findings will be prepared after remaining breast milk samples are processed, and the following points addressed. Samples from the KOALA/BreMil cohort studies can be added to a later manuscript or to replicate findings.

i) difference in breast milk collection, processing and storage methodology. Dr Munblit's BreMil 3-site study, used a standard collection but differing processing/storage methods, can be cited.

ii) regional differences biased by atopic sample from Perth. Dr Palmer will verify if non-atopic samples are available from Perth; Dr Campbell offered to collect new samples from women in Sydney.

iii) if easily available, increase the number of samples from the US WHEALS cohort and add new samples from VietNam, Kazakhstan or La Gambia

iv) correlation between breast milk metabolites. Dr Munblit offered statistical analysis support from Imperial College.

We will submit new proposals to fund additional metabolite analyses (lipidomics, proteomics) in current samples and/or in a new breast milk study to include standardized breast milk collection and storage methods, collection of additional biospecimens (eg. hair), and follow-up for child health outcomes. Possible funding opportunities include:

1) Horizons 2020 budget since the University of Alberta is listed as a collaborator. Funding decision will be made in August.
2) Piggybacked application onto the Horizons 20/20 grant through RFAs for EU-Canada, EU-Australia or EU-Japan
3) country-specific ECRs funding opportunities, such as the Thrasher in the US (eg. Dr. Johnson and Kozyrskyj to follow-up). ECRs, Debbie Palmer and Chrysanthi Skevaki, expressed interest in using data from the pilot breast milk comparison to support a future application.
4) diagnostic companies for reagents and breast milk pumps, which are interested in determining normal ranges in human samples.

## KEY OUTCOMES

1. International comparisons of breast milk from existing cohorts are useful in identifying regional and ethnic variations in breast milk metabolite (sugars, amino acids and derivatives) composition.
2. Regional/ethic differences can be attributed to maternal atopy status and to gender of the fetus, suggesting pregnancy programming of breast milk composition.
3. Breast milk cytokines, growth factors and hormones are additional constituents of maternal "lactotypes" which may predict atopic and metabolic disease in offspring.
4. Pollutants in breast milk may also increase risk of disease in offspring.

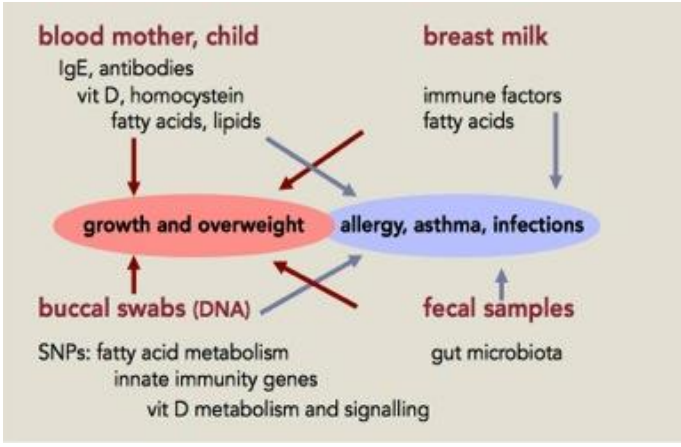
We want to thank everyone who attended the pre-workshop 'Lacto-Active' meeting. Please contact Anita Kozyrsky kozyrsky@ualberta.ca if you would like more information or want to add new aspects to these collaborations.

# Main Meeting

## New Projects and New Friends:

**We welcomed many new collaborators to the *in-FLAME* network this year including new members from the Netherlands (Maastricht), Japan (Chiba), the United States (Detroit, New York and Denver), France (Nantes), Australia (Melbourne) and Italy (Verona). This workshop provided many opportunities to explore synergies and common interests.**

Susan Prescott and Harald Renz opened the meeting, taking the opportunity to restate the philosophies of our network - based on a shared vision, generosity, openness and collaboration. In and new age addressing the biology of complexity, it is of the utmost importance to take a multidimensional approach examining multisystem outcome with open collaboration for mutual benefit. This was followed by a Keynote presentation from Carel Thijs on *The immune system as a central common target for preventing NCDs*, giving valuable examples from the lesson for KOALA cohort. (Dr Thijs had also presented on breast milk lactotypes in the LactoActive program). This set the scene for collaborative discussions, which continued with many other opportunities for new members, including students and early career researchers to discuss their work - and even more informal opportunities to explore new ways of capitalizing on our expertise, cohorts and other assets. There was an important continued focus on harmonizing methods and protocols prospectively, to ensure standardization and long-term capacity. We recognize that this may take many years to build, but that it is important to lay the foundations now. In particular, there was a focus on sharing clinical trial protocols that will allow us to assess the differential impact of various interventions across different (genetic and environmental). This will allow both 'stand-alone' publications as well as comparative Studies and pulled samples for large scale analysis (e.g. genetic studies and studies of complex interactions).


Prof Carel Thijs discusses importance of integrated multisystem approaches, using examples from the KOALA cohort.

# Helminths and allergy in South Africa and Northern Europe

**Randi Bertelsen (Bergen)** presented a new project just funded by the **WUN Research Development Fund (RDF)**. Areas with high prevalence of helminth infections often have a low prevalence of allergic disorders and autoimmune diseases, but rates of most of these conditions have increased as helminth prevalence has fallen. Helminth (parasitic worms) infections stimulate IgE responses, reflecting a strong Th2 expansion – and, thus, most complex than the simple 'Th1 shift' concept of the hygiene hypothesis. Rather, Helminth exposure may reduce the risk of allergy by enhancing immune regulatory responses (i.e IL-10 producing B regulatory cells and anti-inflammatory IgG4 antibodies). Helminth exposure in Europe may be more than we believe.

- *Enterobius* 30%+ (Oslo; children 6-11 yrs)
- *Toxocara* zoonotic infection. 1-30%+ seropositive
- Approx 10% IgE seropositive for Ascaris

## PROJECT QUESTIONS

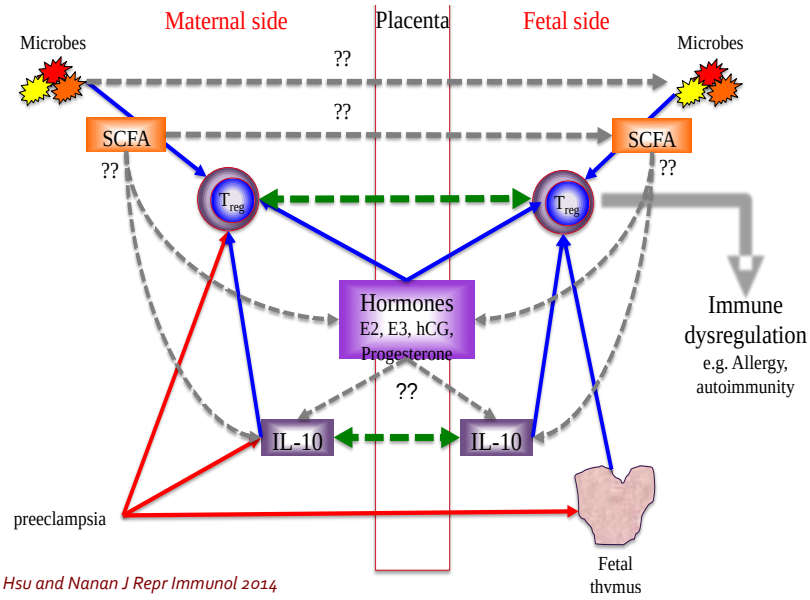
1. How common is exposure to helminths in Northern Europe?
    a. Socioeconomic differences (Norway vs Estonia)
    b. Geographical differences (Northern Europe vs Africa)
2. Does any helminth exposure relate to lower incidence of allergy?

**This project** will analyse Helminth antibodies in relation to allergy markers (clinical disease and IgEs) in cohorts from Norway, Estonia and South-Africa.

**Main Partners:** University of Bergen, Norway (Prof Cecilie Svanes, Dr. Randi J. Bertelsen), University of Cape Town (Dr William Horsnell, Prof Mike Levin), University of Southampton (Prof John Holloway), Aarhus University (Ass prof Vivi Schlunssen), Tartu University (Dr. Rain Jogi)

# Antenatal thymic size – role in predicting disease?

**Ralph Nanan (University of Sydney)** presented fascinating data showing the relationship between fetal thymic size measured by 2-D ultrasounds, and the development of subsequent pre-eclampsia. Fascinatingly, a smaller fetal thymus *preceded* pre-eclampsia in a prospective study, and this was highly significant (P<0.0001), suggesting much earlier immune dysregulation, already evident in the fetus. There newer studies using fetal thymus 3D measurements indicate and approximate 50% reduction of fetal thymic volume in pre-eclamptic pregnancies. This provides novel perspectives on the development of pre-eclampsia and dysregulation at the materno-fetal interface. In collaboration with **Peter Hsu**, they have now demonstrated differences in regulatory T-cell populations with reduced FOXP3+ and FOXP3+/Helios+ populations in pre-eclamptic content to normal pregnancies. This creates enormous opportunities to retrospectively explore antenatal thymic in relation to a range of antenatal exposures (especially nutrition, microbiome and pollutants) and postnatal outcomes.



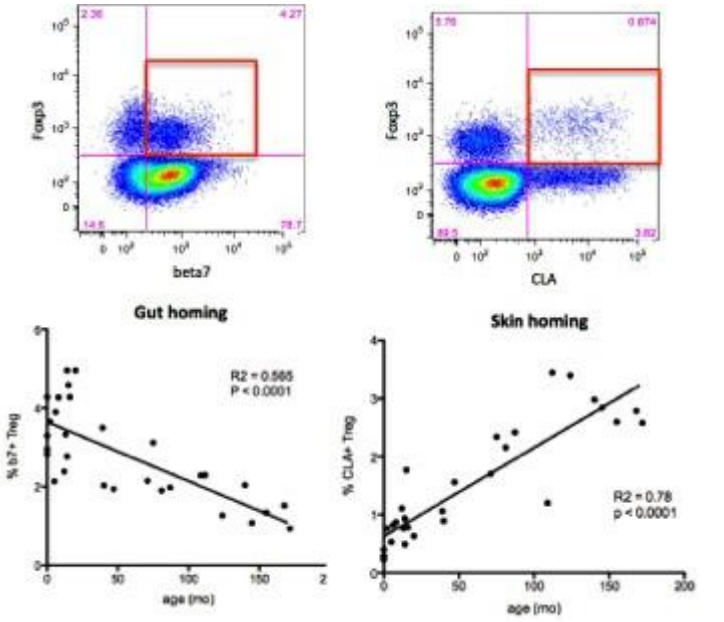

*Hsu and Nanan J Repr Immunol 2014*

## KEY OUTCOMES

1. Automated technologies can now be applied to generate thymic measurements from existing ultrasound data in previous cohorts
2. These measurements maybe correlated with a range of antenatal exposures and postnatal outcomes that may be linked with antenatal immune function
3. Ralph Nanan invited members to make contact if they wish to apply these measurements to their own cohorts.

# Novel studies showing changes in Treg with age.

**Peter Hsu (ECR - University of Sydney)** presented new data on the ontogeny of regulatory T cells in early life. His work demonstrates that with age, and increased antigenic stimulation, Treg cells become more activated and induced in the periphery (as opposed to central thymic induction). He also described significant changes in the pattern of tissue homing. Treg cell 'gut homing' patterns are established prenatally and then decline with age. In contrast, 'skin homing' Treg develop largely in the postnatal period and increase with age. Dysregulation of these processes may have implications for development of both food allergy and eczema.



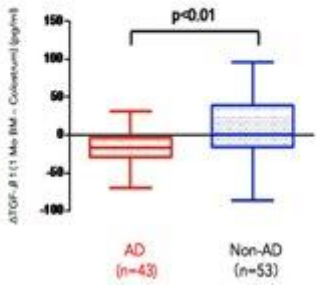

# Interventions that change immunomodulatory characteristics of breast milk may reduce allergy

**Prof Naoki Shimojo (China University, Japan)** presented a number of their studies looking at the immunomodulatory characteristics of breast milk and how this may modify the risk of allergy. Breast milk contains allergy-protective and allergy-promoting immune active substances. Decreased allergy-protective substances such as TGF-beta, soluble CD14 and n-3 PUFA in BM may lead to development of eczema in infancy. Increased allergy-promoting substances such as CoA, cytokines/chemokines and saturated FA/n-6 PUFA in breast milk may lead to development of eczema. Preliminary studies using maternal prebiotic fibre (FOS) dietary supplements in pregnant and lactating women appear to increase allergy-protective immune substances such as IL-27 in breast milk. Larger interventional studies are necessary to substantiate these findings – and are currently being developed as part of the BIRTH EU2020 proposal.

## KEY OUTCOMES

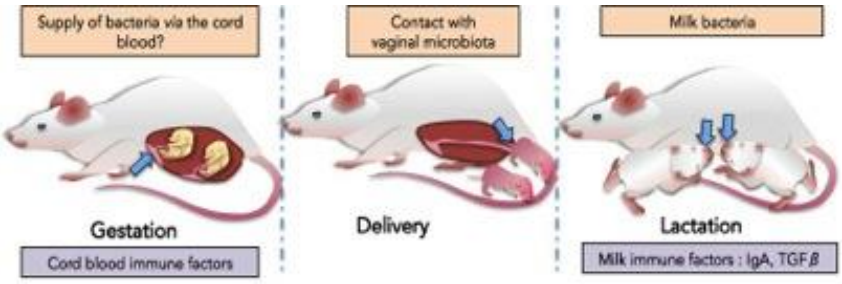
1. Plans to undertake more comprehensive analysis of breastmilk including MicroRNAs, metabolomics, and breast milk microbiome (already discussed as part of the BIRTH EU2020 proposal)
2. Plans to harmonise first clinical trials of prebiotic fibre in pregnancy and lactation – initial RCT in Australia (UWA 'SYMBA Study', Telethon Kids Institute) and Japan (also as part of the BIRTH EU2020 proposal).

TGF-beta1 in BM given to babies who developed AD and those who did not develop AD at 6 mo of age




## Prebiotics: more lessons from animal studies

**Dr Marie Bodinier (Nantes, France)** presented results of new studies demonstrating the multifaceted effects of maternal dietary fibre in protection from food allergy in the offspring. Specifically, administration of GOS/inulin prebiotics supplementation in mothers (gestation and lactation) was associated with the reduction in markers of the allergic phenotype (food specific IgE, IL-4 and IL-5), flavouring Th1 (IFNγ, TGFβ, specific IgG2a and IgA) and tolerance markers (Treg) in the pups. This was associated with favourable effects on intestinal microbiota, reduced intestinal permeability, and reduced in clinical food allergy, *(Bouchaud, Bodinier. et al, in prep).*




### KEY OUTCOMES

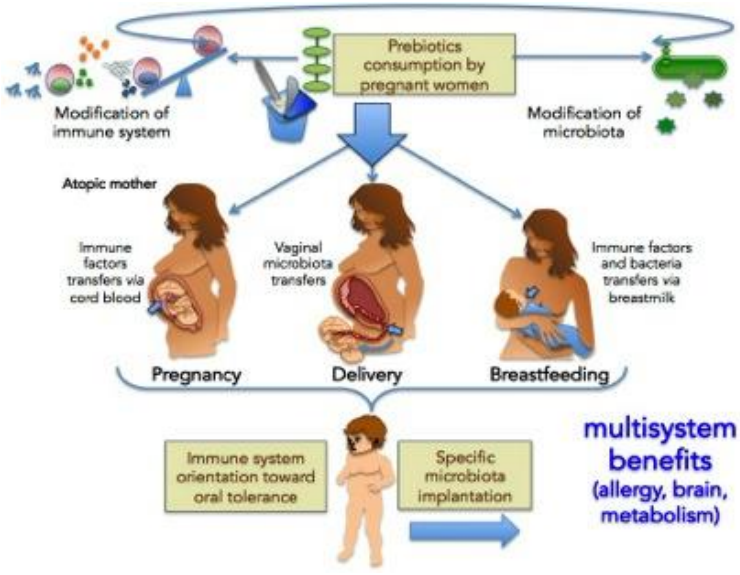
1. Marie will lead an inventory for those working with mouse models of food allergy/allergic march/allergic disease; explore possibility of systems biology approach
2. Marie will spend 12 months in Australia (UWA) in 2016 to work on human trials of prebiotics in pregnancy and lactation, and will contribute to both clinical and laboratory aspects of the SYMBA project (at Telethon Kids Institute).

## The SYMBA study: a clinical trial of prebiotics in pregnancy and lactation – an update

**Dr Debbie Palmer (UWA)** presented an update on progress with the SYMBA study that is planned to commence in September 2016. Seminal studies by our collaborators **(Prof Charles Mackay, Monash)** show that increasing dietary fibre favourably changes gut microbiota, with local and systemic anti-inflammatory effects through production of short chain fatty acids (SCFA) and associated metabolites. To explore this in humans, we will assess effects of a maternal dietary prebiotic intervention (9g/day GOS/FOS) from 16-18 weeks of pregnancy to 6 months of lactation, on eczema and food sensitisation at 12 months of age, and will have capacity to examine metabolic and developmental outcomes. It will commence as the first nested clinical trial within the ORIGINS birth cohort (a collaboration between **Telethon Kids Institute** and **Joondalup Health Campus**).

### KEY OUTCOMES

1. Initial funding (Telethon Perth Children's Hospital Research Fund) will allow this study to commence (n=200) and assess biological outcomes.
2. A current NHMRC application is under consideration to fund the full study (n=650) to examine clinical outcomes.
3. This will also form part of the EU2020 program – with the aim of harmonising with studies in Japan and others.
4. Marie Bodiner will join the SYMBA team in 2016.



# The BOPIA Study: prebiotics in the treatment of peanut allergy

**Prof Dianne Campbell (University of Sydney)** presented the BOPIA clinical trial study to examine a novel strategy in the treatment of children with established peanut allergy. It will which will used a combination of classical oral immune-therapy (OIT) combined with short chain fatty acids (SCFA) as immunomodulating agents. This concept is based on new data from **Charles Mackay's** group which have shown that combining SCFA with food allergens significantly improves tolerance induction in animal models of food allergy in the induction of oral tolerance.

## KEY OUTCOMES

1. This is the subject of a current NHMRC application.
2. Collaborators at Imperial College London up planning a similar study in adults.
3. Denver (Fleisher) and Alberta (Korzyrskyi) are being explored as additional sites
4. Japanese (Shimojo and Suzuki), will explore a similar (protocol using a different SCFA agent.

# Changes in gene expression with administration of synbiotics for oral immunotherapy in children with egg allergy

**Shuichi Suzuki (Chiba University, Japan)** presented pilot data from infants randomised to either oral immunotherapy (OIT) alone (control group) or OIT+synbiotics (test group) containing *Bifidobacterium bifidum* OLB6378 $2\times10^{10}$ cells/day and Fructooligosaccharide 2 g/day. Blood was collected at baseline and after 6 months. Relative to the controls the test group (OIT+ synbiotics) showed relative increase in IFNG and relative decrease in chemokines (CXCR2, CCL4L2, CXCL3, CXCL1).

## Key outcomes

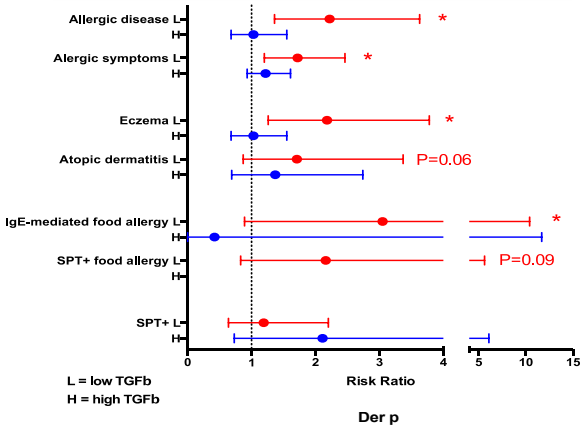
1. These changes suggest that synbiotics facilitate tolerance by suppressing intestinal Inflammation.
2. This group plan future studies with the Sydney group – intending to harmonise with the BOPIA Study.

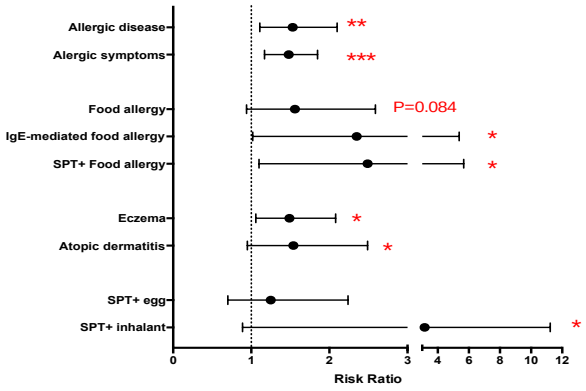
# Allergen and cytokine levels in breast milk modulate allergic risk

**Prof Valerie Verhasselt** and **Dr Meri Tulic (Nice, France)** presented work perfomed in collaboration with **Prof Susan Prescott (UWA, Australia)** and **Jon Genuneit (Ulm, Germany)** using breast milks samples from Perth cohort studies. This was developed in Cape Town and the manuscript is in preparation.

## KEY FINDINGS

1. Der p and OVA allergens are present in human milk in similar levels and are conserved geographically
2. In allergic mothers Der p in BM primes for sensitization at age 1 and is a risk factor for food allergy eczema)
3. Low TGFb in combination with Der p in BM increases the risk of allergic disease.
4. This adds to evidence that variations in BM composition may alter disease risk.



## Aeroallergen levels and the risk of eczema in Japan

**Prof Naoki Shimojo (Chiba)** also presented results from the Chiba High Risk Birth Cohort for Allergy (CHIBA) Study which examined the relationship between environmental house dust mite (HDM) allergen levels (measured as Der1), eczema and HDM sensitization in infancy. Half of children with eczema at 1 year old were sensitized to HDM at 2 years old regardless of HDM concentration. The findings confirm that eczema is a major risk factor for respiratory allergen sensitisation.

### KEY OUTCOMES

1. Der 1 concentration at 3 months of age was associated with subsequent Der 1 sensitization
2. Children with AD at 6 months of age have a low threshold for Der 1 sensitization at 1 year of age

## Mode of delivery, duration of labor, & cord blood inflammation

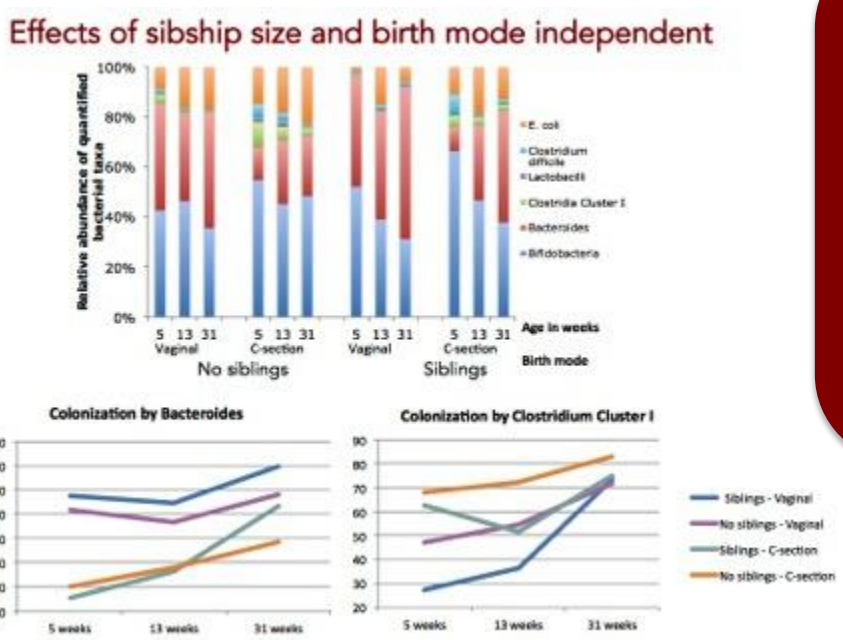
**Dr Jon Genuneit (Ulm, Germany)** discussed their effects of delivery mode and other maternal factors on inflammatory markers in cord blood. The analysis compared non-labor versus active labor deliveries, vaginal assisted included forcep and vacuum assisted deliveries. The duration of labor was derived using mother's self reported onset of labor (recorded by hospital at admission to delivery ward) and time of birth.

### KEY FINDINGS

1. Maternal age, pre-pregnant BMI smoking were associated with cesarean delivery
2. Duration of labor was associated with increased inflammatory markers ( leptin and hs-CRP)

## Effects of sib-ship in the developing microbiome: new perspectives on the hygiene hypothesis

**Dr John Penders (Maastricht University)** showed data from the **KOALA Birth Cohort** Study (n=2834). Recruited between 2000-2002 this is the first large-prospective cohort in which fecal samples were collected for microbiota analysis (subgroup of approximately 1000 children). In a collabation with **Prof Susanne Lau (Charite, Berlin)**, they have also examined stool samples in the **German PAPS cohort**. Collectively these analysis have shown a strong effect of birth mode and birth place (home vs hospital) on microbiota composition, and that early colonization by *Clostridium difficile* (1 month) was associated with increased risk of allergic outcomes. New data show that postnatal colonisation is also independently influenced by the number of older siblings. This provides new insights into the hygiene hypothesis in allergic disease – with mediation of sibship size and birth mode effects through gut microbiota.




### KEY FINDINGS

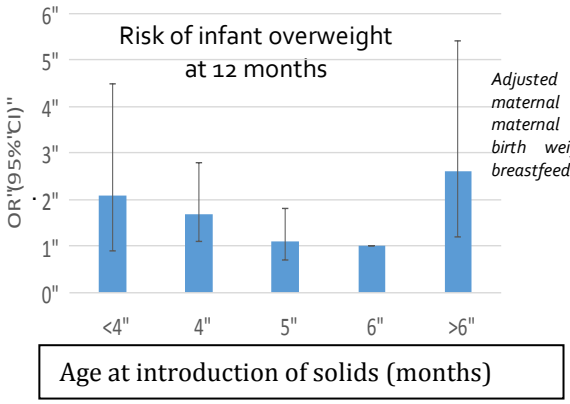
1. Early colonization by Clostridia is a risk factor for allergy
2. Clostridia colonisation is lowest in children with > = 2 siblings and born vaginally and highest in children without siblings born by C-section

### NEXT STEPS

Systems biology approach to examine host-microbial interactions, including metagenomics and plasma metabolomics

## Relation between obesity risk and age of solids introduction

**Dr Jennifer Koplin (ECR - Melbourne, Australia)** discussed data the population-based cohort (**The Healthnuts Study, n=5300**) as an opportunity to assess the impact of infant feeding practices on multiple non-communicable disease outcomes. They have previously observed that introduction of egg at 4-6 months of age associated with less egg allergy (Koplin et al. JACI 2010), and that there has been a change in infant feeding behaviour since new ASCIA guidelines in 2008 - with allergenic foods and solids introduced earlier. Tey et al. JACI 2014. These changes in feeding practice were examined in relation to the risk of overweight at 12 months of age.




*Adjusted for socioeconomic status, maternal place of birth, any siblings, maternal smoking during pregnancy, birth weight, and prematurity and breastfeeding duration*

### KEY FINDINGS

1. Solids >6 months or < 4 months was associated with infant overweight (.
2. There suggests that feeding between 4-6 months of age (allergy guidelines) does not increase the risk of overweight.

## Understanding the allergy protective characteristics of raw milk

**Prof Johan Garssen (Utrecht, Netherlands)** discussed the mechanisms of the allergy protective effects of unpasteurized farm-milk. Proteins, including allergens, are highly versatile and subject to modification by temperature, pH, matrix ,Preliminary evidence that heat treatment may modify antigen uptake (differences in uptake of native versus pasteurised ß lactoglobulin in dendritic cells. Enzymatic cross-linking of ß lactoglobulin may also increase IgE sensitisation and modify allergenicity. Raw milk treatment before senstizatizing mice with an irrelevant food antigen decreased the acute allergic skin response, and prevented allergen-specific IgE.

### KEY OUTCOMES

1. Collaboration with John Sinn (Sydney) to examine allergen modification
2. Funding for 1 PhD student to focus on tolerogenic effects of raw milk
3. Will investigation enzymes such as alkaline phosphatase in protection from mucosal inflammation.

## Vitamin D status and challenge proven IgE-mediated food allergy in the Barwon Infant Study

**Dr John Molloy (ECR - Melbourne, Australia)** discussed consistent evidence of a latitude gradient for various proxies of food allergy prevalence, and the need for longitudinal studies. He presented the recent findings of his PhD relating directly measured maternal and infant vitamin D to subsequent challenge proven IgE-mediated food allergy in the Barwon Infant Study cohort (BIS).

### KEY FINDINGS

1. There was no evidence of a longitudinal association between maternal and/or infant vitamin D status and IgE-mediated food allergy at 1 year.
2. This cohort was predominantly Caucasian with a high rate of maternal vitamin D supplementation.

## Vitamin D supplementation in Africa: a new RCT

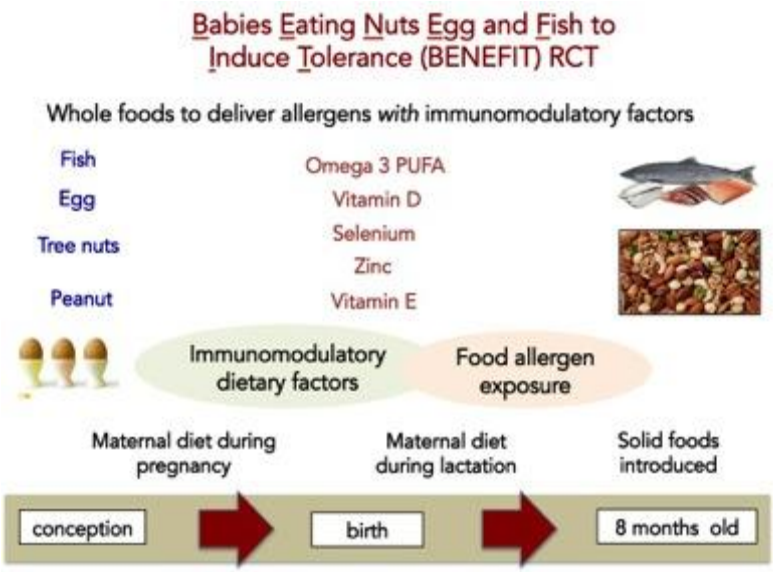
**Dr Rose Kamena (Nairobi, Kenya)** discussed the higher rates of maternal and infant vitamin D deficiency in Africa. She presented a proposal for a new clinical trial of vitamin D supplementation in African infants. This will be done in collaboration with investigators in Perth (UWA) using a similar protocol in Western Australian infants.

### KEY OUTCOMES

1. This will establish new collaborations between Aga Khan University Nairobi, UWA (Australia), and UCT (South Africa))
2. We intend to explore Australia Africa research grant opportunities – which may facilitate exchange programmes for students and staff from Africa.

## The BENEFIT Study: a new 'whole foods' clinical trial

**Dr Debbie Palmer (UWA Perth, Australia)** discussed evidence that the immune conditions that lead to fail oral tolerance and food allergies are frequently initiated prior to 4 months of age – during lactation or even in pregnancy. Earlier interventions are needed to promote tolerance during initial allergen exposure, which is likely to begin in utero. She proposed a new clinical trial taking a 'whole foods' approach which will deliver the most allergenic foods together with their natural immunomodulatory constituents, beginning in pregnancy and continuing through lactation – aiming to 'set the scene' for tolerance by the time these foods are introduced to the infant diet.




### KEY OUTCOMES

1. Collaborators in Sydney (Campbell and Hsu) expressed interest in developing this project in partnership
2. An NHMRC proposal will be considered the 2016. Commercial funding will also be explored – in particular suppliers of fresh produce.

## Immunosuppressive and transgenerational effects of *Schistosoma mansoni* on asthma

**Dr Clarissa Prazeres da Costa (Technische Universität München)** presented data on how maternal schistosomiasis affects offspring allergy susceptibility. Her data showed that infection induced inflammation alters the placental milieu and is essential to mediate protection from allergic outcomes.

### KEY FINDINGS

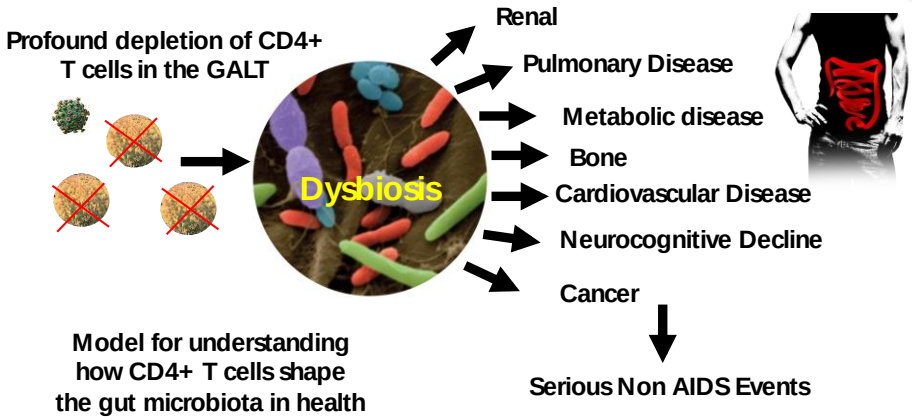
1. Maternal schistosomiasis changes susceptibility in offspring
2. Infection during pregnancy affects placental gene expression and cytokine milieu phase-dependently: major role for maternal IFN-g and VDR regulation

# Intestinal dysbiosis: the nexus between HIV and NCDs

**Prof Alan Landay (Rush University)** discussed the role of intestinal dysbiosis in the morbidity and susceptibility to effects of HIV. HIV is associated with increased pro-inflammatory bacteria (e.g. Prevotella) and decreased protective species (e.g. Bacteroides). Evidence that maintenance of mucosal barrier, lack of microbial translocation may protect from HIV comorbidities and disease progression.

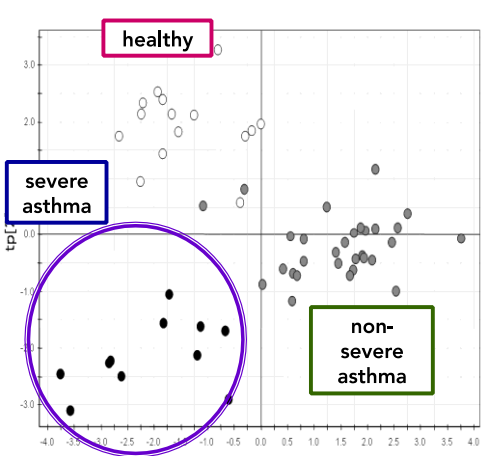
## KEY FINDINGS

1. HIV may promote intestinal dysbiosis as a result of $CD_4$ / Treg depletion
2. Simple dietary interventions (prebiotics) may play a role in preventing dysbiosis in HIV infection through anti-inflammatory effects of SCFA.

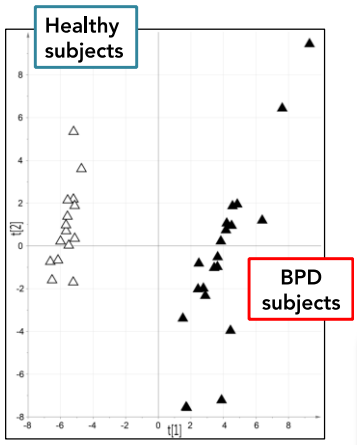



# Metabolomic approach to pediatric respiratory diseases:

**Dr Silvia Carraro (ECR - University of Padova, Italy)** discussed the use of metabolomics to investigate paediatric respiratory diseases. Together with **Prof Eugenio Baraldi** she has been applying a 'breathomics' approaches to explore metabotypes in various disease phenotypes. So far this has revealed different 'breathoypes' in the context of asthma and bronchopulmonary dysplasia (BPD). In addition, a retrospective retrospective study of women undergoing amniocentesis between the 21st and 28th weeks of gestation (n=32) show independent clustering of metabotype amniotic fluid according to subsequent preterm labour and the development of BPD.




*Carraro, Allergy. 2013;68:110-7.*




*Carraro, J Pediatr. 2015;166:234-9.e1.*

## KEY FINDINGS

1. Metabolomics applied to amniotic fluid can predict pediatric respiratory conditions.
2. This may provide a novel approach in the study and predisposition of respiratory diseases.

# Immunomodulatory effects of breast milk on intestinal epithelial cells in the context of microbial stimuli

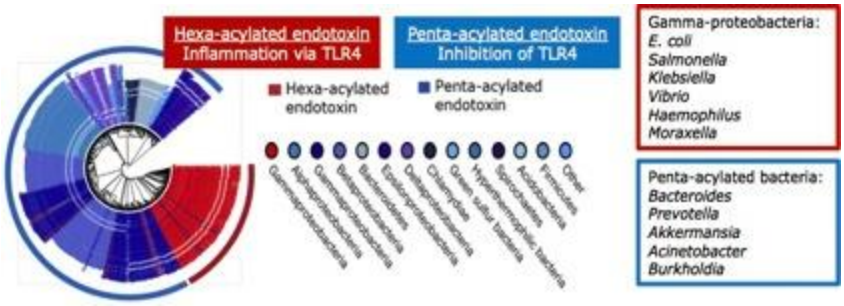
Dr Sofia Nordlander (ECR, Professor Eva Sverremark Ekström group, University of Stockholm).
Early colonisation with lactobacilli decreases the risk of allergy and inhibit pro-inflammatory planet cytokine responses to Staphylococcus aureus. Early bacterial profile correlate with response patterns (IL-4/IL-5 and IFNg) at two years of age. Sofia is performing new studies using caco-2 cells in transwell studies to examine the in vitro effects of various bacteria strains on cytokine production by epithelial cells and how this is modulated by breast milk and other factors. So far results suggest that the presence of breast milk increases production of IL-8 in vitro.

## FUTURE DIRECTIONS

Ongoing studies to determine if epithelial integrity challenged by damage can be modulated by factors present in the infant gut.

# Endotoxin variants within the lung microbiome of asthmatics

Susanne Brix (COPSAC,Technical University of Denmark) described two different endotoxin variants in whole-genome sequenced bacteria – a penta-acylated endotoxin which inhibits inflammation and downregulates Th1 and Th2 mediated inflammation, and a Hexa-acylated endotoxin which promotes inflammation through TLR4.




## KEY OBSERVATIONS

1. Variations in the patterns of the lung microbiome result in differential production of these endotoxin variants.
2. Altered lung microbiome with increased abundance of γ-proteobacteria and Hexa: penta-acylated ratio may contribute to inflammation in asthma.

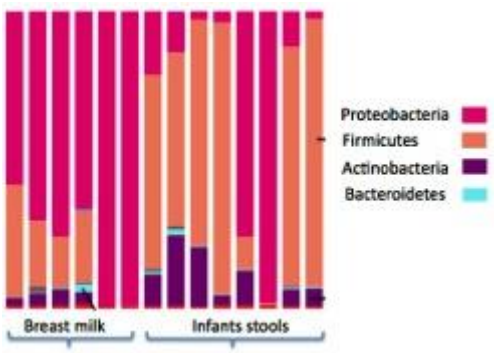
# Strategies to promote favourable infant colonisation after C-section

Dr Aveni Haynes (ECR, Telethon Kids Institute, UWA) discussed how to address the disruption of postnatal colonisation following C-section deliveries. She discussed the role and limitation of using probiotic supplements in this risk group. Group discussions centred around the optimal strategies to test in a clinical trial.

## KEY OUTCOMES

1. Potential proposals included a trial to test the practice of "seeding" - the growing trend of mothers/midwives swabbing the mouths/nostrils of infants born by C-section delivery with maternal vaginal fluid
2. Collaborators from Alberta, Umea, Linkoping will discuss further.

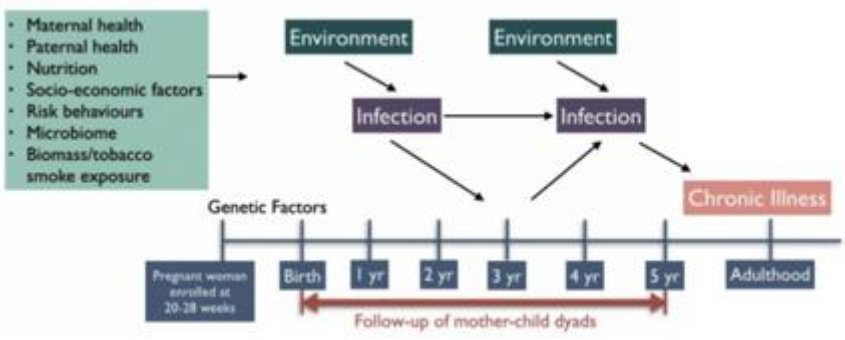
# The Drakenstein Child Health Study: the microbiome, metabolites and allergy and early onset NCDs in South Africa




**Dr Eloise Du Toit (ECR, University of Cape Town, South Africa)** discussed the extensive data and sample collection from the Drakenstein child health study, and the proposals for microbiome and metabolic analysis of breast milk, serum and stool sample is in relation to the early environment and subsequent risk of disease. Opportunities for collaborations with other cohort studies were discussed.

## KEY OUTCOMES

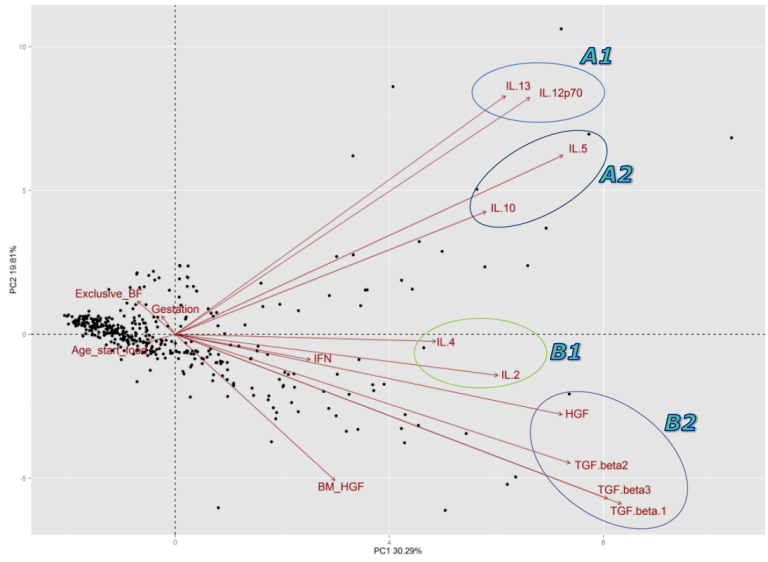
1. Preliminary international comparative studies (with other cohorts in Australia, Japan, Canada and Europe) have already commenced
2. Urine and breast milk samples have already been sent for comparative metabolic / pollutant analysis.




# Determinants of colostrum and breast milk immune 'lactotypes' and consequences for infant health

**Dr Daniel Munblit (ECR, Imperial College London)** discussed a ***prospective*** multicenter ***cohort study of mother/infant Pairs from*** *London (N=105)*, *Moscow (N=200)* and *Verona (N=80)*. The aims were to examine colostrum/breast milk composition for distinct patterns or clusters of immune mediators to characterize them into specific "lactotypes". This revealed several key **lactotypes"** : A1 (IL5 and IL10), A2 (IL12 and IL13), B1 (IL2 and IL4), B2 (TGFβ1,2,3 and HGF). Mothers from London tend to fall into cluster B "lactotype" groups significantly more often in comparison to women from Moscow and Verona. They then assessed if any "lactotype" was associated with maternal or environmental exposures and/or immunological outcomes in the infants at 6 and 12 months of age.

Defining Lactotypes by PCA




## KEY FINDINGS

1. Levels of cytokines and growth factors in colostrum declined rapidly over time
2. Significant differences in breast milk composition between countries
3. Speciifc "lactotypes" (A1 and A2) were associated with reduced incidence of itchy rash, cough or wheeze and any reported immunological outcome at one year of age

## Parasites and the Placenta

**Prof Cathy Thornton (Swansea University)** has been working on a collaboration with **Bill Horsnell (University of Cape Town)** and **Karl Hoffmann (Aberystwyth University)** since the 2014 meeting in South Africa. The trematode flatworm that causes schistosomiasis affects over 200 million people worldwide (including around 40 million women of reproductive age. The main goal this collaboration is to understand the impact of parasitic infection on the placenta and implications for the next generation.

### PROGRESS

This work is still ongoing and will examine in vitro models using placental samples. And examined by invasion and production of inflammatory cytokines in response soluble (endotoxin free) adult worm antigen and soluble egg antigen. Results are anticipated by the next meeting

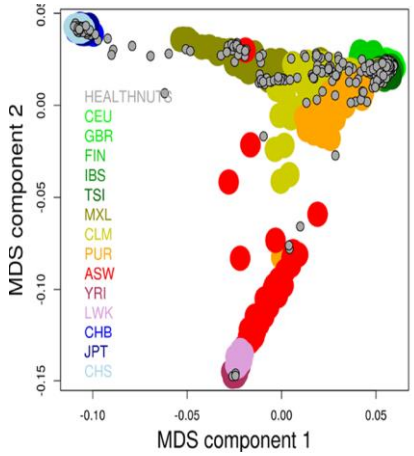
## Using genetic admixture mapping to understand pathways and susceptibility to food allergy

In Australia food allergy is 2-3 times higher in children of East Asian decent. The paradox is, that food allergy rates in Asia are much lower than Australia. **Dr David Martino (ECR - Murdoch Children's Research Institute)** explored the hypothesis that the frequency of particular genotypes could be more prevalent in Asian populations. These genotypes may have been selected over the course of evolution to be optimal for a particular type of immune response that is optimal against factors endemic in the East Asia region (e.g. particular bacterial populations), but they may come at a cost of suboptimal responses to other exposures. Because chromosomes are inherited in chunks, the offspring of ancestral populations that have interbred have chunks of chromosomes from both ancestral populations, and that's why they lie half way between the European and Asian clusters. So we can capitalize on this using a genetic mapping technique known as Admixture mapping. Admixture mapping is a way of localizing chromosomal regions of excess ancestry with respect to the phenotype. David and collaborators are now applying this to the HEALTHNUTS population.

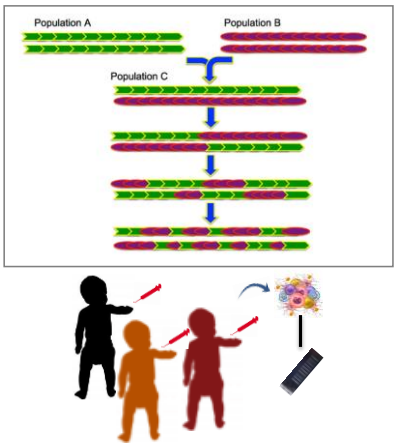
### KEY POINTS

1. 'Admixture mapping' is a powerful approach that leverages ancestral data to identify chromosomal segments linked to disease.
2. These methods may provide unique insights into the initiating pathways of food allergy
3. Ultimately this may enable us to identify risk populations and target therapies in a more effective manner

Population structure




Genetic admixture




# Report: Epigenetic Collaboration

Our epigenetics collaboration established in 2012 has been one of the most productive aspects of the in-FLAME network, with several projects examining maternal nutrition in pregnancy on both DNA methylation and acetylation in cord blood CD4+ T cells. This has provided opportunities for ECR leadership and PhD student exchanges – largely between Germany and Australia. Currently the major partners are (more welcome):

| | |
|---|---|
| University of Western Australia | Manori Amarasekera (ECR), Paul Noakes, Susan Prescott |
| Philips University Marburg | Hani Harab (ECR), Dorthe Kesper, Harald Renz |
| Telethon Institute for Child Health Research | Deborah Strickland |
| Murdoch Children's Research Institute | David Martino (ECR) Sarah Ashley (ECR) Richard Saffery |
| University of Adelaide | Antonio Ferrante |

## KEY FINDINGS

1. Genome-wide DNA methylation profiling identified a folate-sensitive region of differential methylation upstream of *ZFP57* imprinting regulator
2. Hypomethylation at this region replicated in an independent sample set. This data suggests that exposure to folate has effects on the regulation of DNA methylation during fetal development, and this may be important for health and disease.

## Effects of maternal folate status in pregnancy on neonatal genome-wide DNA methylation profile

**Presented by Dr David Martino (ECR):** Folate intake during pregnancy may affect the regulation of DNA methylation during fetal development. The genomic regions in the offspring that may be sensitive to folate exposure during *in utero* development have not been characterized. Using genome-scale profiling we investigated DNA methylation in two immune cell types ($CD4^+$ & antigen presenting cells, APCs) isolated from neonatal cord blood, selected on the basis of in utero folate exposure. High (HF, n=11) and low folate (LF, n=12) groups were selected from opposite extremes of maternal serum folate levels measured in the last trimester of pregnancy. A comparison of these groups revealed differential methylation at seven regions across the genome. By far, the biggest effect observed was hypomethylation of a 923bp region 3kb upstream of the *ZFP57* transcript, a regulator of DNA methylation during development, observed in both cell types. Levels of H3/H4 acetylation at *ZFP57* promoter and *ZFP57* mRNA expression were higher in $CD4^+$ cells in HF group relative to the LF group. Recently published (Amarasekera et al. FASEB in press).

## Maternal fish oil supplementation: effects on Epigenome-wide analysis of neonatal $CD_4$ T-cell

**Presented by Dr David Martino (ECR):** Supplementation of fish oil rich in omega-3 polyunsaturated fatty acids (n-3 PUFA) during pregnancy has been shown to confer favorable health outcomes in the offspring. In a randomized controlled trial, we have previously shown that n-3 PUFA supplementation in pregnancy was associated with modified immune responses and some markers of immune maturation. However, the molecular mechanisms underlying these heritable effects are unclear. To determine whether the biological effects of maternal n-3 PUFA supplementation are mediated through DNA methylation, we analyzed $CD_4$ T-cells purified from cryo-banked cord blood samples from a previously conducted clinical trial. Of the 80 mother-infant pairs that completed the initial trial, cord blood samples of 70 neonates were available for genome- wide DNA methylation profiling. Our analysis of the microarray data did not suggest strong modifying effects of in utero n-3 PUFA exposure on $CD_4$ T-cell methylation profiles, and no probes on the array met our criteria for further validation.

### KEY FINDINGS

1. Comparison of DNA methylation profiles between the supplement and control groups did not reveal significant differences in CpG methylation, at the single- CpG or regional level.
2. Tests for association between methylation levels and key n-3 PUFA parameters, DHA, EPA or total n-3 PUFAs were suggestive of dose-dependent effects, but these did not reach genome-wide significance.
3. Other epigenetic mechanisms may be more relevant mediators of functional effects induced by n-3 PUFA.

## A miniaturized, validated method to assess histone acetylation

**Presented by Dr Hani Harb (ECR):** In order to understand the cause – effect-relationship between environmental exposures and disease development, methods are needed to assess epigenetic regulation even in large cohort studies. For this purpose, we have developed and validated a protocol which allows assessment of histone acetylation, in a quantitative fashion in candidate genes.

### KEY OUTCOME

The method of chromatin immunoprecipitation (ChIP) has been validated for a minimum requirement of $1 \times 10^5$ target cells (e. g. $CD_4$+ T cells). This method was then applied to assess $H_3$ and $H_4$ histone acetylation changes in neonatal samples from an established cohort (below).

## Relationship between maternal folate levels and histone acetylation of neonatal $CD_4$ T cells

**Presented by Dr Hani Harb (ECR):** This new method (above) was applied to sampled from a large prospective cohort. 23 neonates were selected from the two extremes of maternal serum folate levels. DNA methylation and histone acetylation were analyzed in genes associated with allergy development in $CD_4^+$ T cells. Cytokine production was measured following c stimulation of cord blood mononuclear cells (CBMC) with ovalbumin (OVA). The whole cohort DNA methylation and histone acetylation profile revealed a bias towards a Th2 phenotype. Maternal serum folate levels are likely influencing the developing fetal immune system by modifying epigenetic marks.

### KEY FINDINGS

1. The whole cohort DNA methylation and histone acetylation profile revealed a bias towards a Th2 phenotype.
2. $H_3$ and $H_4$ acetylation at *GATA3* and $H_4$ acetylation at *IL9* was increased in children born to mothers with high serum folate, together with decreased acetylation at the *IFNG* locus.
3. High folate levels promote a transcriptional permissive chromatin state particularly at Th2 loci and might thus affect disease development in later life.

## Neonatal PKC zeta determines T-cell maturation and is epigenetically modified by maternal fish oil intake

This collaboration (Marburg, Adeliade and Perth) investigated the relative immaturity of the neonatal immune system and its relationship to allergic predisposition, together with how this is modified by fish oil supplementation during pregnancy. We demonstrated that the basis for low production of IFNγ in cord blood mononuclear cell (CBMC) cultures lies not only in an intrinsic deficiency in the T cells, but also with the accessory cells, which show low production of IL-12 and elevated production of IL-10. Addition of either anti IL-10 antibody or recombinant IL-12 increased IFNγ production. The development of CB T cells towards maturity and Th1 polarisation was dependent on PKCζ levels. Knocking down PKCζ in CB T cells using shRNA nucleofection hindered this development. Using a mouse model of neonatal T cell maturation, we show that low PKCζ at birth normalises towards adult levels by the end of the neonatal period.

### KEY FINDINGS

1. An increase in PKCζ levels in neonatal T cells after maternal fish oil was associated with $H_3$ and $H_4$ acetylation, suggesting a nutrient induced epigenetic regulation of PKCζ expression and protection against allergic diseases.
2. Immaturity of expression of PKCζ in neonate may ensure phenotypic plasticity during early immune development

# Report: Mental health and nutrition

A/Prof Felice Jacka joined our network to discuss the role of nutrition in the treatment and prevention of mental health disorders. She gave a keynote presentation highlighting the importance of dietary patterns in mental health and has a particular interest in nutritional approaches in pregnancy to improve cognition and long term mental health of the next generation.

Her work has shown that the impact of early life nutritional exposures extends from physical to mental health. Studies of dietary patterns in over 23,000 mother-babies pairs, revealed that higher intake of 'unhealthy foods' by mothers during pregnancy is associated with increased externalising behaviours in children. In children both higher intake of 'unhealthy foods' and lower intake of 'healthy' foods increase the risk of early internalising and externalising behaviours Jacka et al. (2013) JAACAP. Similar observations have now been made in other cohorts (Generation R, Steenweg de Graaf Clin Nutr 2014), with poorer cognitive function and emotional dysregulation in offspring of mothers who had low intake of healthy (Mediterranean type) foods compared with 'unhealthy foods'. These effects are independent of postnatal depression, postnatal nutrition and a large range of socio-demographic and familial factors. Even in maturity there is growing evidence that dietary improvements can reduce inflammation, and the risk for depression.

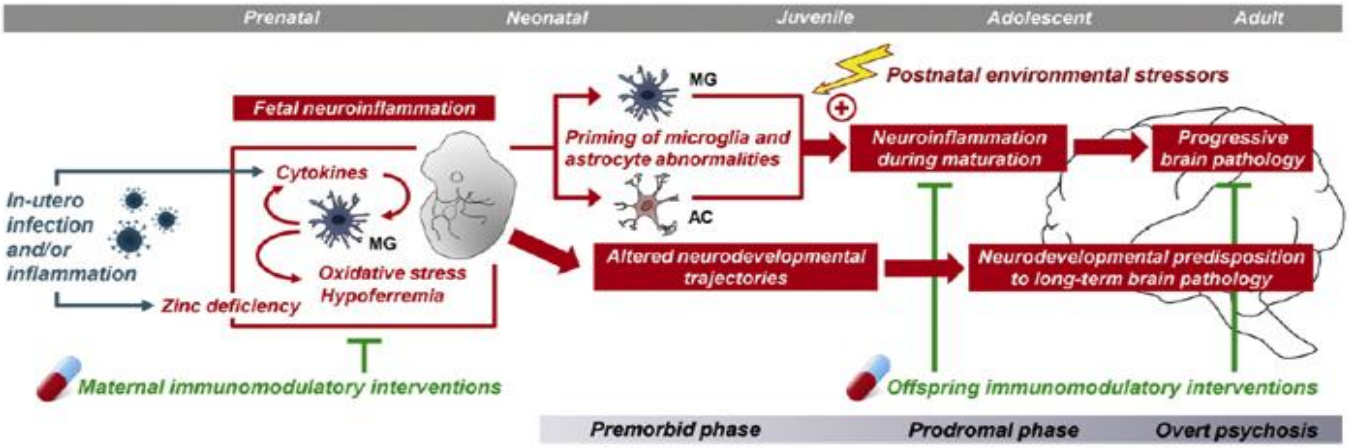
The mechanisms of these effects are likely to be complex and include epigenetic effects on key developmental pathways, perturbations in the serotonergic system, altered sympathetic nervous system activityand upregulation of inflammatory and oxidative stress pathways and mitochondrial dysfunction, as demonstrated in many animal models. These studies also demonstrate a strong link between inflammation and mental health – which may be mediated or abrogated through diet.

Another important avenue of effect is via the 'gut=brain axis'. Individual 'enterotypes' are determined by long-term dietary patterns, particularly protein and animal fat (Bacteroides) versus carbohydrates (Prevotella). New human studies demonstrate differences in the microbiota composition in individuals with major depressive disorder (MDD). *Bacteroidetes* and *Proteobacteria* were significantly more abundant in MDD subjects compared to HC, whereas theproportion of *Firmicutes* was lower (Jiang et al. Brain, Behavior and Immunity 2015).

Controlled feeding studies show that microbiome composition changes detectably within 24 hours of initiating a high-fat/low-fiber or low-fat/high-fiber diet, although enterotypes remain stable (Wu et al. Science 2011). Microbial diversity is also associated with improved working and reference memory and reduced anxiety behaviour (Li et al. JPhysio Behav 2009). Other experimental evidence shows altered gut microbiota in animal models of depression (Park AJ, 2013) and early life stress (O'Mahony SM, 2009), and that probiotics can reverse anxiety-like behaviors in animal models of IBD (Bercik P et al.2010). *Lactobacillus rhamnosus (JB-1)* has been shown to reduce anxiety and depression related behaviors and also altered expression of GABA receptors (Bravo JA et al.2011). Furthermore, 'swapping' intestinal microbiota of mice can switch their behavioral phenotypes (Bercik P et al. 2011). In humans probiotics have been shown to ameliorate psychological distress and reduce cortisol in healthy volunteers (Messaoudi et al.2011), and improved psychological symptoms in patients with chronic fatigue syndrome (Rao et al.2009). Fermented milk products with probiotics also appear to modulate brain activity in humans (Tillisch et al. 2013).

There is also growing evidence that maternal diet and stress in pregnancy are important factors in later risk of neurodevelopmental and mental disorders. Maternal immune activation in pregnancy appears to be a risk factor for both autism spectrum disorder and schizophrenia. In animal models, inflammation in pregnancy has been linked to intestinal permeability (Leaky gut) and the autism behavioural phenotype (social, communicative, stereotypic) – which can be ameliorated with administration of bacteroides fragilis (Hsiao et al. Cell 2013). This is also relvant to humans. In a recent epidemiological study, mothers of children with autism

spectrum disorder reported greater frequency and severity of vaginal bacterial infections during pregnancy (Zerbo et al., 2013). Chronic stress during pregnancy alters vaginal host immunity and resident bacteria composition. This suggest that body composition, diet, infection, antibiotic treatment and stress may all influence the developing brain, and subsequent mental health. They are also logical targets for prevention.




*Urs Meyer Progress in Neuro-Psychopharmacology & Biological Psychiatry (2013)*

In her summary Felice highlihhted the potential of dietary manipulation in the periconception and perinatal period as a key strategy for improving mental health outcomes in children. She also outlined her poropsal doe a new clinical trial to assess the benefits of a whole foods diet and stress management (mindfulness) on infant development. '*As such, a low-risk strategy for attempting schizophrenia prevention may consist of delivering health education and nutritional and lifestyle interventions to women during the antenatal period'* she concluded.

## NEW STUDY:

HEALTHY PARENTS, HEALTHY KIDS a preconception study whole of diet approach:

1. Diet (gut focused)
2. Stress (mindfulness)
3. Control

OUTCOMES: Microbiota and metabolites Epigenetics, Inflammation and cortisol (initial pilot study will assess feasibility)

4.

# Report: Pollutants and the Built Environment

Modern environmental change is a major factor in changing disease profiles, and in particular increase in noncommunicable diseases (NCDs). Impact of human activity on the natural environment, is in turn affecting our own health, through effects on the quality of our food and water supply and our air quality. This is interrelated to the effects on micro-biodiversity in the external and internal human environments. There is also growing awareness that declining interaction with natural 'green space' is an independent factor influencing our physical and mental wellness.

## Natural environments, ancestral diets and microbial ecology

Dr Alan Logan (New York) was invited as a keynote speaker, to discuss the evidence and the importance of 'Natural environments, ancestral diets and microbial ecology' in the changing patterns of human health. Alan is a recognised authority on the influence of 'Green Space' on cortisol stress responses, resilience, perceived stress and cognition. These effects appear independent of physical activity. Sadly, his flight was cancelled because of bad weather and we will have to wait until 2016 to welcome him to our next workshop.

The goal of this session was to highlight the interconnectedness of the built environment with other aspects of our mental and physical health and behaviour, as we move away from traditional diets and traditional environments. In particular we need to understanding impact of 'Dysbiotic Drift' in Developed Nations with expanding Western Industrial Grey Space. This is complex and likely to be associated with psychological stress, more processed and ultra-processed foods, additives advanced glycation end products (AGEs), absence of phytochemicals, inadequate essential fats, inadequate vitamin D and magnesium, antibiotic contamination, expansion of potentially pathogenic bacteria, lack of physical activity/excess indoor screen time, tobacco and alcohol use, sleep disturbances and disruption of circadian rhythms, overcrowding, climatic stress and environmental toxins. Importantly one third of the food and beverages consumed within many global traditional diets are fermented. This includes fermentation of cereals, legumes, dairy, vegetables, fruits, fish, seafood, and meats as a significant part of ancestral dietary practices. In addition to preservation, preservative effects, this also has positive effects on nutritional quality taste/texture and the microbiota. All of these influences clearly begin very early in life, with pre-conception health of the mother, and ongoing effects in pregnancy and early childhood.A newly published study of on 21,294 urban residents from 34 European nations demonstrates the importance of neighborhood environments in socioeconomic inequalities in mental well-being (Mitchell et al. Am J Prev Med 2015). This stresses the need to explore more 'equigenic environments' - and that more recreational/green areas may disrupt the usual conversion of socioeconomic inequality to health inequality. We are looking forward to welcoming Alan to Maastricht in 2016 to discuss this further.

## Early life exposure to pollutants – implications for immune and metabolic health

Prof Merete Eggesbø (Oslo, Norway) set the scene for the pollutants workshop, presenting further data from several of the European studies she has been leading (also see page 12). Synthetic persistent organic pollutants (POPs) have been extensively employed for electrical insulators and pesticides until their production and use were banned in the US in the late 1970s and in Europe in 2001. However, they persist in the environment for many years and due to their high lipophilicity and biomagnifying properties they are still being detected in human samples in most parts of the world. Fetuses and neonates are exposed to these compounds via placental transfer and through breastfeeding, and due to their relatively immature organs and detoxification mechanisms they are considered to be especially vulnerable to their adverse health effects.

Early exposure to POPs through breastfeeding significantly increases children's body burden and is thought to be the primary determinant of children's blood levels until at least 7 years of age. The impact of post-natal intake of POPs on child development has been assessed in epidemiologic studies but results are inconsistent, possibly because of limitations in methods used to assess exposure.

She presented a validated toxicokinetic model used to estimate children's blood POP levels in early to mid-childhood. Estimates can be used in epidemiologic studies to evaluate the impact of exposure during hypothesized postnatal periods of susceptibility on health. Overall, breastfeeding-related parameters has a greater relative influence on simulated POP levels from 6 months up to 45 months. For example, nearly half the variability in simulated blood POP levels at 6 months of age can be attributed to the influence of duration of breast-feeding. As expected, weight gain during pregnancy has a greater influence on simulated levels at 6 months than at 16 or 45 months, whereas postpartum weight changes became more influential over time. This model also demonstrates that estimations based solely on breast milk levels and duration of breastfeeding may lead to exposure misclassification (Figure above).

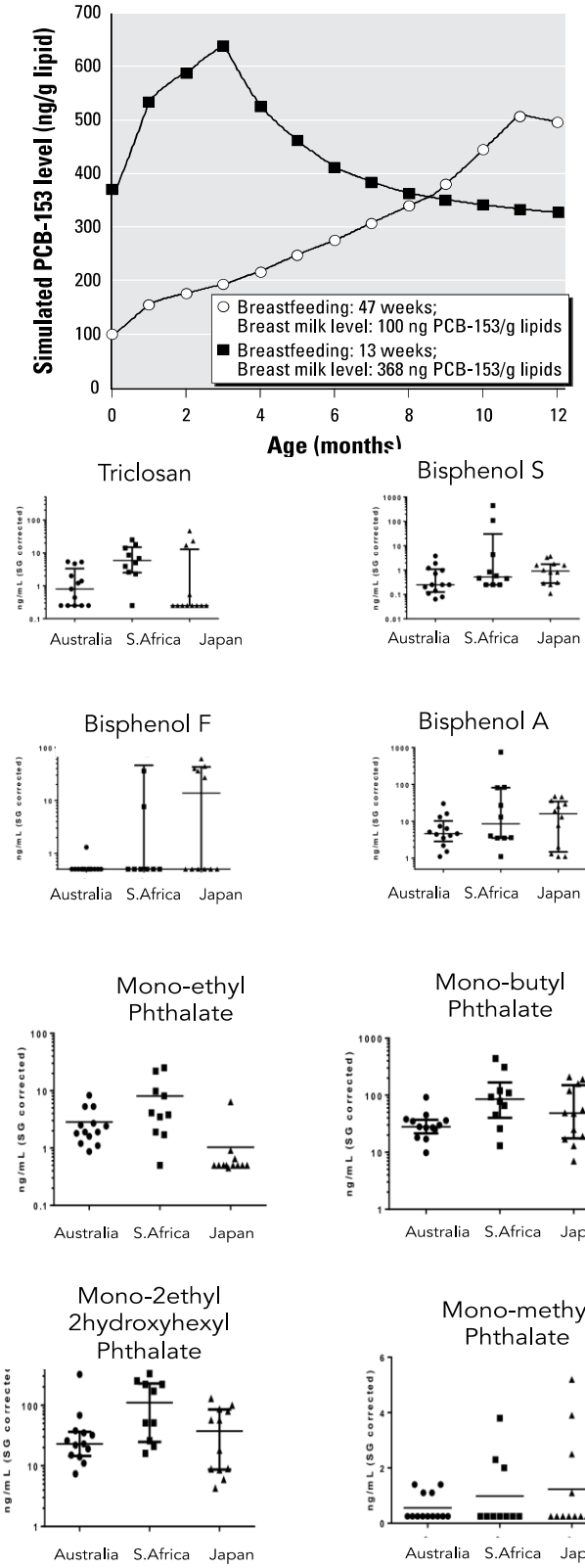
She also highlighted the importance of being aware of limitations and confounding factors in POPs analysis, including the effects of specimen collection, preservatives, and handling (e.g which may affect concentration of bisphenol A in urine).

There is a continuing need for a standardized approach to collection of samples (e.g. breast milk). Accurate recording of mothers weight is also essential for calculating toxicants transfer to the baby. Exposure estimates are also influenced by Interindividual and intraindividual differences in fat content of human milk. There are many issues that need to be addressed – and could be further developed through the in-FLAME collaborations.

# Preliminary data: infant pollutant levels in in-FLAME cohorts

**Dr Amana Wheeler, Edith Cowan University/ Telethon Kids Institute** has been leading a preliminary study to compare pollutant levels in various in-FLAME cohorts - so far results for triclosan, phthalates and bisphenol analyses.

**The samples**: Initial analysis of approximately 10 infant urine samples per cohort. Samples were collected at 12 months of age (this was selected as a common time point for most studies). All samples corrected for Specific Gravity




**The cohorts (others pending):** child health cohorts from **Australia** (Telethon Kids Institute, UWA) **Japan,** Kawatetsu-Chiba Hospital, University of Cape Town, **South Africa** and the CHILD study, **Canada.**

Some pollutants were not detected in any samples including some phthalate metabolites MOP (mono-n-octyl phthalate), MNP (Mono-iso-nonyl phthalate) and MiDP (Mono-isobutyl phthalate). Only one S. Africa sample had detectable levels of MCHP (Mono-cyclohexyl phthalate).

So far, these results suggest variability both between and within countries. This is very preliminary. Although the highest levels of some phthalates were seen in S. African infants (log-scale) there was a wide range demonstrating that some infants are exposed but others not. This may reflect differences in maternal levels, breastfeeding duration, dietary patterns and local environments. In the discussions **Prof Ruby Pawankar** indicated that high levels of pesticides on vegetables and fruits may be an issue in some regions, and another factor which could influence the developing microbiome. This could also be relevant to the project proposed by **Dr Shuichi Suzuki (Japan)** to investigate the relationship between POPs the microbiome and enterotypes (page 35). Even this initial observational data is valuable in highlighting the fact that many babies are still exposed to a variety of POPs despite regulations to reduce these.

## NEXT STEPS:

1. Additional baby urine samples and additional centres will be added to the analysis present (initially Canada)
2. The samples will also be analysed for metals analyses by ICP-MS
3. New collaborator Merete Eggesbø will add a valued dimension to this project.
4. Donna Geddes (UWA, Australia) is also planning to undertake POPs analysis on breast milk a larger sample of well characterized Perth familes – This will allow POPs levels to be examined in relation to material diet and other exposures, as well as immune, metabolic and clinical parameters in the children.

# Report: from the Microbiome Group

A/Prof Tina West (Umea, Sweden) gave an update on general progress in the last 12 months. The short-term goals of the group have been on a) developing an inventory of stool samples collected across centres, b) generating pilot data c) developing our profile through a number of key position papers in leaving journals. There has been steady progress in each of these domains. The longer term goals are to develop prospective harmonised studies.

The main challenges to large-scale analysis of existing samples have been in the considerable heterogeneity between studies (particularly in terms of sample collection methods, ages of collection, and outcome measures available), the considerable cost of microbiome analysis, and the changing technology. For this reason the focus will be on capitalising on future opportunities rather existing cohorts - with the exception of specific opportunities where samples are well harmonised.

## Gut microbiome and innate immune response patterns in IgE-associated eczema

The results of our first collaborative studies (Sweden and Australia) have been accepted for publication (West et al. Clin Exp. Allergy 2015). We examined gut microbiome development in the first year of life in relation to innate immune responses and onset of IgE-associated eczema over the first 2.5 years in children of allergic mothers. Microbial composition and diversity were analyzed with barcoded 16S rRNA 454 pyrosequencing in stool samples in pregnancy and at 1 week, 1 month and 12 months ages of age in infants who developed IgE-associated eczema (n=10) and infants who remained free of any allergic symptoms at 2.5 years of age (n=10). Microbiome data were analyzed in relation to immune responses to TLR 2 and 4 ligands at 6 months of age. There were inverse relationships between relative abundance of immunomodulatory bacteria at 1 week of age and TLR responses (SEE KEY FINDINGS). This relationship persisted at 1 month, with inverse associations between the relative abundance of *Enterobacteriaceae* (within the Protebacteria phylum) and TLR4 induced TNF-α (rs=-0.697, p=0.038) and *Enterobacteriaceae* and IL-6 (rs=-0.709, p=0.035). These findings suggest that reduced relative abundance of potentially immunomodulatory gut bacteria (particularly Gram-negative Enterobacteriaceae and Gram-positive Ruminococcaceae) is associated with exaggerated inflammatory cytokine responses to TLR ligands and subsequent development of Ige mediated eczema.

## New developments

There is obviously considerable overlap between the interests of this Working Group and the Nutrition and Metabolism Working Group. Indeed, there are now a series of clinical trials and animal models that are testing dietary strategies (such as prebiotics) which specifically target the microbiome for immunomodulation (e.g SYMBA and BOPIA). This will generate the series of opportunities to analyse both the microbiome composition and it's metabolic activity. This reflects the evolving interest in understanding not just 'who is there' in the gut, but also 'what they are doing' through microbial metabolites such as short chain fatty acids (SCFA). Metabolomics studies also offer a cheaper and more functional measure of microbiome activity.

### KEY FINDINGS

1. Mothers of infants with IgE-associated eczema had lower α-diversity of Bacteroidetes
2. The relative abundance of Gram-positive *Ruminococcaceae* was lower at 1 week of age in infants developing IgE-associated eczema.
3. The relative abundance of *Ruminococcus* was inversely associated with TLR2 induced IL-6 and TNF-α, and here was also an inverse association between the abundance of Proteobacteria and TLR4 induced TNF-α
4. At 1 year, α-diversity of Actinobacteria was lower in infants with IgE-associated eczema compared with controls

The LactoActive collaboration (page 10) lead by **Prof Anita Kozyrskyj (Alberta)** has been another important initiative arising from this group. The effects of maternal nutrition in pregnancy and lactation is of growing interest in immune programming, through

- effects of the maternal microbiome
- direct metabolic effects on developing fetus
- establishment of the pioneer microbiome (pre and postnatal)
- and effects on breastmilk composition

In addition to previously established microbiome studies (discussed elsewhere in this report) there were a number of other opportunities discussed during the meeting including

a new collaboration was established through Prof Annika Scheynius (Sweden) to analyze fecal samples collected at early time points in the ALADDIN birth cohort – in particular a collaboration with Dr Susanne Brix Pedersen, Denmark, to investigate the different variants of LPS in relation to life style and allergic outcome in the children.

A new collaborative project was suggested, where the influence of solid food introduction on gut and oral microbiome development will be studied in different regions of the world. Profs Tina West and Maria Jenmalm will develop this project in collaboration with working group members that are interested in participating.

Prof Alan Landay also suggested to include **adult stool samples** for collaborative projects on NCD development, and informed about the *cloud-based Human Microbiome Project resource*.

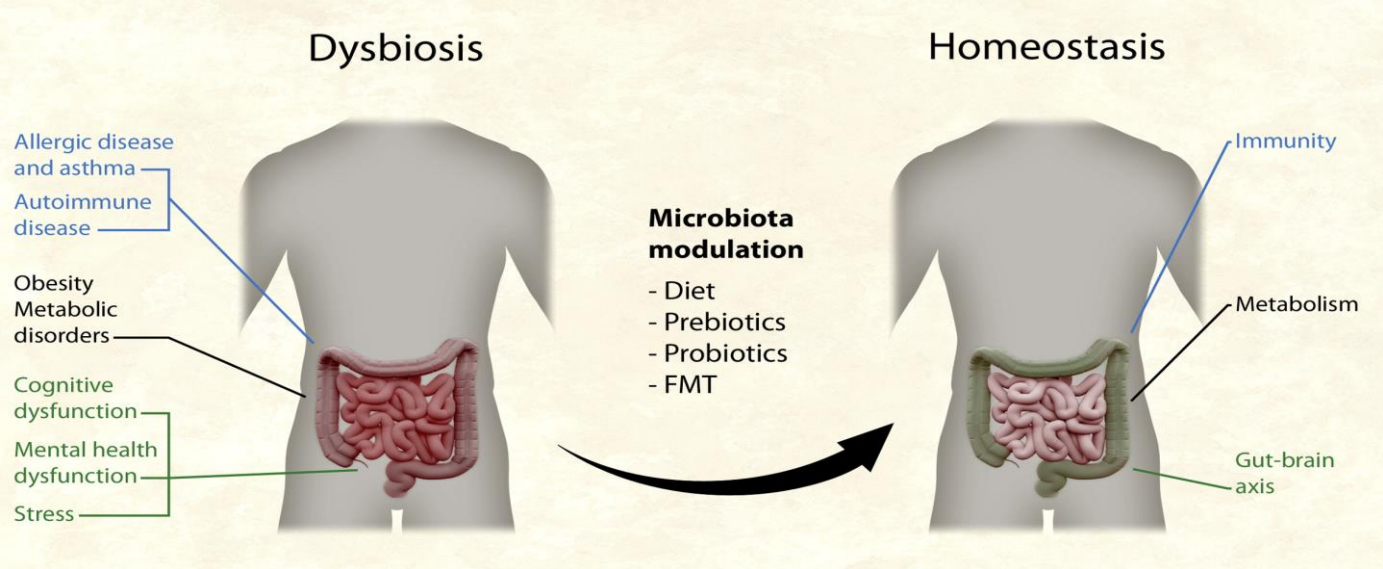
There was also a novel proposal by Dr Shuichi Suzuki (Japan) to investigate the effects of POPs on the microbiome whether different enterotypes are a) more susceptible to the effects of POPs, or b) may moodify the metabolism/bioavailabilty of ingested pollutants. This will be explored further with Dr John Penders and Dr Koen Venema (Maastricht) in a validated model of the colon (TIM-2).

There are ongoing discussions between Dr Aveni Haynes (Telethon Kids Institute), Profs Tina West and Maria Jenmalm (Sweden) about the "best" or "biologically logical" choice of intervention for babies born by C-section would be maternal vaginal fluid +/- perineal swab for exposure to maternal fecal flora. This is anticipated to be acceptable to mothers in Sweden, and Aveni will conduct community/ focus groups in Australia to assess acceptability of this approach.

As it is important to develop **harmonised methods (SOPs)** for sampling in future collaborative studies John Penders volunteered to provide sample collection and storage SOPs to the working group. These SOPs will be posted on the new in-FLAME website.

## Publications:

There have been a significant number of review papers from the in-FLAME microbiome group, including several 'perspectives' in JACI (see full publication list). Our goal is now to focus on more original research papers.



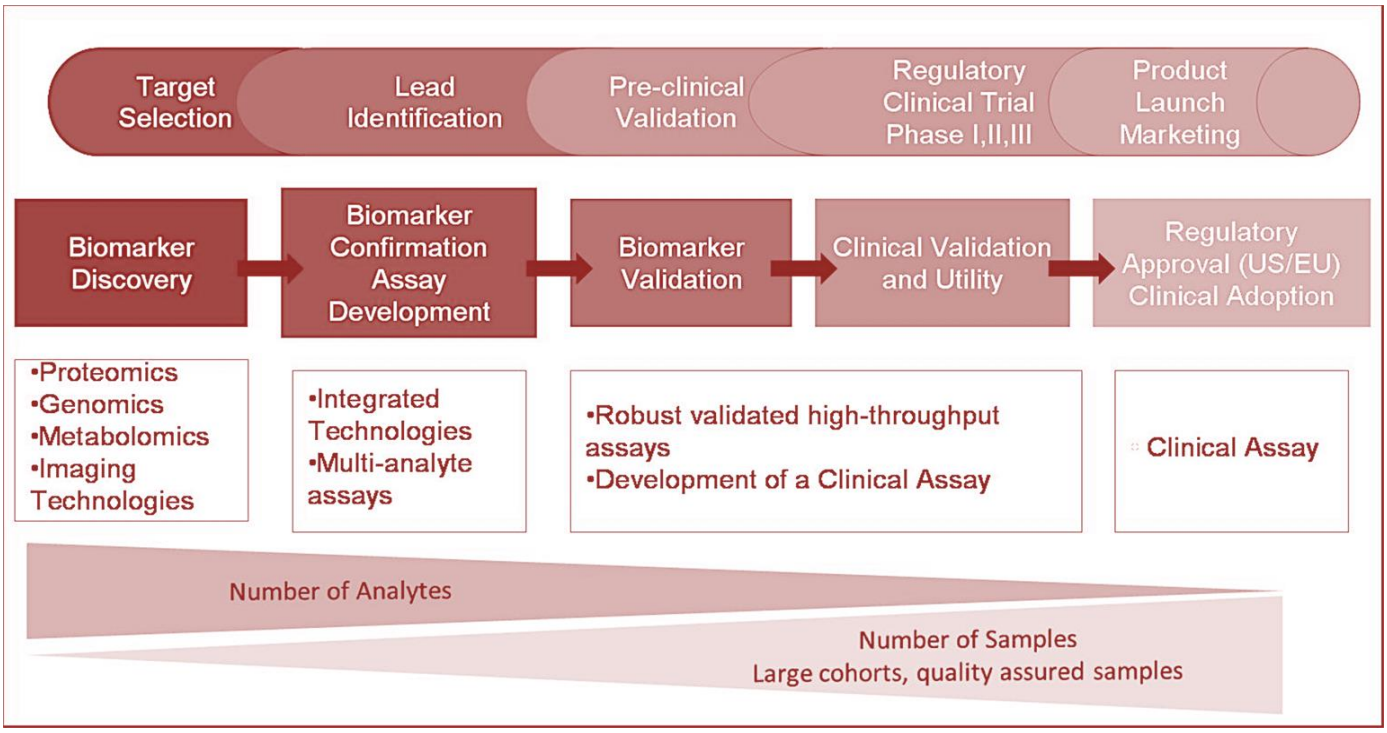
# Report: Metabolism, Nutrition and Immunomodulation

**Prof Cathy Thornton (Swansea)** submitted the following Report from *Metabolism and Immunomodulation Working Group*, In-FLAME meeting Marburg June 2015-06-16

**In attendance/expressed interest to be involved:** Phil Calder, Cathy Thornton, Chrysanthi Skevaki, Marie Bodinier, Johan Garssen, Jon Genuneit, Carel Thijs, Bianca Schaub, Silviaa Carraro, Aveni Haynes

| New Project | Lead | Action |
|---|---|---|
| Autoimmunity project | Chrysanthi Skevaki | – screen existing cohorts for autoantibodies: review inventory once updated; liaise with Mike Levin and Randi Bertelsen Jacobsen as they are undertaking a similar project for helminths; explore funding opportunities and practical issues (e.g. volume of sample needed). |
| Explore possibilities of systems biology approach in human cohorts: | Carel Thijs and Johan Garssen; | A. Identify existing cohorts that have sufficient data or could be enhanced by supplemental data that is straight forward to collect (e.g. maternal BMI) and consider alternative outcomes to allergy (e.g. weight). |
| | Bianca Schaub | B. Develop a strategy to eventually pool data from multiple cohorts for systems biology approach. |
| Animal model inventory | Marie Bodinier and Johan Garssen | Conduct an inventory for those working with mouse models of food allergy/allergic march/allergic disease; explore possibility of systems biology approach. |
| Biomarkers Review | Chrysanthi Skevaki and Marie Bodinier | Review of biomarkers in mouse versus human |
| Gestational diabetes project | Cathy Thornton and Jon Genuneit | – incorporate study of maternal/infant microbiome into any ng/planned projects related to gestational diabetes. |
| Short chain fatty acids | Cathy Thornton and Phil Calder | – gather information about who is doing what in this area in terms of host response to SCFAs – functional effects; gene expression; polymorphisms, etc. through newsletter/email |
| What about fathers? | All members | All of in-FLAME to ensure as much data as possible about the fathers collected |

There were also other additional projects to emerge in the meeting, including a collaboration between **Catherine Thornton** and **Susanne Brix Pedersen (Denmark)** to look at LPS antagonism in placenta/cord blood. From general discussion it was concluded to investigate the effects of SCFAs on placental and cord blood immune responses.




Catherine Thornton also outlined the proposal that has been accepted for review in JACI titled 'Immune biomarkers in the spectrum of childhood non-communicable diseases' authored by Skevaki C, Van den Berg J, Jones N,.....Thornton CA for the inFLAME Network. The paper will discuss NCDs and inflammation, including the common mechanisms and ontogeny of inflammation, both general and in obesity and childhood-specific diseases. It will then focus on potential biomarkers to monitor inflammation in general and in specific childhood NCDs. It will explore the **benefits and challenges of biomarkers which vary with** sample type (saliva, urine, blood, exhaled breath tears, nasal secretions, sputum), sample processing and methodologies (discovery & verification strategies), data handling andinformatics. It will examine biomarkers in pre-clinical and clinical development (including reference to progress in drug development and safety monitoring), and conclude with future directions.

# Our commitment to students and early career researchers:

One of the highlights of our meeting was the career researcher 'rapid fire' bus-stop presentations. This was very stimulating and highly interactive, and gave the VCR and opportunity to network with senior researchers and with other ECR. A number of important collaborative projects developed as a result.

A key outcome of our meeting will be to establish a more formal Early Career Cesearcher Network within in-FLAME, and we are encouraging the ECR to lead this process.
Another important outcome was the proposal for a formal Mentoring Program, that will pair early career researchers with more senior members, to help provide additional career development opportunities. Again, it is intended that this process will be driven by the ECR (by Dr Daniel Munblit and others), and will be overseen at a more senior member, initially Prof Anita Kozyrskyj (Alberta). There has been an application (after the meeting) for administrative to funding to facilitate this process.

# Sustainability and Strategy:

Prof Katie Allen (Melbourne) discuss strategies to build capacity and sustainability and around disease prevention and early life research using '*LifeCourse* Initiative' at the Melbourne Children's Campus as an example of how to better capitalize on existing resources, and build a more integrated collaborative vision. She spoke on taking a pragmatic view in a flat funding environment that is increasingly competitive. The objective of *LifeCourse* is to maximise the value of state-based longitudinal studies of child health and development with the aim of generating robust evidence on the burdens, causes, and consequences of childhood diseases. This is a collaborative venture hoping to link with external institutions and made also be a model for other centres to consider. There are likely to be many opportunities of collaboration with the in-FLAME network

Prof Dianne Campbell Allen (Sydney) gave an update on the in-FLAME network website development at http://www.wuninflame.org. Which we hope will provide a forum for sharing documents, data and discussions. Passwords will be distributed to members.

Prof Susan Prescott (Perth) table to proposal for our next meeting to be held in Maastricht, Netherlands in April 2016 in conjunction with WUN general meeting. There was general support that this concert and the proposed dates are April 1-3 2016. Susan will work with the WUN secrateriat and members from Maastricht to secure A local venue at the University (Prof Carel Thijs and Dr John Penders). In the coming weeks we will submit grant applications to support the meeting. In the meantime labour also be exploring a number of avenues to support specific research projects – and ask that everybody explores new opportunities with us.

We look forward to welcoming you to Maastricht next year!

# Some of your comments and feedback after the meeting:

After the meeting there was a flurry of very positive feedback and we thought it would be valuable to record this here. Just a few of the comments are listed:

## Comments from ECR and PhD students:

- *"I just wanted to thank you for the recent WUN-inFLAME meeting - it was the best meeting I have yet attended, with such good discussion, support for ECRs and a true sense of joint vision and collaborative effort for preventing disease. I feel very fortunate for having had the opportunity to attend, meet so many senior and inspiring researchers and now to have the chance to build collaborations for some exciting new projects. Thank you again" (*ECR, from UWA, Perth)
- *"Many, many thanks for this wonderful meeting! Looking forward to Maastricht 2016!" (*ECR, Imperial College, London)
- *"Thank you for the very interesting presentations and discussions during last week. As a student in the field of research it has been an very rewarding experience and I am looking forward to future meetings!"* (PhD student, Cape Town S. Africa).
- *"What an inspiring and interesting meeting in such a beautiful setting! Perfect for setting up new collaborations and getting new ideas. I am already looking very forward to meeting next year!"* (PhD student, UWA, Perth)

## Comments from Senior members:

- *'It was truly great; one of the best meetings I have attended in a long time! Such an open and collaborative atmosphere among very knowledgeable people (and in a beautiful setting).I already look forward to the next meeting!"* (Oslo, Norway)
- "Thank you for a great meeting with stimulating discussions and long-lasting scientific goals important for global health". (Karolinska, Sweden)
- *"Thanks so much for this truly inspiring and fun meeting - looking beyond the "normal research questions", and this warm welcome in InFLAME ! It was wonderful to meet you all and I am looking very much forward to future collaborations. I am very happy to contribute to current and future projects."* (New member, Munich Germany)
- *"It was the first time for us and what a great experience and warm welcome! A really productive meeting and such an enthusiastic group of people with so many collaborations going on. I am really looking forward to welcome you all in Maastricht next year,"* New members, Maastricht.
- *"We from Detroit echo these positive comments and thanks. We were excited about our first meeting and we certainly were not disappointed! Truly a collaborative group!"* New members from Detroit, USA
- *"Thanks for a truly memorable and productive meeting. They just keep getting better and better. I was at WHO today and they are looking to start a range of pre-pregnancy cohorts to look at an "early healthy start to life" around the world. I suggested speaking to our inFLAME group would be valuable!" (*Melbourne, Australia)

# A taste of other projects/ideas that are being developed as a result of *in-FLAME* collaborations

In addition to the many new projects formally presented there were an even larger number that developed organically during the meeting as a result of the new relationships formed. This summary captures a number of these – based on what members have reported back so far.

| Project | Who | What |
|---|---|---|
| GENERAL BIOINFORMATICS SUPPORT | Fiona Brinkman | Expertise available to those who are interested - bioinformatics and data integration for inflammatory disease/innate immunity/allergy and asthma, with data integration projects involving the Canadian AllerGen NCE ***Please feel free to contact Fiona if you need assistance* |
| Impact of oligosaccharides supplementation on breast milk mediated prevention of allergy | Valérie Verhasselt | A collaborative project with Johan Garssen's team on impact of oligosaccharides on oral tolerance induction through breast milk (following Cape Town meeting). Will include both immune as well as physiological measurements in offspring animals focussing on gut and lung function. |
| Plans for new breast milk collaborations on mother-child cohorts | Valérie Verhasselt | Working with cohorts run by<br>Carel Thijs (Maastricht, Holland)<br>Naoki Shimojo (Japan)<br>Debbie Palmer (Australia)<br>Maria Jenmalm (Sweden) |
| Allergy risk factors in breast milk | Valérie Verhasselt | Mechanistic studies in collaboration with:<br>Clarissa Prazeres da Costa (Germany) (Marburg)<br>Harald Renz and Hani Harb ( Cape Town- Marburg) |
| LPS variants in ALADDIN | Annika Scheynius<br>Susanne Brix Pedersen | During the meeting a new collaboration was established with, Copenhagen, Denmark, to analyze fecal samples collected at early time points in the birth cohort ALADDIN (see attachment). A special focus is to investigate the different variants of LPS (as Susanne presented) in relation to life style and allergic outcome in the children. |
| Epigenetics in the placenta | Annika Scheynius<br>Harald Renz | A pilot study was initiated with Harald Renz, Marburg, to investigate possible epigenetic differences in the placenta specimens collected in ALADDIN (see attachment). Based on the results from the pilot study indicating a difference for several genes associated to lifestyle of the mother we decided at the meeting in Freiburg to expand the analyses with additional 128 placenta samples. |
| New microbiome collaorations in Sweden | Annika Scheynius<br>Christina West and Maria Jenmalm | Planning for a new microbiome study and where the ALADDIN cohort might be included. |
| Autoimmune studies (1) | Aveni Haynes<br>Chrysanthi Skevaki | Exploring idea of doing a study using existing cohorts to investigate the association between risk factors and inflammatory markers with the development of autoantibodies in childhood (Type 1 diabetes, Coeliac disease, Hypothyroidism, Juvenile Arthritis etc) |
| Autoimmune studies (2) | Aveni Haynes<br>Jon Genuneit<br>Cathy Thornton | Develop study ideas using Jon's cohorts to investigate the association between maternal HbA1C in mother's with gestational diabetes and inflammatory/metabolic markers in the offspring |

| Project | Who | What |
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| Gestational Diabetes | Aveni Haynes<br>Susan Prescott<br>Rae Chi Huang | Investigate the programming effect of GDM in the ORIGINs cohort perhaps? In particular, are there programming effects of GDM per se which are independent to BMI and excessive gestational weight gain? Or can these be modified by exercise in women with GDM etc |
| Food Allergy (1) | Aveni Haynes | Ongoing discussions on potential collaboration |

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| | Katie Allen<br>Jennifer Koplin | |
| Food Allergy (2) | Daniel Munblit<br>Diego Peroni (Naoki Shimojo Alexander Pampura Audrey Dunn Galvin | Plans of collaboration on food allergy QoL questionnaires validation in Russia, Italy and Japan with subsequent study on FA children QoL (UK, Russia, Japan, Italy, Ireland) |
| FADS collaboration | Berthold Koletzko<br>Karen Simmer<br>Suzanne Meldrum<br>Rae Chi Huang<br>Susan Prescott | This is an ongoing collaboration exploring FADS gene analysis in relation to the PUFA studies (Nina di Vaz, Susan Prescott). The results will be analysed in relation to<br>• allergy<br>• neurodevelopment<br>• metabolic measures on these cohorts<br><br>(Several manuscripts anticipated)<br>Berthold Koletzko's group in Munich have also performed metabolomic analyses of the blood samples of Western Australian subjects. Currently Sebastian Rauschert is in Perth for a 6 months research fellowship to collaborate with the team at the Telethon Kids Insitute to analyse the data and prepare the publications (supported by the EU FP7 EarlyNutrition Project). |
| Fatty acids (2) | Carel Thijs<br>Others in metabolism group | -replication of results of immune components and fatty acids in breast milk in existing cohorts and extension to new hypotheses and new biomarkers |
| BIRTH consortium | Multiple members (see EU 2020 information) | - submission of application to Horizon 2020 grant with Daniel Munblit as coordinator: collaboration initiated in Milan, made concrete in Cape Town - currently searching for other potential grant proposals (e.g. Newton-Al Farabi foundation) - Planning fundraising for non-communicable diseases research |
| Breast milk and appetite | Donna Geddes | Breast milk appetite hormones across the first 12 months of lactation and their relationship with maternal and infant body composition (measured by BMI, bioimpedance and ultrasound skin folds). Ability to run large sample sets for fat, protein, lactose, lactoferrin, lysozyme, BBSL, ALP, Na, K with very small amounts of milk. Appetite hormones are also being currently worked up (leptin in whole milk, adiponection, grehlin and looking at resistin, obestatin. |
| POPs in breast milk | Donna Geddes<br>With potential to collaborate with Canadian and Noregian members | Identification of pollutants in breastmilk: Validation of a sensitive technique requiring only 1mL of milk. Cross sectional cohort (n=50, Perth, 2, 5, 9, 12 months lactation), longitudinal cohort (n=15, Perth, 2, 5, 9, 12 months lactation), 24 hour milk profiles that allow accurate calculation of dose rather than an estimation. |

| Project | Who | What |
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| Review on the importance of natural environments and biodiversity in NCDs | Alan Logan<br>Susan Prescott<br>Jeff Craig | Will review evidence that is emerging on 'green space' and early outcomes, including evidence that the benefits may begin before birth. Hope to also explore new projects on this. |
| Peruvian breast milk analysis | Donna Geddes | We are poised to run 21 micronutrients in milk hopefully within a month on a large sample set from Peru (>500 samples). |
| Pollutants and mental health in children | Felice Jacka<br>Merete Eggesbø | Will be meeting in Oslo in Sept/October to begin planning for collaborative projects examining the possible relationship of early life exposure to pollutants and mental health outcomes in children. We hope to utilise data from more than one cohort study. |

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| Diet and inflammation | Felice Jacka<br>Susanne Brix Pedersen | We hope to collaborate on a project examining dietary exposures and their relationship to inflammatory markers and gut microbiota, as well as child outcomes in COPSAC cohort study. Susanne will also advise us on the analysis of some of the Australian data. |
| Nutrition and 'Leaky gut' hypothesis | Felice Jacka<br>Meri Tulic | Preliminary plans to share mucosal biopsy data with to examine 'leaky gut' hypotheses (using Ussing Chambers). Pending funding (currently submitted). |
| Biosensor for detecting allergic risk in newborns | Vicki Clifton | Has been developing and continue to develop the biosensor for detecting allergic risk in newborns. This is a collaboration with Tanya Monro, Peter Hoffmann, Andrew Tai. It is currently a PhD project conducted by Nurul Zainal. This was initiated from attendance at the Washington In-FLAME meeting |
| Thymic Studies on various cohorts | Ralph Nanan<br>Peter Vuillermin<br>Dianne Campbell<br>Anita Korzyrskyj | Collaboration to analyse thymic size in relationship to outcomes and exposures in various cohorts (including CHILD (Canada), Barwon Infant Study (Melbourne) and ORIGINS (Perth) and others. |
| BOPIA (Peanut and SCFA) | Dianne Campbell<br>Paul Turner<br>David Fleisher<br>Anita Kozyrskyj<br>Naoki Shimojo<br>Shuichi Suzuki | Collaborations with Imperial college, Denver, Alberta/ Edmonton, Chiba (see main report) |
| Effects of vitamin D on Treg in protection from atopic dermatitis | Clarissa de Costa<br>Ralph Nanan<br>Pete Vuillermin | Potential collaboration to examine vitamin D effects on Treg protection against allergy and function in AD. |
| Effects of labour and delivery method in immune phenotypes | Jon Genuneit<br>Peter Vuillermin<br>Susanne Brix Bianca Schaub | Proposed collaboration to investigate the effects of duration of labour and of delivery mode on immune phenotypes in cord blood in 5 cohorts from Copenhagen, Munich, Melbourne, Ulm. The first teleconference planned for end of 07/2015. This will build on finding in BIS suggesting that a relationship between nTreg and FA is modified by exposure to labour. |
| New allergens, raw milk research program (see main report) | Johan Garssen<br>John Sinn. | Idea initiated in Cape Town. Funding for 1 PhD student who will focus on raw milk and its tolerance inducing capacity. Besides allergens/antigens we will focus on milk enzymes such as alkaline phosphatase which might be highly relevant in inflammation management. |
| SCFA in sperm and success of IVF (in vitro fertilisation) | Johan Garssen<br>Philip Calder | Sperm contains unique non-digestible oligosaccharides that are very similar to those in breastmilk. During the coming year we will analyse non-digestible oligosaccahrides in sperm samples and might link this to success of the IVF therapy. |

| Project | Who | What |
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| 'Natural' toxins in foods | Johan Garssen<br>Jon Genuneit | As discussed during this Marburg meeting and in Cape Town we will focus on natural toxins present in foods/cereals/rice etc. such as mycotoxins (DON). In preclinical studies we discovered that low levels of mycotoxins (levels that are allowed according to international safety guidelines) can serve as adjuvant for food allergens. In Asia, especially China mycotoxin levels in milk and food and even breast milk are relative high and might be responsible at least in part for the increase in incidence/severity of allergies. Together with Jon Genuneit we will analyse breastmilk samples for mycotoxins/DON. Using food questionairres we are already estimating the dosage consumed in Asia. |
| Collaborative Animal models (SCFA and fibre) | Johan Garssen<br>Marie Bodinier | Marie is running almost similar studies with pregnant/lactating animals as they do at Utrecht University. Will exchange samples |

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| | | (stool/blood/breastmilk) for collaborative experiments. |
| Suggestion:<br><br>that we make a coordinated international appeal to preserve the assets that we have around the world: for further discussion | Tim Takaro | Suggested that WHO/other entities may be interested in keeping alive existing cohorts with deep exposure assessment, genetics, epigenetics, diet, microbiome, psych-soc. assessment and multiple biological samples? I know of at least one (CHILD) where our babies are reaching the 5 year clinical endpoints without funding to analyze samples or keep the cohort together past five. New cohorts can be very interesting, but It makes a lot of sense to keep previous investments alive too! |
| Comment: | Katie Allen | I was at WHO after the meeting and they are looking to start a range of pre-pregnancy cohorts to look at an "early healthy start to life" around the world. I suggested speaking to our inFLAME group would be valuable! It is having networks like ours that bring knowhow together which i think are really valuable. It means we can be ready when opportunities arise. |
| Vitamin D in Africa (similar protocol to Australian study) | Rose Kamenwa<br>Debbie Palmer<br>Kristina Rueter<br>Susan Prescott<br>Mike Levin | In this double blind placebo controlled randomised trial 120 infants with a family history of allergic disease receive 400 IU vitamin D supplementation/day or placebo for 6 months. In this study a UV dosimeter will also be worn by the infants from birth to 6 months of age to measure actual UVB exposure. |
| BENEFIT RCT (whole foods approach to allergy prevention) | Debbie Palmer<br>Kristina Rueter<br>Dianne Campbell<br>Susan Prescott<br>Daniell Munblitt | This study aims to look at the influence of a mixed 'whole food' diet delivering allergenic foods in the context of immunomodulatory nutritents (PUFA, prebiotics, vitamin D etc in whole foods). After a fruitful discussion we came to the<br>agreement that this study should be limited to a maternal dietary intervention only (from 20 weeks of gestation until cessation of breastfeeding). Apart from nuts, egg and fish the diet will also include balsamic vinegar. Groups will be divided into 'high consumption" of thesefoods versus "low consumption" (specific amount still needs to be determined).Discussions are ongoing and other collaborators are welcome. |

| Project | Who | What |
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| Pet ownership and endotoxin variants | Peter Vuillermin<br>Susanne Brix | Will investigate the hypothesis that the protective effect of pet ownership on allergic outcomes is mediated by enteric colonisation of the mother and/or infant gut with penta-acetylated endotoxin producing bacteria. This will add substantial value to the NHMRC Pete was awarded last year for 16s and SCFA measures in BIS. |
| Maternal diet, microbiome and thymic size | Peter Vuillermin<br>Ralph Nanan | Plan to investigate the hypotheses that a maternal gut microbiome that produces lower levels of SCFAs during pregnancy is associated with (a) pre-eclampsia, and (b) reduced thymic size and nTreg in the offspring. Again, adding substantial value to funded work underway in BIS. |
| Mechanisms of early oral tolerance – sharing samples and data from infant feeding RCT | Peter Hsu<br>Debbie Palmer<br>Dianne Campbell<br>Susan Prescott | Treg studies and other collaborative analyses on samples from infant feeding studies (especially STAR, STEP. BEAT and QUEST). Will form a new partnership between Telethon Kids Institute (Perth) and Kids Research Institute (Sydney). Opportunities for PhD student exchange. We hope to link this with the EU project (iFAAM) lead by Clare Mills. |
| Larger scale comparison on POPs and other pollutants in in-FLAME cohorts – in relation to specific outcomes | Merete Eggesbø<br>Chrysanthi Skevaki<br>(Amanda Wheeler, Donna Geddes, Tim Takaro TBC) | Initiative to explore future use *in-FLAME* cohorts for a combined analysis within immunology, the details not yet decided on. |
| Interactions between POPs and the microbiome | Shuichi Suzuki<br>John Penders<br>Koen Venema | Shuichi had some interesting ideas about investigating the link between the microbiome and the metabolism/bioavailabilty of ingested pollutants and I thought there might be some possibilities to examine this within the TIM-system. Currently being explored further. Recommend also including Merete Eggesbø in the discussions. |

# *In-FLAME* Publications:

## 2015 – accepted or now published (*original research)


1. * D Martino D, T Dang , A Sexton-Oates, S Prescott , ML Tang ML, S Dharmage, L Gurrin L, J Koplin, AL Ponsonby, KJ Allen, R Saffery; HealthNuts study investigators. Blood DNA methylation biomarkers predict clinical reactivity in food-sensitized infants. J Allergy Clin Immunol. 2015. 135(5):1319-28.

2. *CE West, P Rydén, D Lundin, L Engstrand, M Tulic, SL Prescott. Gut microbiome and innate immune response patterns in IgE-associated eczema. Clin Exp Allergy. 2015 May 5. [Epub ahead of print]

3. *H Harb, M Amarasekera, S Ashley[3] MK. Tulic, PI Pfefferle, DP. Potaczek, H Renz, D Martino, DA. Kesper and SL. Prescott. Establishment and Validation of a chromatin immunoprecipitation method for cohort studies and their application Clinical Chemistry accepted October 2014.

4. S Ashley' T Dang, J Koplin, D Martino and S Prescott Food for thought: Progress in understanding the causes and mechanisms of food allergy. Current Opinion in Allergy & Clinical Immunology. Curr Opin Allergy Clin Immunol. 2015 Apr 16. [Epub ahead of print]

5. D Campbell, RJ Boyle, CA Thornton, SL Prescott. Mechanisms of Allergic disease - Development of immune responses in infancy and early childhood. Clin Exp Allergy **2015** May;45(5):844-58. PMID: 25772780

6. RC Huang, SL Prescott, KM Godfrey, EA Davis. How to assess cardiometabolic risk in children in population studies – underpinning DOHaD birth cohort studies. Accepted J Nutrition Science J Nutr Sci. 2015 Apr 10;4:e12.

7. K Rueter, SL Prescott and J Palmer. Nutritional approaches for the primary prevention of allergic disease: an update, Journal Paediatrics and Child Health, J Paediatr Child Health. 2015 Jul 2. [Epub ahead of print]

8. CE West, HD Renz. MC Jenmalm, AL Kozyrskij, KJ Allen, P Vuillermin, SL Prescott, The gut microbiota and inflammatory non-communicable diseases: Associations and potentials for gut microbiota therapies. 2015 Jan;135(1):3-13;

9. CE West, MC Jenmalm, SL Prescott. The gut microbiota and its role in the development of allergic disease: a wider perspective. Clin Exp Allergy. 2015 Jan;45(1):43-53

10. SL Prescott, "Origins: Early Life Solutions to the Modern Health Crisis" First published in 2015 by UWA Publishing Crawley, Western Australia 6009, Copyright © Susan L. Prescott (ISBN 978-1-74258-670-0 (based on the philosophies of the WUN in-FLAME network)

11. K Rueter , A Haynes, SL Prescott. Developing primary prevention strategies to prevent allergic disease, Curr Allergy Asthma Reports (in press)


## 2015 – submitted or under revision


12. *Mathilde Turfkruyer, Akila Rekima, Patricia Macchiaverni, laura Le Bourhis, Gijs Van den Brink, Vanesa Duncan, Meri Tulic and Valérie Verhasselt. Allergy prevention by oral tolerance is inefficient in neonates due to physiological vitamin A deficiency. Mucosal Immunology (under revision)

13. *Meri K Tulic , Mylene Vivinus-Nebot, Nathalie Vergnolle, Chrystelle Bonnard , Hai Ning Shi, Allan Walker, Thierry Piche and Valérie Verhasselt. Commensal house dust mite allergen: a contributor to gut inflammatory disease? Gut (under revision)

14. *H Harb, M Amarasekera, S Ashley[3] MK. Tulic, PI Pfefferle, DP. Potaczek, H Renz, D Martino, DA. Kesper and SL. Prescott. Folate status as a modifier of epigenetic profile in human neonatal $CD4^+$ T cells. Submitted

15. *AR Tuck, SM Edwards, L Grzeskowiak, A Osei-Kumah, Z Saif, A Tai, SL Prescott, MK Tulic, R Saffery, VL Clifton Distinct sex-specific gene expression changes in the placenta in association with childhood allergy at 2 years. (Submitted)

16. Van den Berg J, Skevaki C, Jones N….Thornton CA for the InFLAME Network. Immune biomarkers in the spectrum of childhood non-communicable diseases. J Allergy Clin Immunol (Outline has been accepted by JACI – now in preparation, as per report by Metabolism Working Group).

17. A. Khan, D.Palmer SL. Prescott. In utero exposure and the evolving epidemiology of paediatric atopy. Current Opinion in Allergy & Clinical Immunology. Under review May 2015

18. MC Jenmalm, CE West, SL Prescott, AL Kozyrskij, A 'Probiotics in allergy prevention: time to revisit recommendations?' Clin Exp Allergy (in preparation)

19. MC Jenmalm, CE West, SL Prescott, AL Kozyrskij, The use of probiotics for the prevention and treatment allergic diseases and asthma' Expert Review of Clinical Immunology (in preparation)

20. P Vuillermin, J Genuneit, C West, D Campbell, K Allen, SL Prescott et al, The potential link between dietary intake of fermentable fibre, the production of short chain fatty acids by gut microbiota and asthma and allergic disease. In preparation.

21. AC Logan, SL Prescott, JC Craig. Journal of Physiological Anthropology. Natural Environments, Nature Relatedness and the Ecological Theatre: Connecting Satellites and Sequencing to Shinrin-yoku (submitted July 2015)

22. *Nour Baiz, Patricia Macchiaverni, Meri Tulic, Akila Rekima, Antonio Condino Neto, Isabella Annesi-Maesano* and V. Verhasselt*: respiratory allergen in human breast milk are risk factor for allergy (in preparation)

23. *Meri Tulic, Akila Rekima, Jon Genuneit, Christelle Bonnart, Nathalie Vergnolles, Hani Harb, Samara Medeiros, Samantha Zanelli, Harald Renz, Susan Prescott and Valérie Verhasselt: protease mediated priming of food allergy in early life by house dust mite allergen (in preparation)

24. Valérie Verhasselt, Philip Calder and Daniel Munblit : new insights on breast milk long term health beneficial effects *(in preparation – authors order, contributors and exact title not yet finalized)*

25. Munblit D, Sheth S, Abrol P, Treneva M, Peroni DG, Chow L-Y, Boner AL, Pampura A, Warner JO, Boyle RJ Exposures influencing total IgA level in colostrum. J DOHaD (*under revision*)

26. *Munblit D, Treneva M, Peroni DG, Colicino S, Chow L-Y, Dissanayeke S, Pampura A, Boner AL, Boyle RJ and Warner JO. Colostrum and breast milk of mothers from London, Moscow and Verona: determinants of growth factor levels and cytokines detectability. European Journal of Nutrition (*In preparation*)

27. Munblit D, Treneva M, Peroni DG, Colicino S, Chow L-Y, Dissanayeke S, Pampura A, Boner AL, Boyle RJ and Warner JO Colostrum immune composition and immunological outcomes assessment using Principal Component analysis (PCA). (*In preparation*).

## 2014 publications

28. *M Amarasekera, D Martino, S Ashley, H Harb, D Kesper, D Strickland, R Saffery and SL Prescott. Genome-wide DNA methylation profiling identifies a folate-sensitive region of differential methylation upstream of *ZFP57* imprinting regulator in humans. FASEB J. 2014 Jun 2. pii: fj.13-249029. [Epub ahead of print]

29. *Macchiaverni P, Rekima A, Turfkruyer M, Mascarell L, Airouche S, Moingeon P, Adel-Patient K, Condino-Neto A, Annesi-Maesano I, Prescott SL, Tulic MK, Verhasselt V. Respiratory allergen from house dust mite is present in human milk and primes for allergic sensitization in a mouse model of asthma. Allergy. 2014 Mar;69(3):395-8

30. JM Craig, SL Prescott Non-communicable diseases: Early life is key to disease risk *Nature* 512, 28 (07 August 2014) doi:10.1038/512028d.

31. Martino, D., Kesper, D.A., Amarasekera, M., Harb, H., Renz, H., Prescott., S.,Epigenetics in immune development and in allergic and autoimmune diseases, J Reprod Immunol. 2014 Oct;104-105:43-8.

32. DJ Palmer, RC Huang, JM Craig, SL Prescott. Nutritional influences on epigenetic programming: asthma, allergy and obesity. Immunol Allergy Clin North Am. 2014 Nov;34(4):825-37.

33. K. Rueter, A. Siafarikas, SL Prescott, D Palmer. In-utero and postnatal vitamin D exposure and allergy risk. Expert Opin Drug Saf. 2014 Dec;13(12):1601-11.

34. *Macchiaverni P, Rekima A, Turfkruyer M, Mascarell L, Airouche S, Moingeon P, Adel-Patient K, Condino-Neto A, Annesi-Maesano I, Prescott SL, Tulic MK, Verhasselt V. Respiratory allergens in human milk: potential impact on susceptibility to allergic airway disease. Clin Transl Allergy. 2014 Feb 28;4 (Suppl 1 3rd Pediatric Allergy and Asthma Meeting (PAAM)Publi):P1. Doi

35. SL Prescott, Disease Prevention in the age of convergence – the need for a wider, long-ranging and collaborative vision. Allergology International, 2014 Mar;63(1):11-20.

36. *M Amarasekera, P Noakes, D Strickland[2], R Saffery, DJ Martino, SL Prescott. Epigenome-wide analysis of neonatal $CD4^+$ T-cell DNA methylation sites potentially affected by maternal fish oil supplementation. Epigenetics. ePub 2014 Dec 7:0.

37. D Munblit, RJ Boyle, JO Warner. Factors affecting Breast Milk composition, and potential consequences for development of the allergic phenotype. Clin Exp Allergy 2014 Jul 31. doi: 10.1111/cea.12381. [Epub ahead of print]

38. A Jones, N D'Vaz, S Meldrum, D Palmer, G Zhang, SL Prescott 25-hydroxyvitamin $D_3$ status is associated with developing adaptive and innate immune responses in the first 6 months of life. Clin Exp Allergy, ePub Nov 6 2014

## 2012-2013 publications

39. *SL Prescott, R Pawankar, KJ Allen, DE Campbell, JK Sinn, A Fiocchi, HA Sampson, K Beyer, BW Lee. A global survey of changing patterns of food allergy burden in children. World Allergy Organ J. 2013 Dec 4;6(1):21.

40. SL Prescott. Early life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases (NCDs). J Allergy Clin Immunol 2013 Jan;131(1):23-30

41. D Geddes. SL Prescott, Journal of Human Lactation. *Developmental Origins of Health and Disease: the role of breast milk in preventing disease in the 21st century* J Hum Lact. 2013 May;29(2):123-7.

42. Palmer DJ, Metcalfe J, Prescott SL. Preventing disease in the 21st century: The importance of maternal and early infant diet and nutrition. J Allergy Clin Immunol. 2012 Sep;130(3):733-4

43. D Martino, SL Prescott. Progress in understanding the epigenetic basis for immune development, immune function and the rising incidence of allergic disease. Current Asthma and Allergy Reports (2013) Feb;13(1):85-92.

44. SL Prescott, D Palmer. Early events in the development of allergic disease. Curr Opin Allergy Clin Immunol. Accepted Jan 2013

45. PI Pfefferle, SL Prescott, M Kopp *Clinical evidence from microbial influence on tolerance development.* J Allergy Clin Immunol (2013) 131(6): 1453-63

46. Ferrante A, Prescott SL. Immunological Immaturity of the Neonate, Protein Kinase C Zeta and Allergy. J Neonatal Biol 2013 3:e106. doi: 10.4172/2167-0897.1000e106

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Welcome to the

# 5th Annual *in-FLAME* workshop Maastricht April 1-3, 2016

*in-*FLAME