

Phenotyping of multiple biofluids for liquid biomarkers for diagnostics and personalized medicine

By Allan Stensballe; PhD. (ATV)



AALBORG UNIVERSITY
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Phenotyping for enhanced diagnostics and personalized medicine – A focus on autoimmune diseases & Inflammation

- ▶ More than 80 diverse autoimmune diseases affect 6.4% percent of women vs. 2.7 % of men
- ▶ Researchers don't know exactly what causes many autoimmune diseases.
- ▶ Genetics, diet, infections, and exposure to chemicals might be involved.
- ▶ Many subtypes remain excessively difficult to validate and no single test can diagnose most autoimmune diseases.
- ▶ Low grade inflammation is central in most pathologies.



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Personalized and precision medicine are key a focus in the future

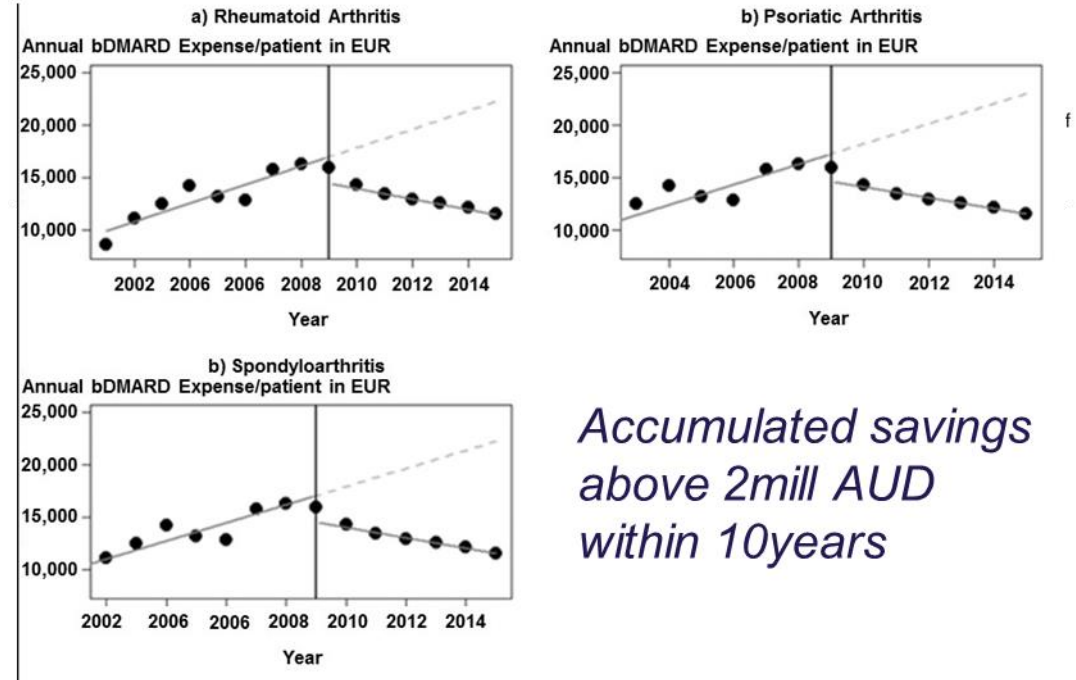


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Personalized Rheumatic Medicine through Dose Reduction Reduces the Cost of Biological Treatment – a retrospective intervention analysis

MK Meyer^{1,2,3,4,5}, M Andersen^{1,2,3}, T Ring^{3,6,7}, GN Andersen^{1,2,4}, LH Ehlers⁷, C Rasmussen^{1,2,5,7}, A Stensballe^{3*}



Accumulated savings above 2mill AUD within 10years

IMPRECISION MEDICINE

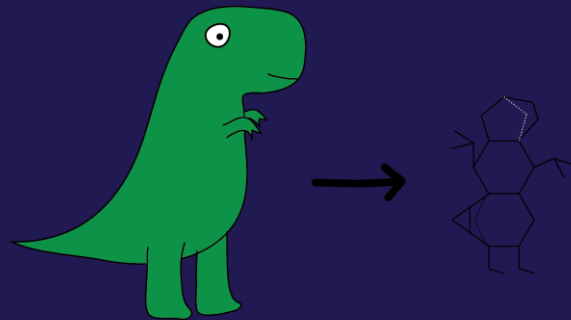
For every person they do help (blue), the ten highest-grossing drugs in the United States fail to improve the conditions of between 3 and 24 people (red).

Drug	Indication	Helped (Blue)	Not Helped (Red)
1. ABILIFY (aripiprazole)	Schizophrenia	10	3
2. NEXIUM (esomeprazole)	Heartburn	10	24

Improved and truly translational biomarkers are needed

- ▶ Enabling technologies including all Omics technologies paves the way to understanding disease etiology.
- ▶ Key to insight remains the ability to find good answers to relevant clinical questions
- ▶ Why responders and non-responders?

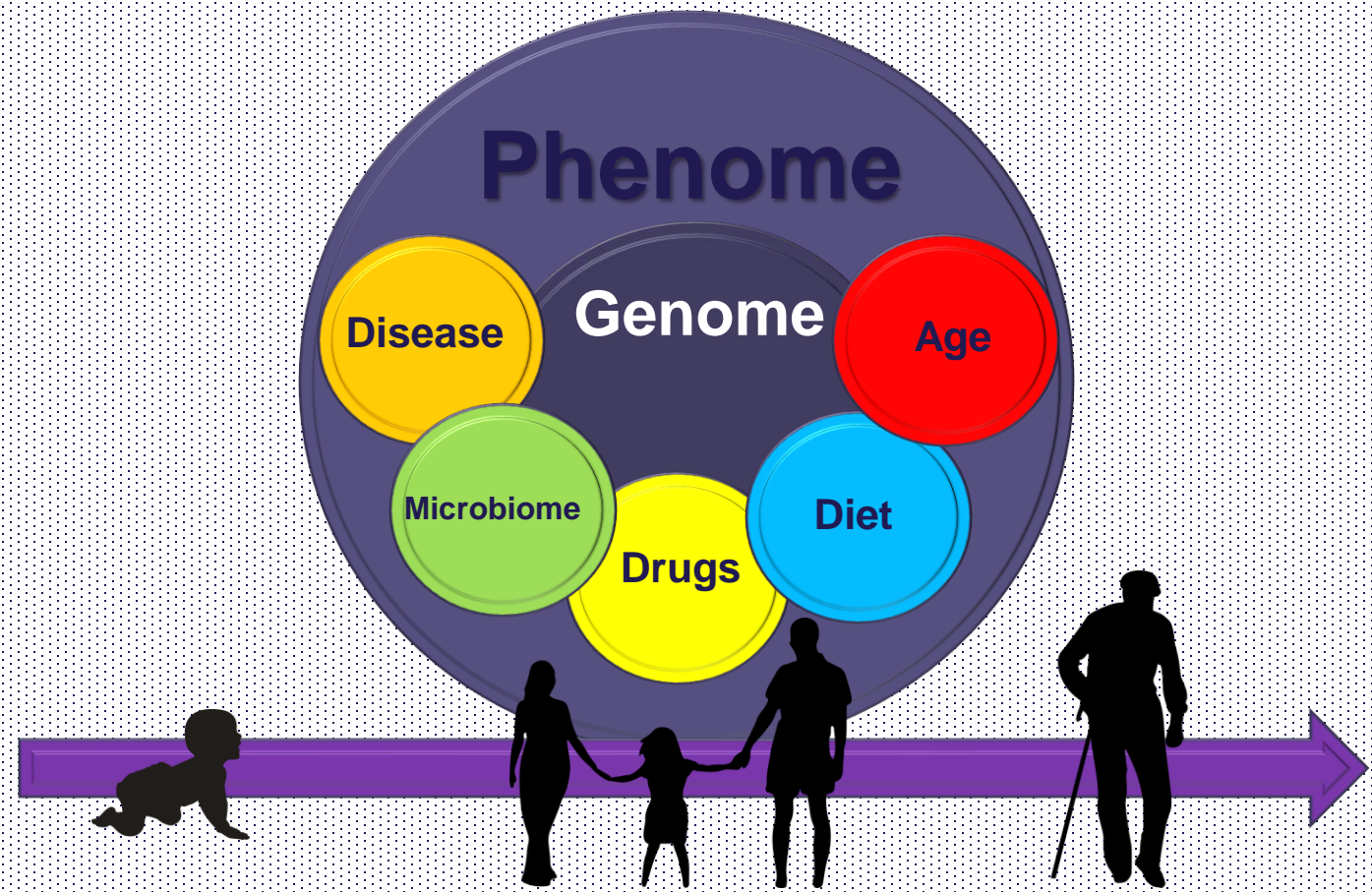
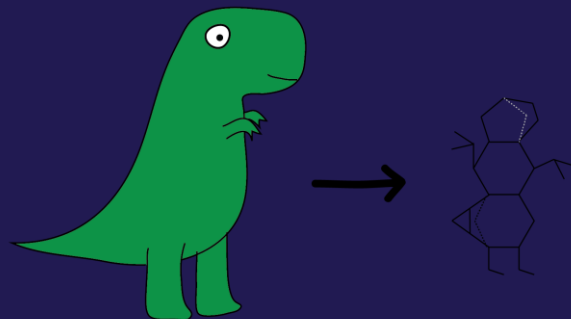
BIOMARKER



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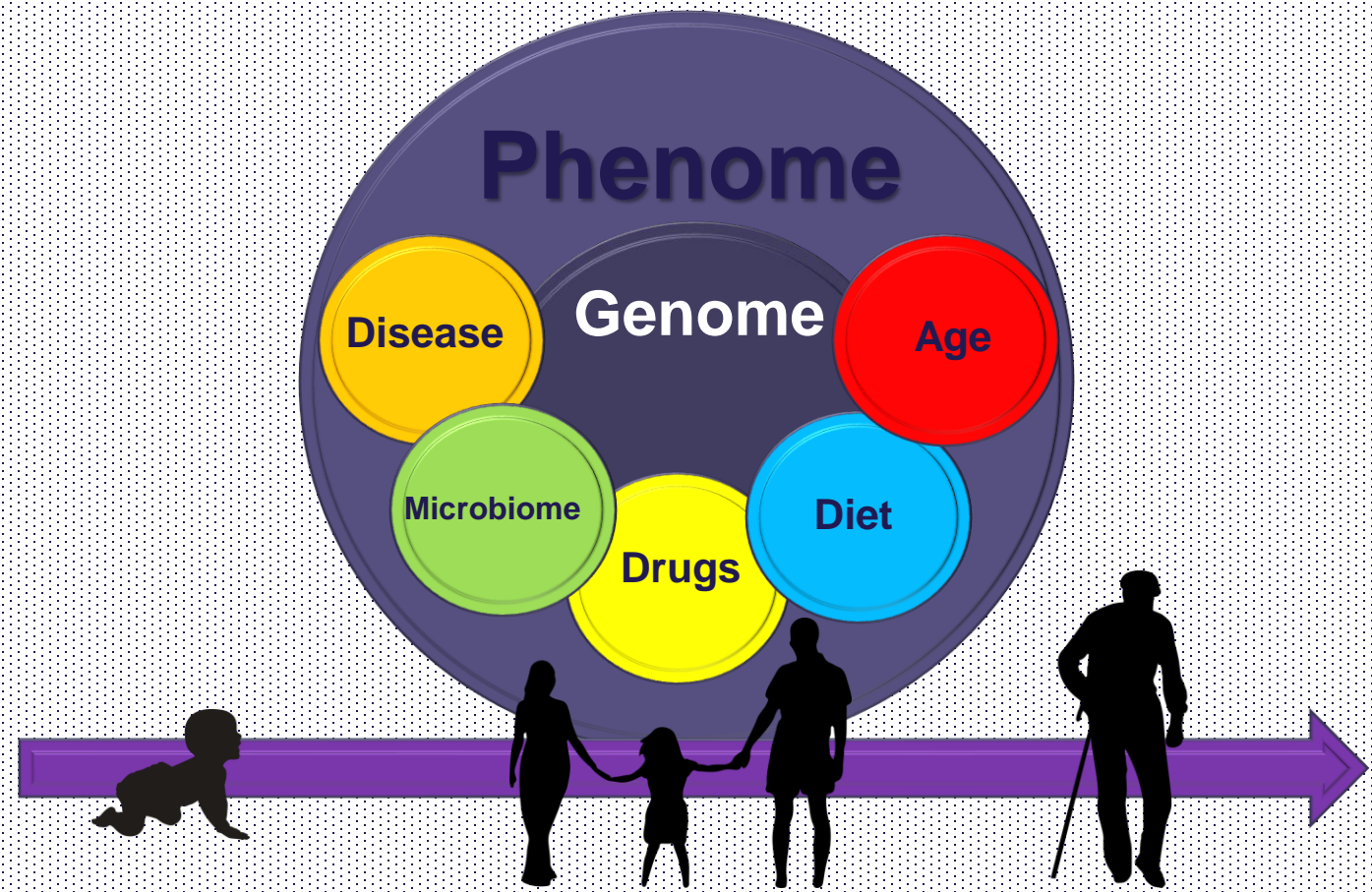
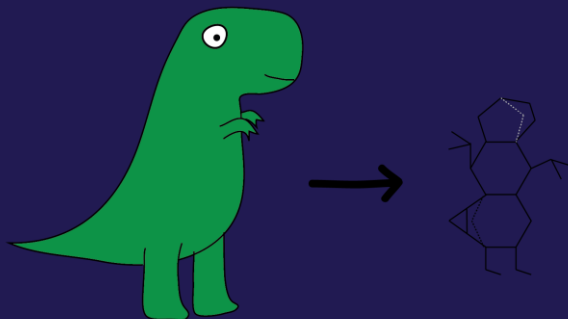
Disease risk
(prognosis/Diagnosis/
prevention)



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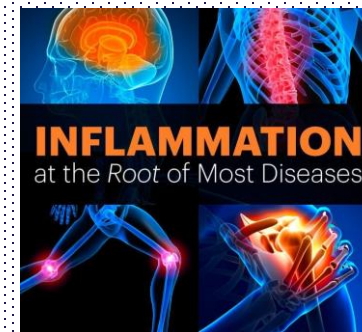
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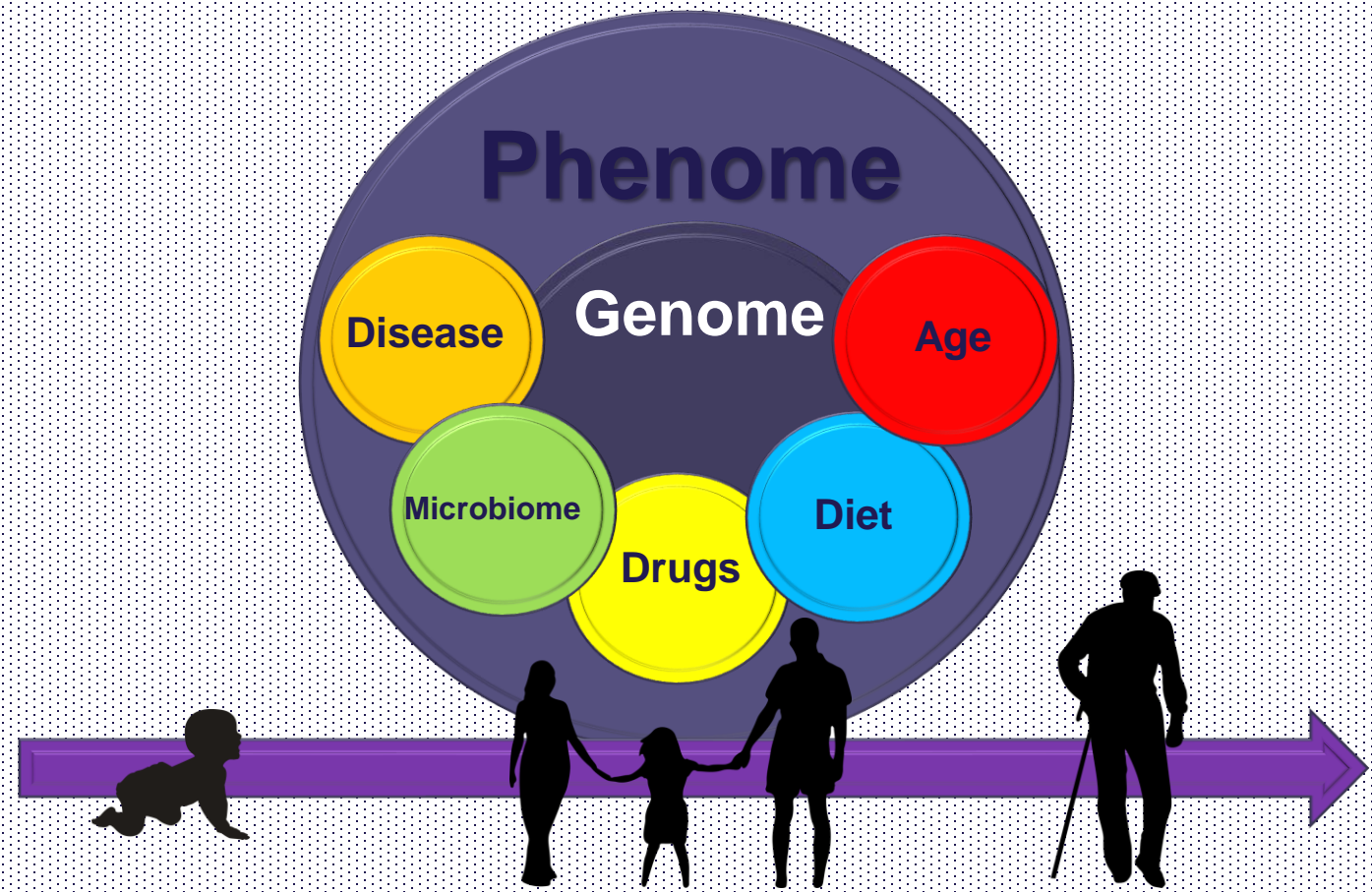
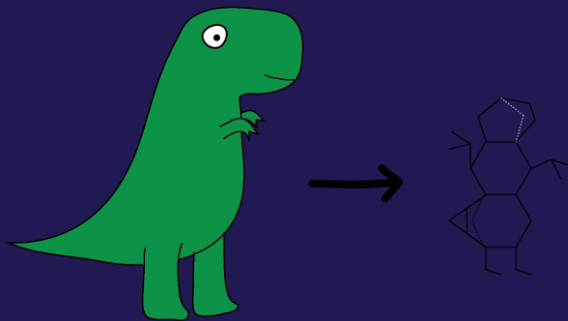
Patient stratification
(Translational biomarkers)



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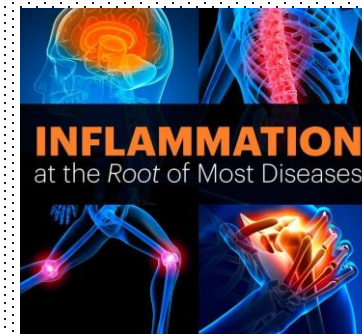
BIOMARKER



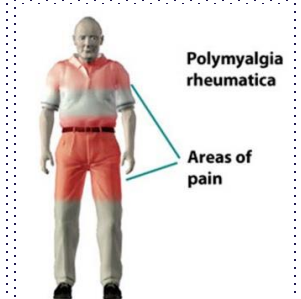
Disease risk
(prognosis/Diagnosis/
prevention)



Patient stratification
(Translational biomarkers)



Treatment response
(Prediction/monitoring)



The transition at birth and the preterm problem



Tik Muk



Azra
Karamehmedovic



Preterm infants are susceptible to sepsis, which in turn may lead to neurodevelopmental disorders



Milk and microbiota effects on immunity, gut and brain development is poorly understood

The transition at birth and the preterm problem



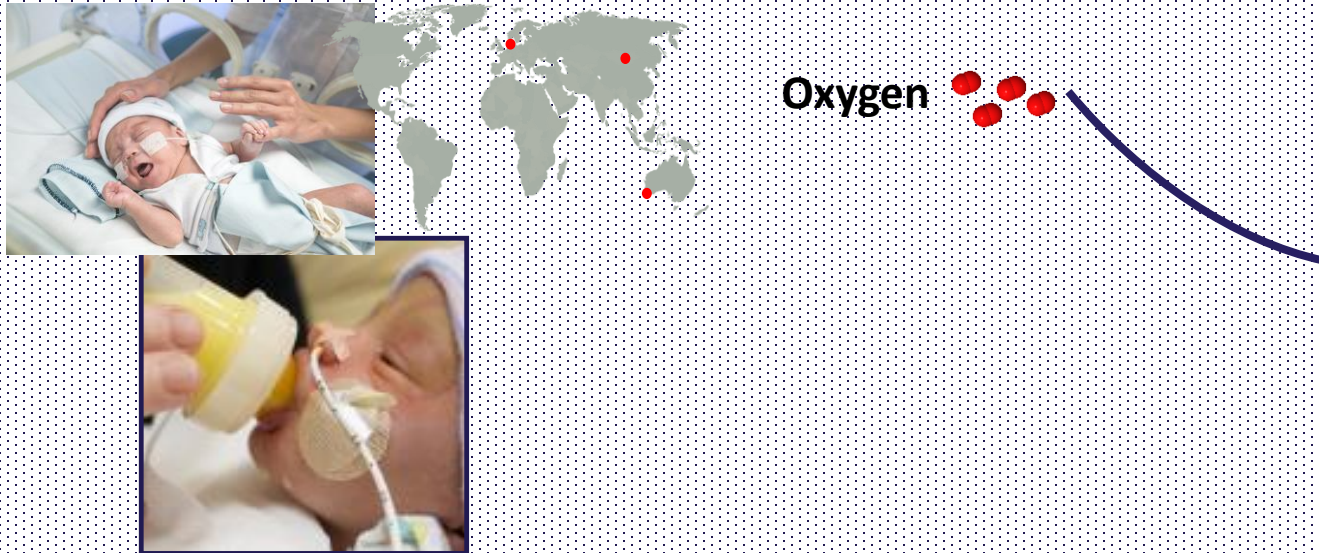
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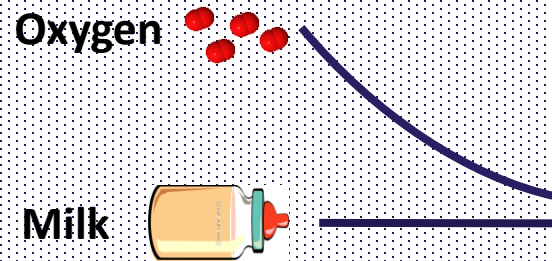
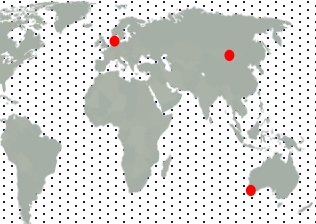
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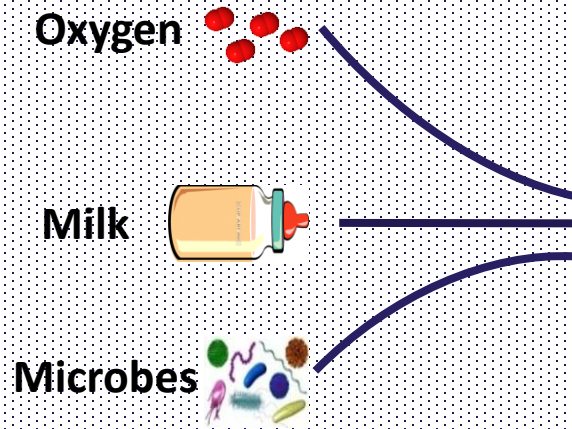
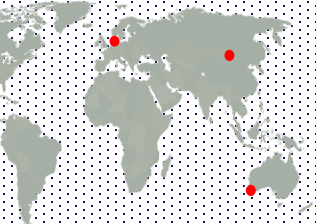
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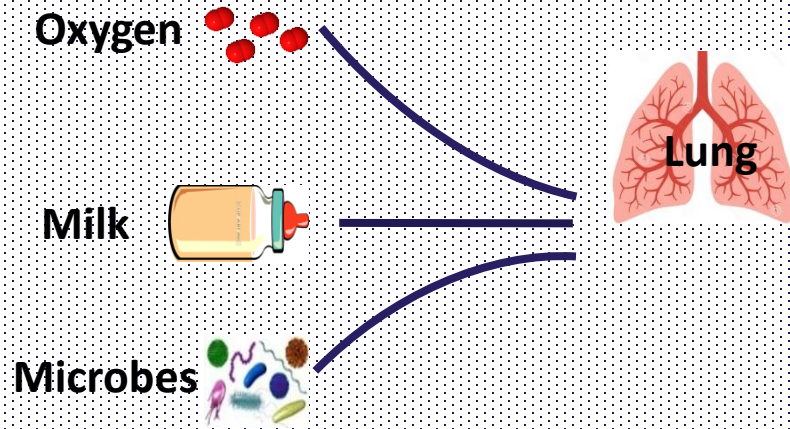


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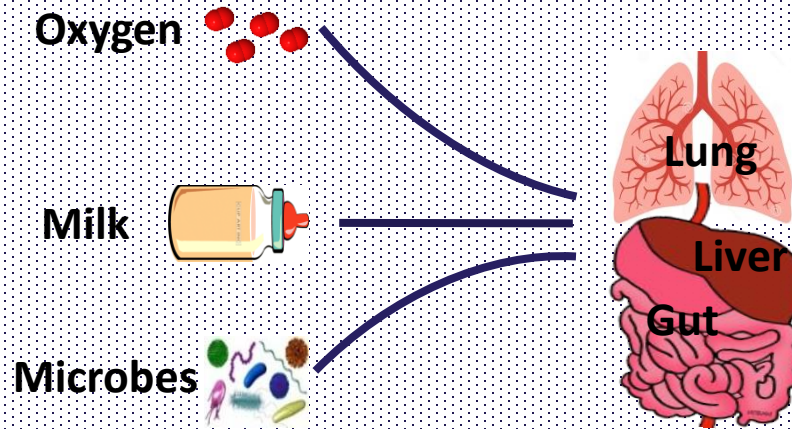
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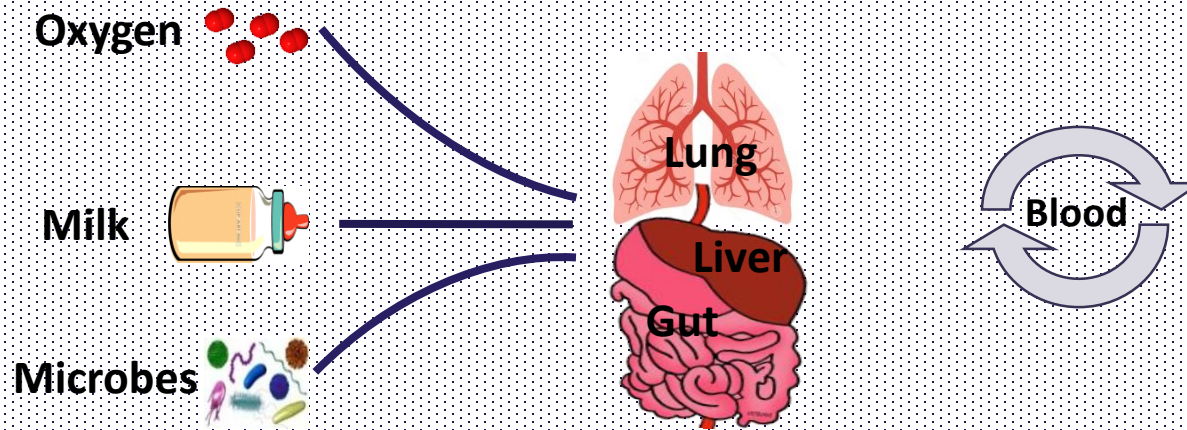


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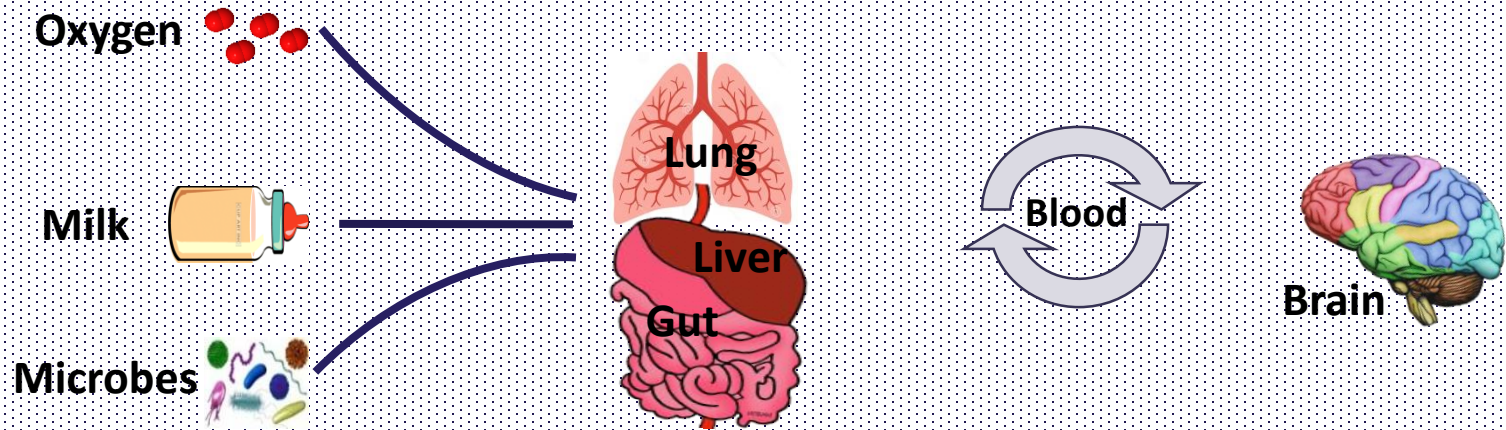


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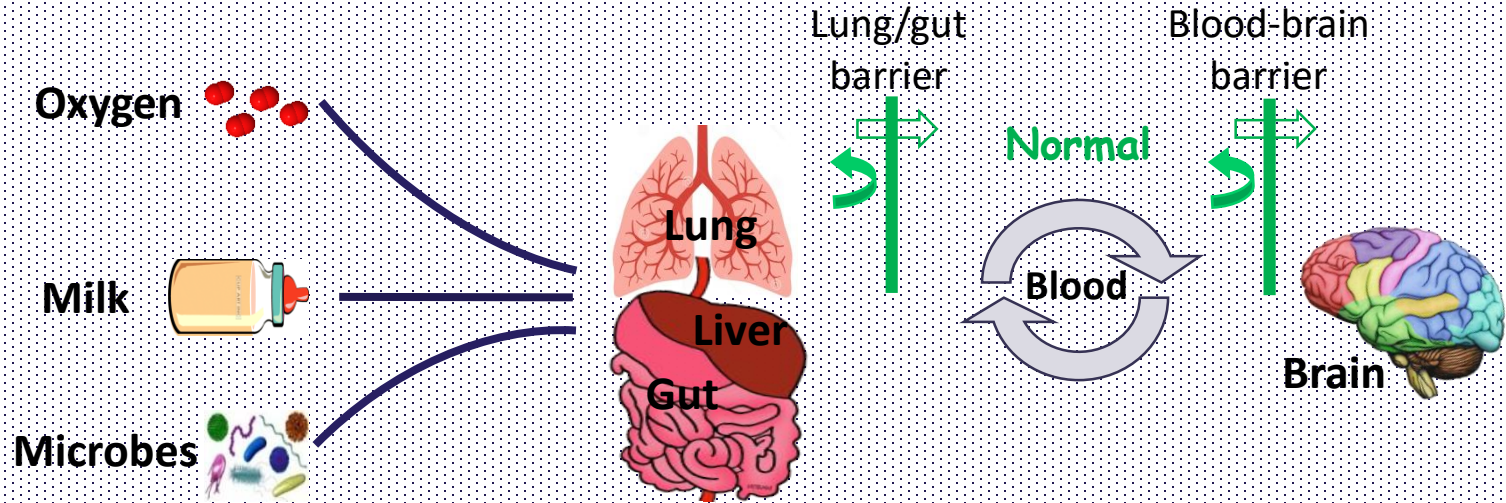


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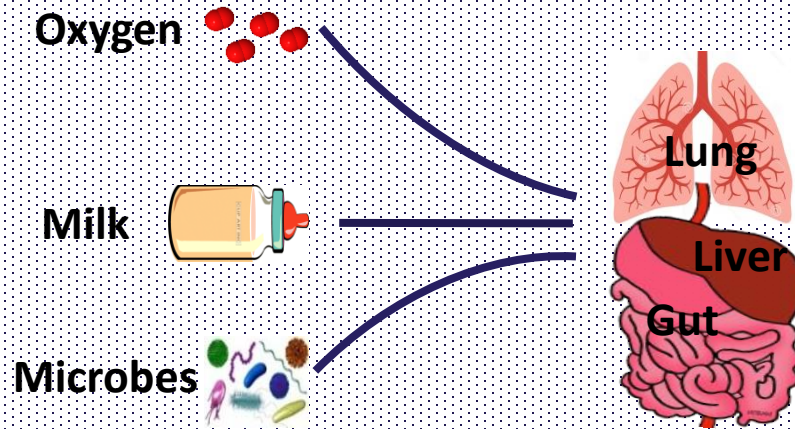


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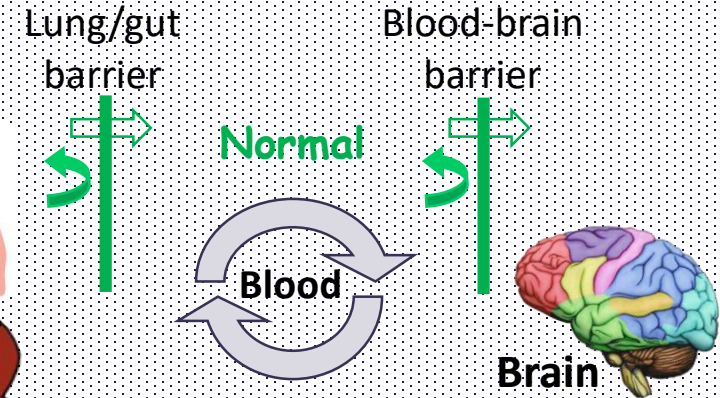


NEOMICS

Preterm infants are susceptible to sepsis, which in turn may lead to neurodevelopmental disorders



Protected environment



Milk and microbiota effects on immunity, gut and brain development is poorly understood

The transition at birth and the preterm problem



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NEOMICS

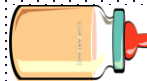
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Oxygen



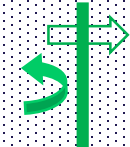
Milk



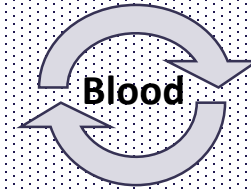
Microbes



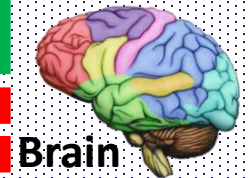
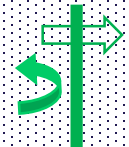
Lung/gut barrier



Normal



Blood-brain barrier



Preterm: Inflammation



Milk and microbiota effects on immunity, gut and brain development is poorly understood

The transition at birth and the preterm problem



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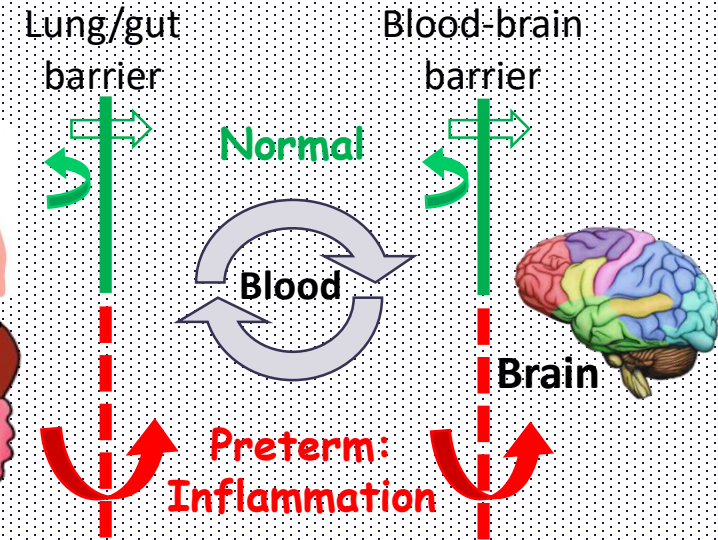


Milk and microbiota effects on immunity, gut and brain development is poorly understood

Oxygen
Milk
Microbes



Protected environment



Challenged environment:

- *Oxygen variability*
- *Milk mal-digestion*
- *Bacterial dys-colonization*

The transition at birth and the preterm problem



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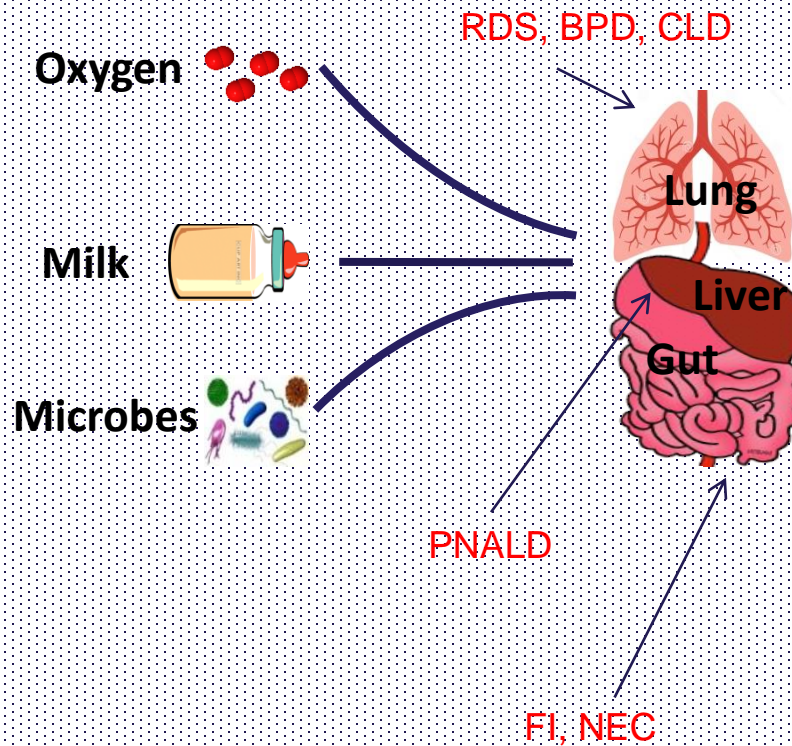


NEOMICS

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Protected environment

Lung/gut barrier

Blood-brain barrier

Normal

Blood

Brain

ND, PVL, CP, IVH, WMD

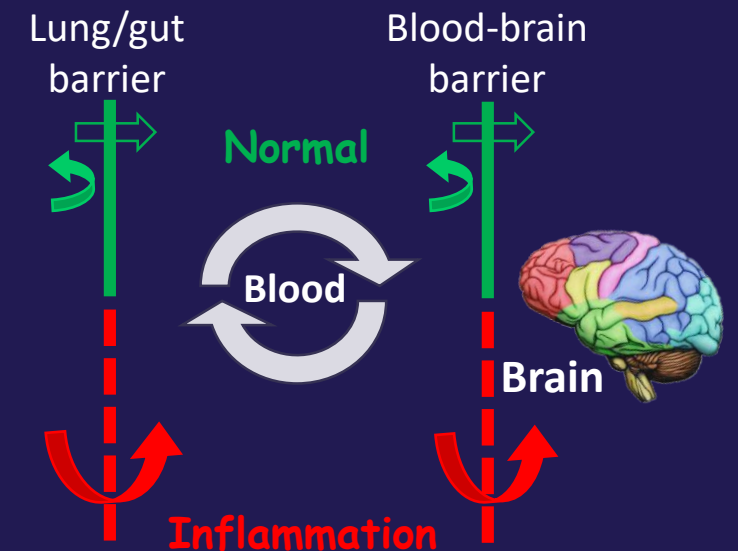
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LOS, DIC

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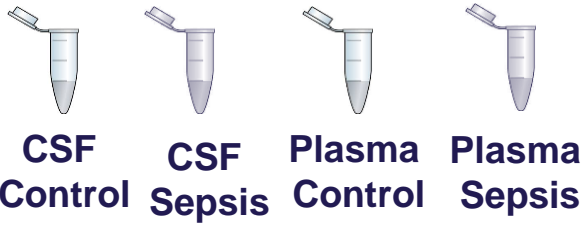
Sepsis-induced changes in cerebrospinal fluid affect proteome of the developing hippocampus - correlation to systemic changes



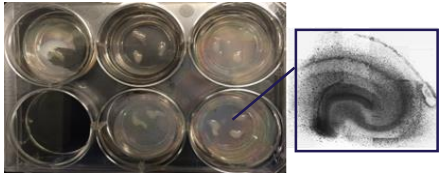
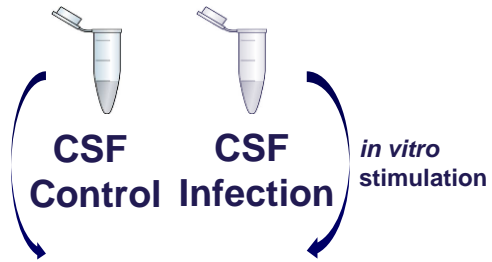
- *How systemic infection affects brain development and functions is poorly understood.*
- *Good biomarkers for early diagnosis and treatment for sepsis-induced brain injury are lacking.*



Preterm infant (GA<32 wk)



Formula-fed 5d preterm pigs (90% GA)



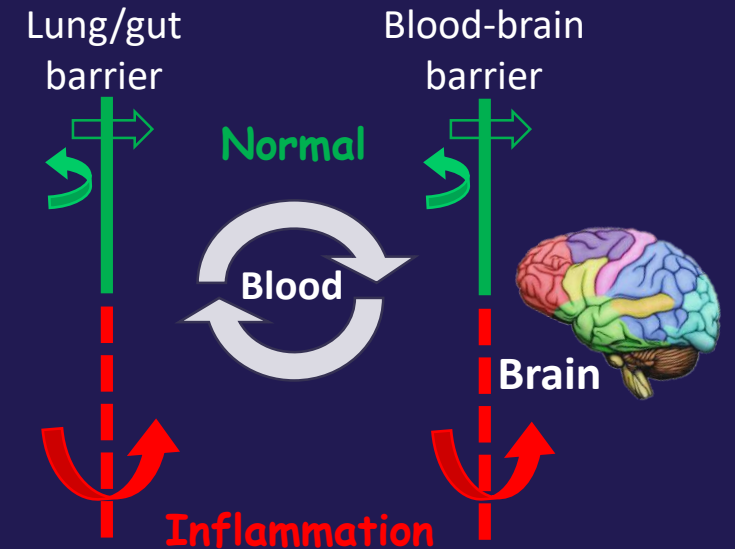
Primary hippocampal slices derived from preterm pigs



Collect culture medium and tissue

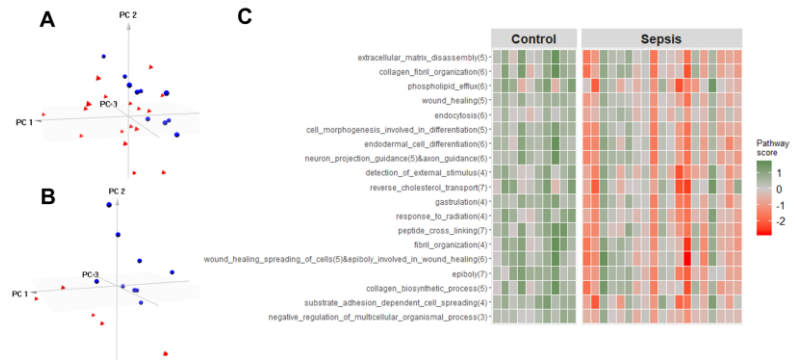
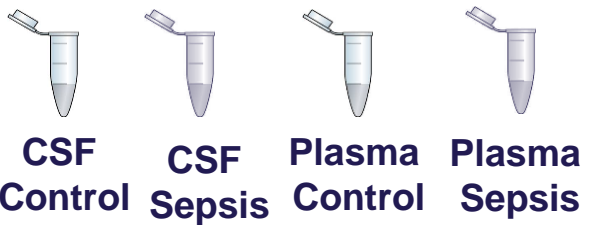


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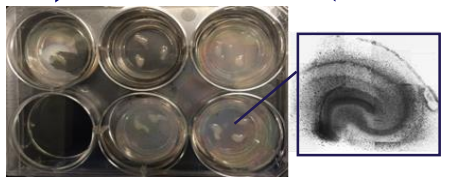
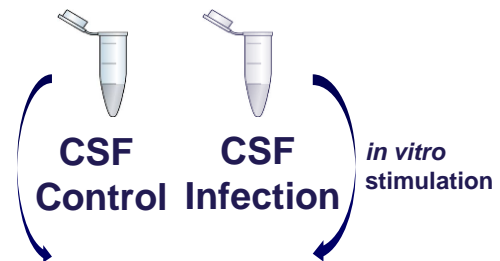
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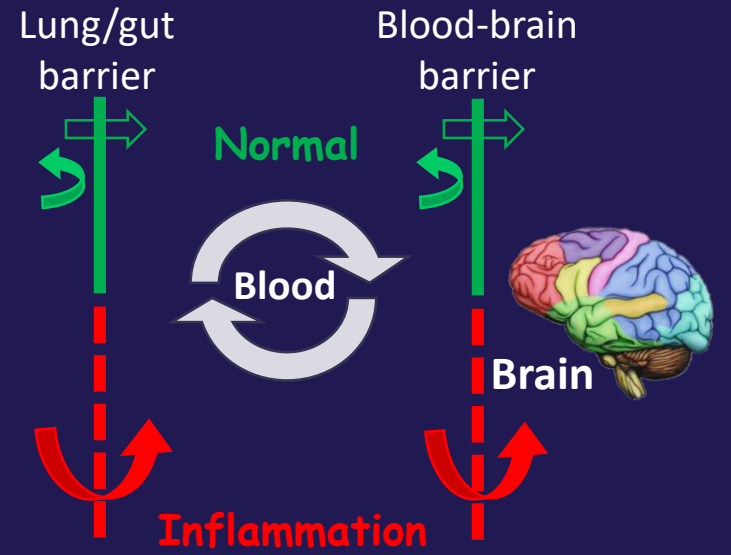
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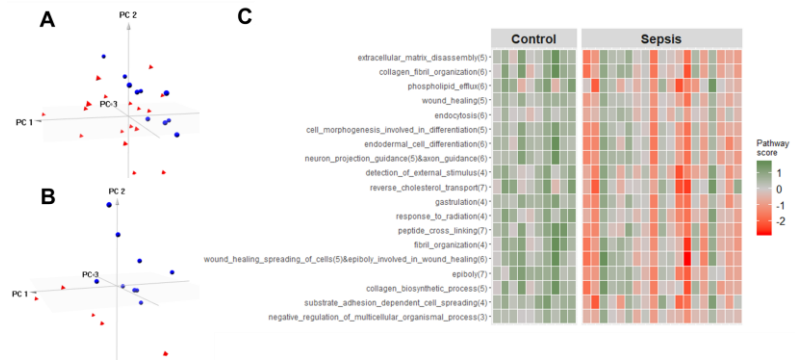
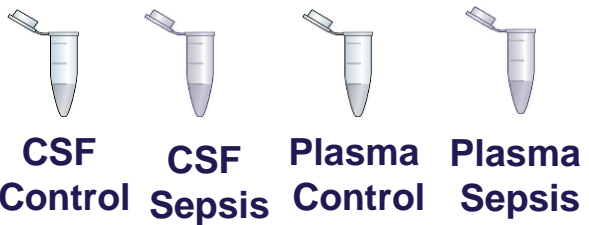


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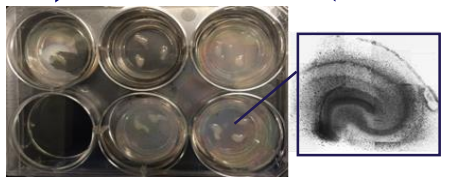
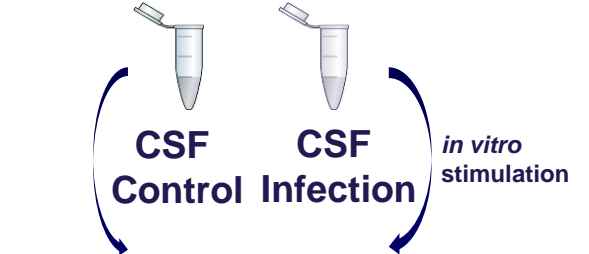
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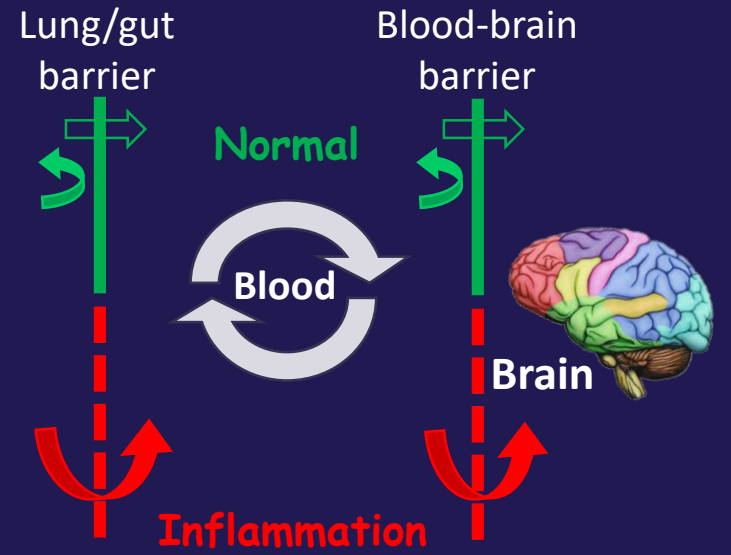
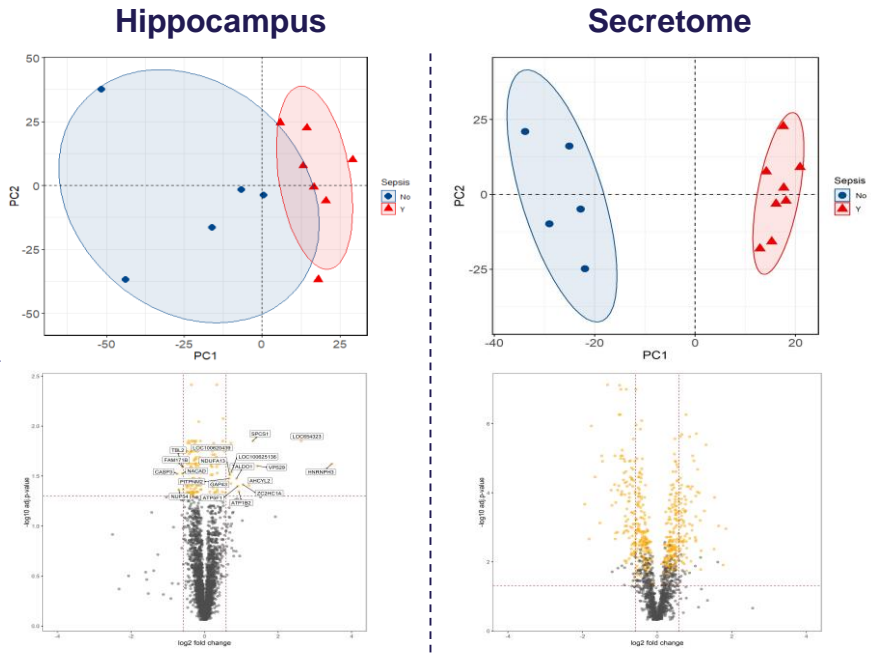
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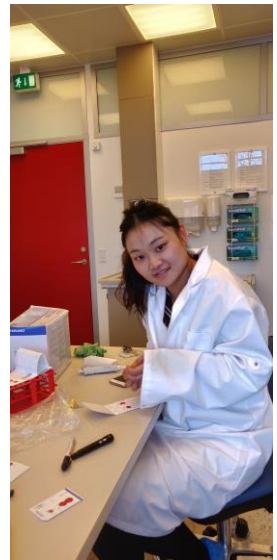
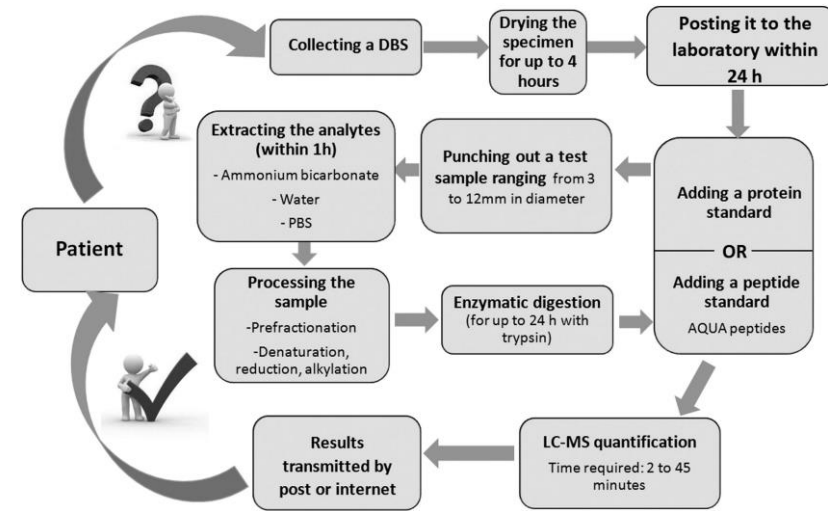


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Omics analysis of Dried whole blood samples – Whole blood analysis

- *Dried blood spots offer many advantages as a sample format including ease and safety of transport and handling.*
- *Dried blood spots are a potentially rich source of protein biomarkers, an area that has been overlooked.*

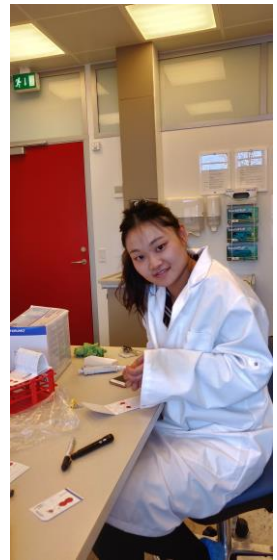
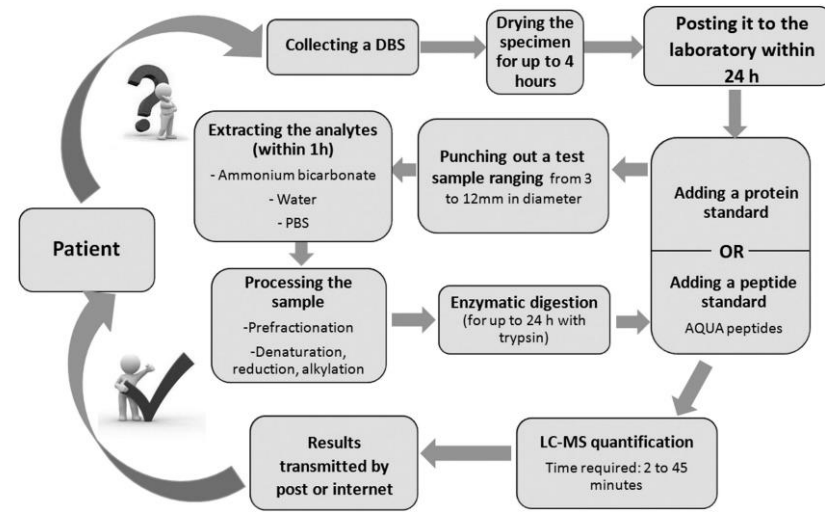


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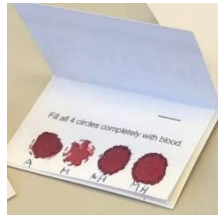
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Finger puncture whole blood



Pig Human



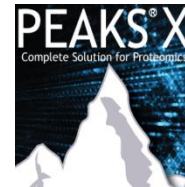
FASP extraction



5% SDC
S-TRAP



Single column setup (25cm Ionoptiks) and Bruker timsTOF PRO PASEF



Data analysis



Omic analysis of Dried whole blood samples – Whole blood analysis

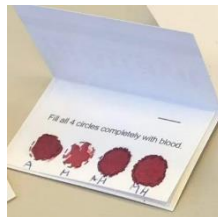
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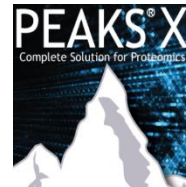


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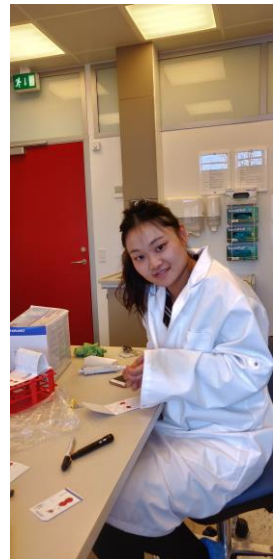
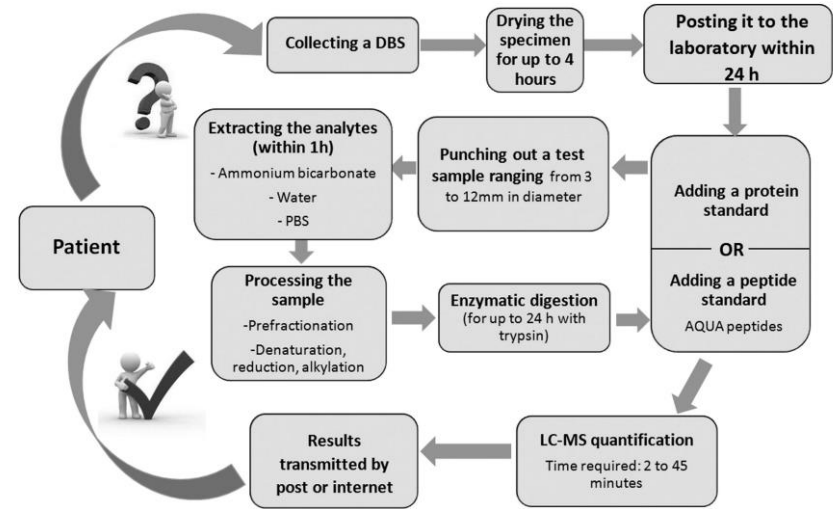
S-TRAP



Single column setup (25cm Ionoptiks) and Bruker timsTOF PRO PASEF



Data analysis



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Accession	-10lgP	Coverage	Con
P02768 ALBU_HUMAN	699.93	95%	
tr A0A0C4DGB6 A0A0...	697.05	94%	
tr B7WNR0 B7WNR0_H...	660.45	94%	
tr HOYA55 HOYA55_HU...	658.12	98%	
rows identified peptides R2_HU...	643.05	92%	
P04114 APOB_HUMAN	566.65	60%	
P01024 CO3_HUMAN	557.08	90%	
P68871 HBB_HUMAN	649.59	100%	
P69905 HBA_HUMAN	716.46	100%	
P02549 SPTA1_HUMAN	560.99	82%	
P01023 A2MG_HUMAN	539.93	84%	
tr G3V1N2 G3V1N2_HU...	703.30	100%	
P11277 SPTB1_HUMAN	529.64	74%	
P02042 HBD_HUMAN	528.02	100%	
P02787 TRFE_HUMAN	497.14	88%	
P16157 ANK1_HUMAN	540.23	72%	
P02647 APOA1_HUMAN	467.12	95%	
P0C0L5 CO4B_HUMAN	512.95	68%	
P0C0L4 CO4A_HUMAN	511.77	68%	
tr A0A0G2JPRO A0A0G...	510.96	68%	
tr A0A2R8Y7C0 A0A2R...	629.68	66%	
tr A0A0G2L54 A0A0G...	510.46	68%	



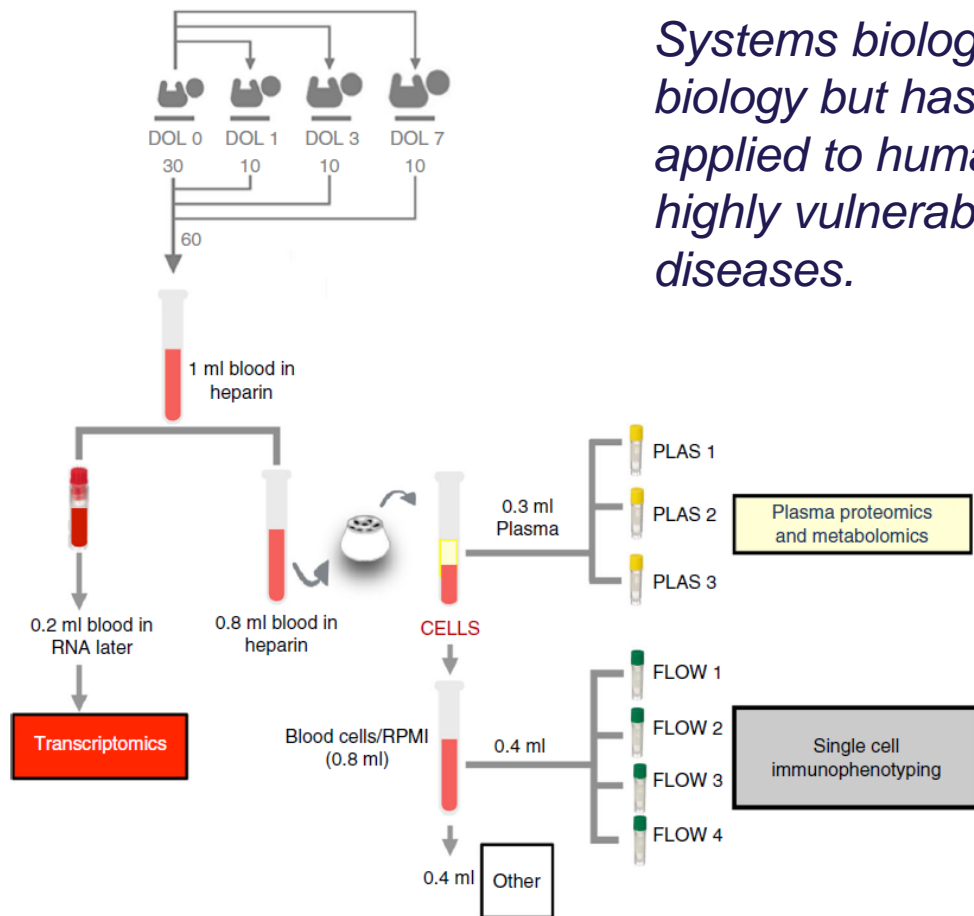
Deep proteome coverage reducing missing values for diagnostics and prognostics

Dynamic molecular changes during the first week of human life follow a robust developmental trajectory



Tue B. Bennike

Systems biology can unravel complex biology but has not been extensively applied to human newborns, a group highly vulnerable to a wide range of diseases.

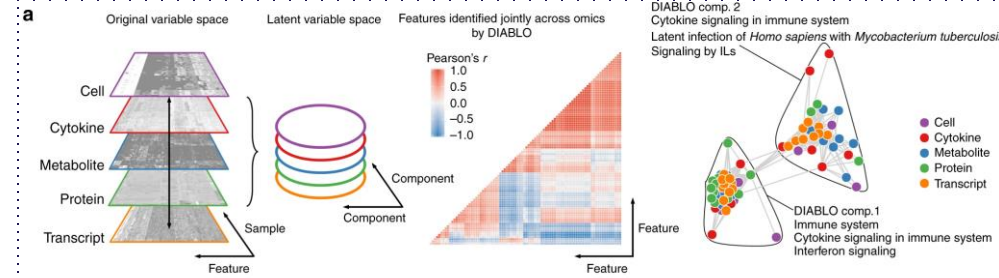
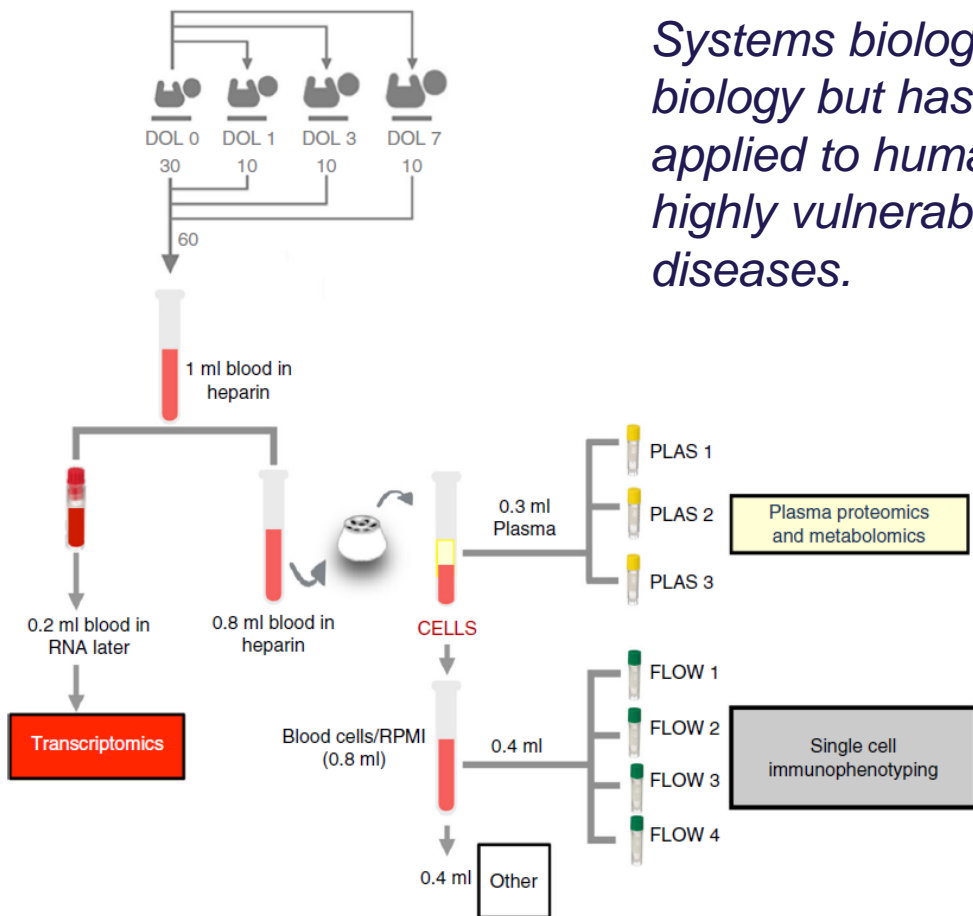


Dynamic molecular changes during the first week of human life follow a robust developmental trajectory



Tue B. Bennike

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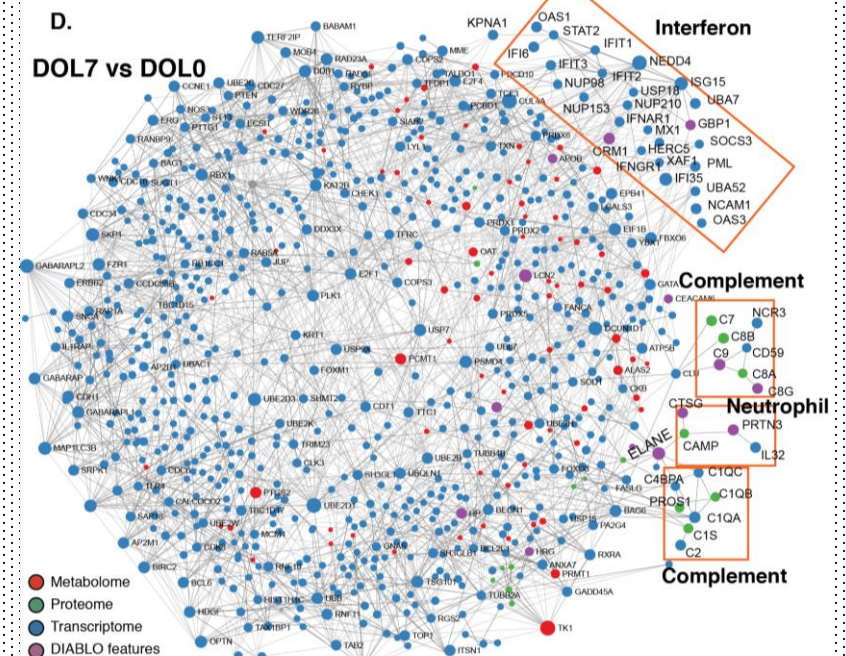
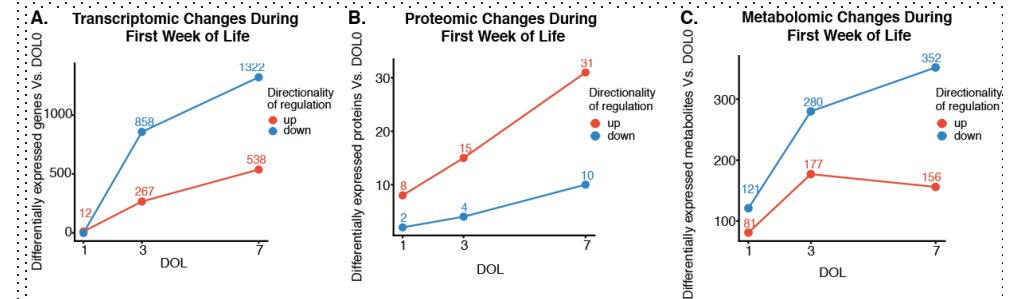
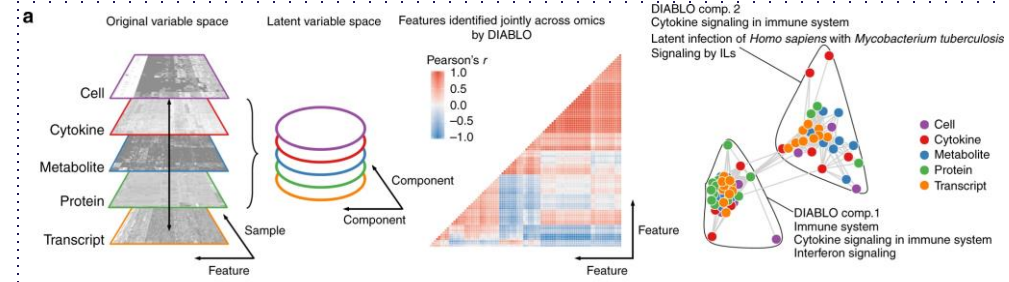
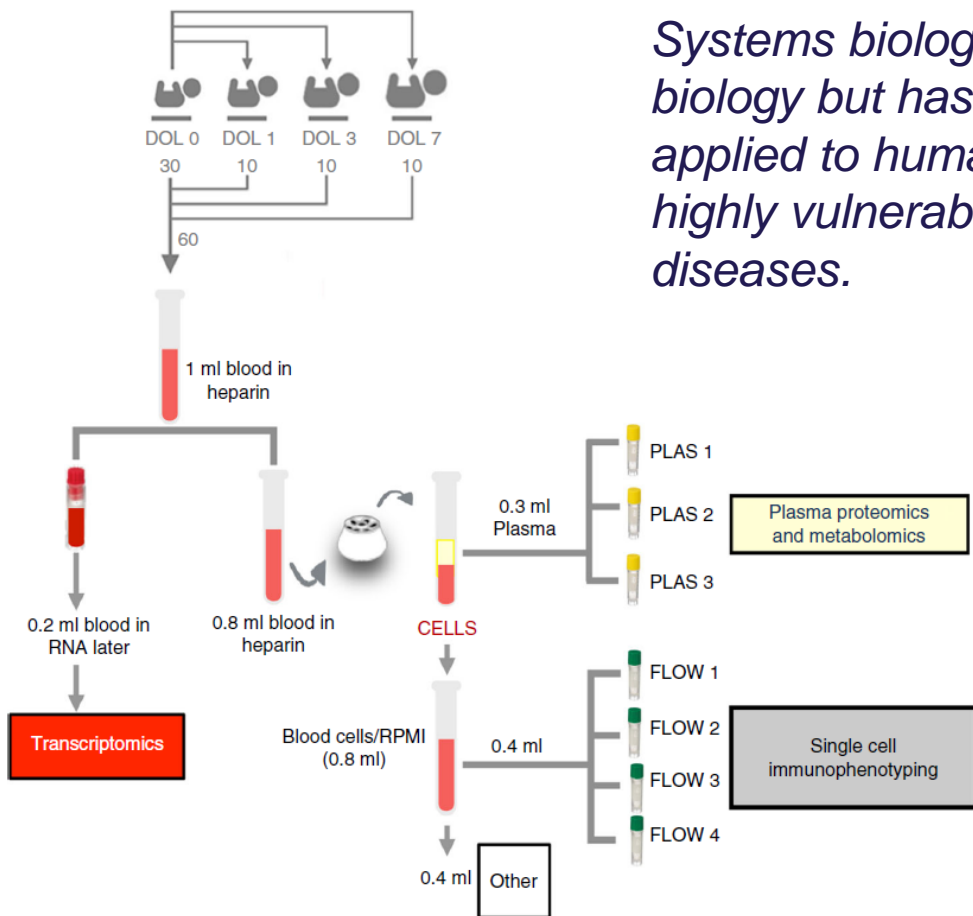


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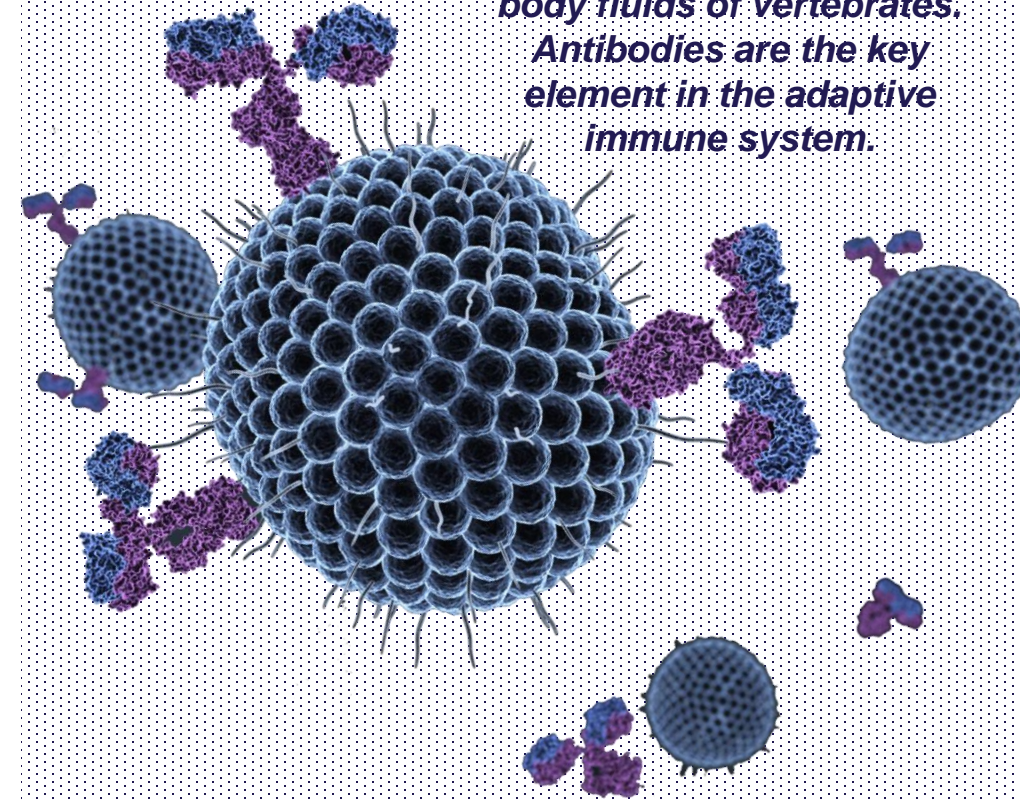
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Improved phenotyping of disease subtypes by Immunome array

- Elevated levels of autoantibodies are present before the clinical diagnosis of auto immune diseases



Antibodies (also called immunoglobulins) are large Y-shaped proteins. They are found in the blood or other body fluids of vertebrates. Antibodies are the key element in the adaptive immune system.



Proportion of Patients with Positive Antibody Tests Relative to the Time of Diagnosis or Appearance of the First Clinical Manifestation of Systemic Lupus Erythematosus

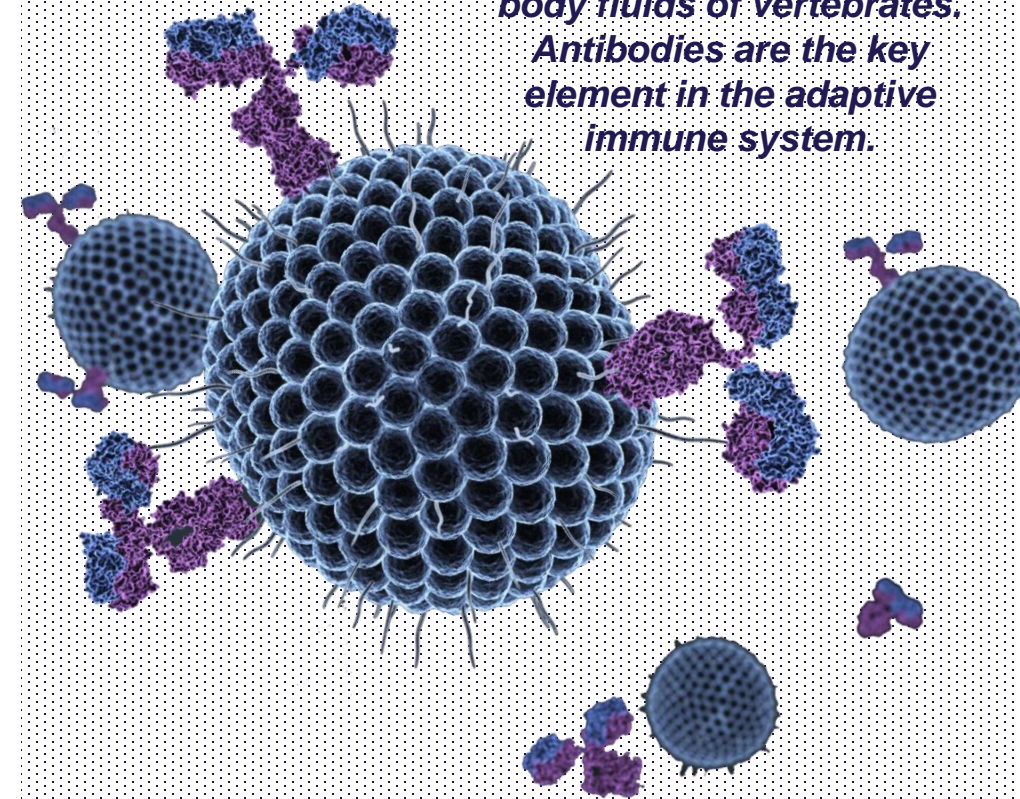
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- **Rheumatoid Arthritis – Up to 16yr**
- **Multiple sclerosis - Up to 14yr**

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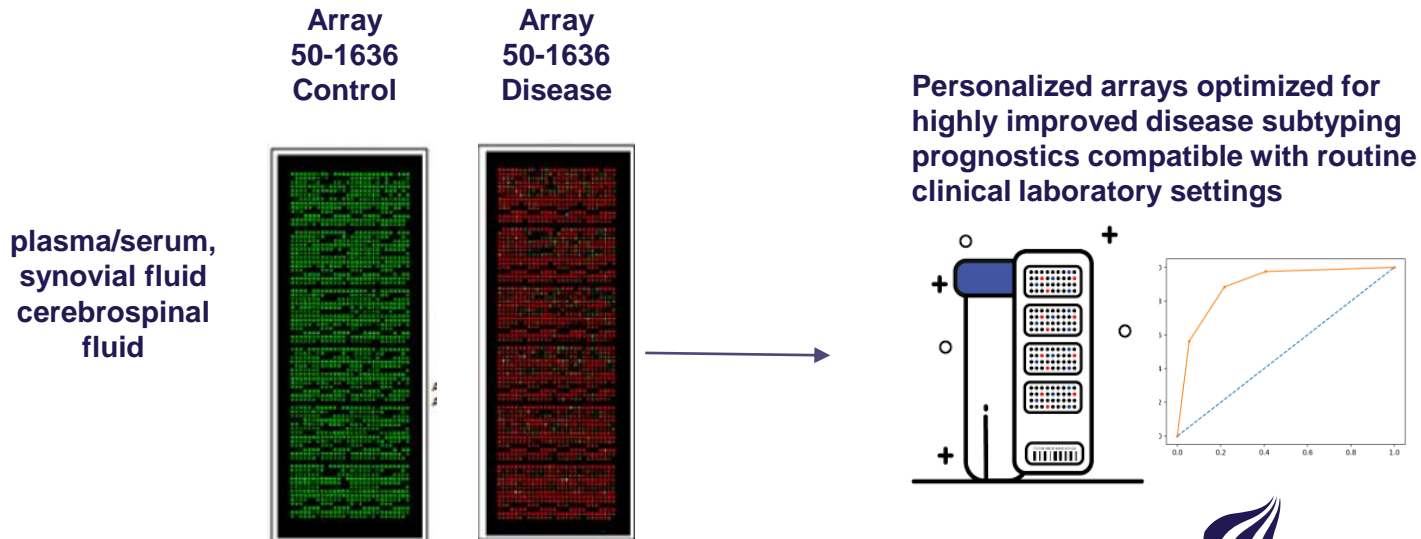


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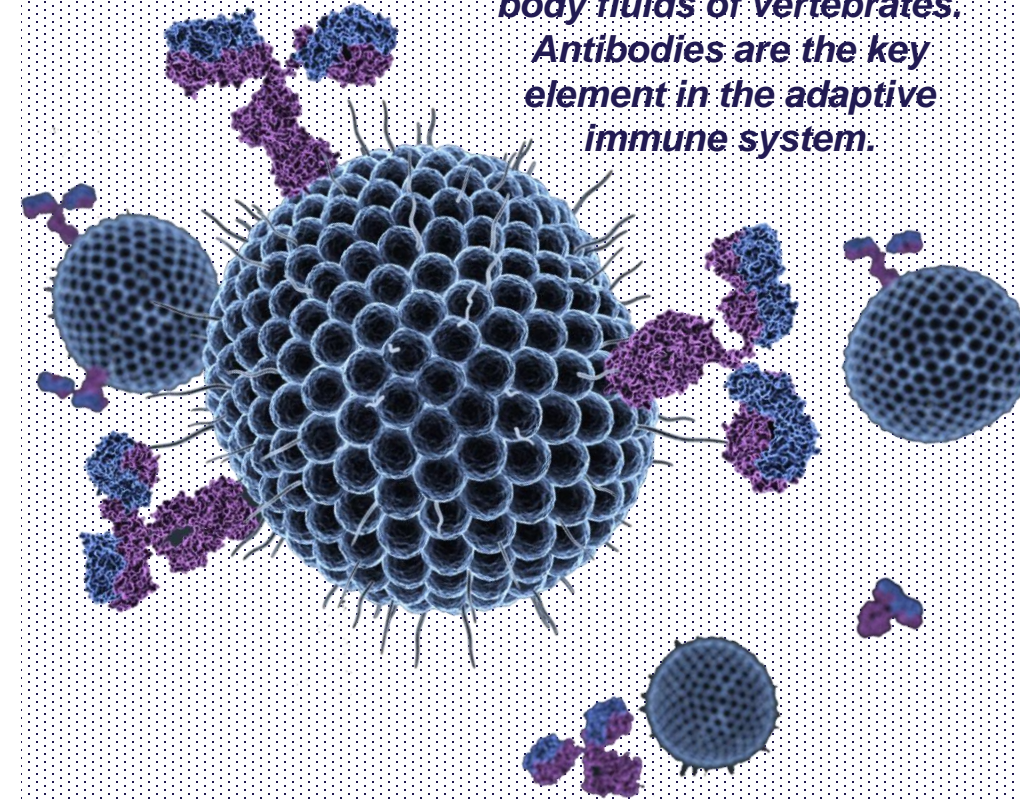
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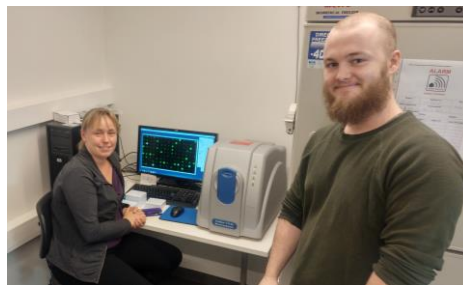
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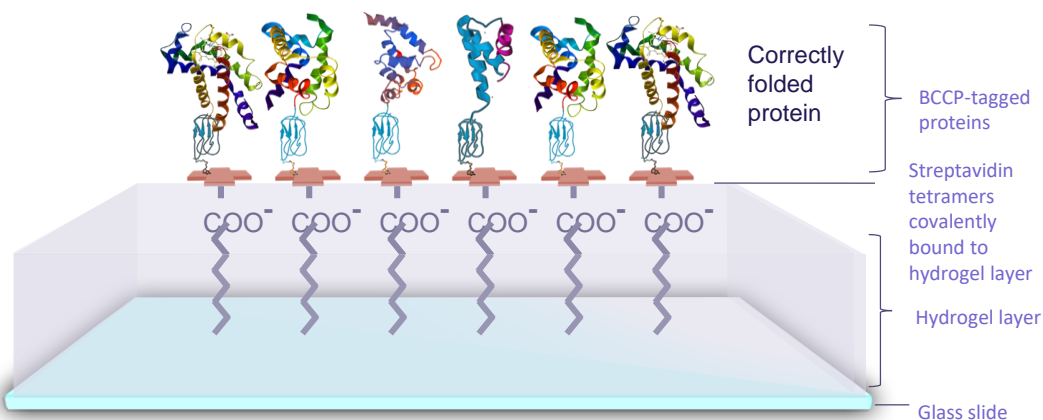
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Digging deeper into the autoantibody-ome in biofluids



Thomas BG Poulsen
Malene M. Jørgensen

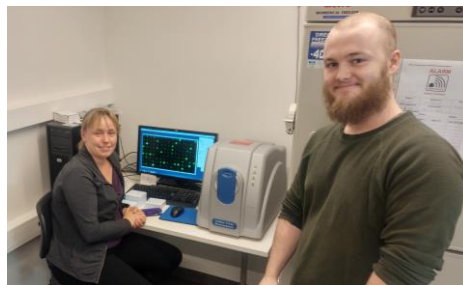
- ▶ IMMUNOME Discovery Array is a protein array which utilizes the patented Sengenics KREX™ functional proteomics technology.
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KREX technology enables autoantibody panels from single to 1600+ individual antigens.

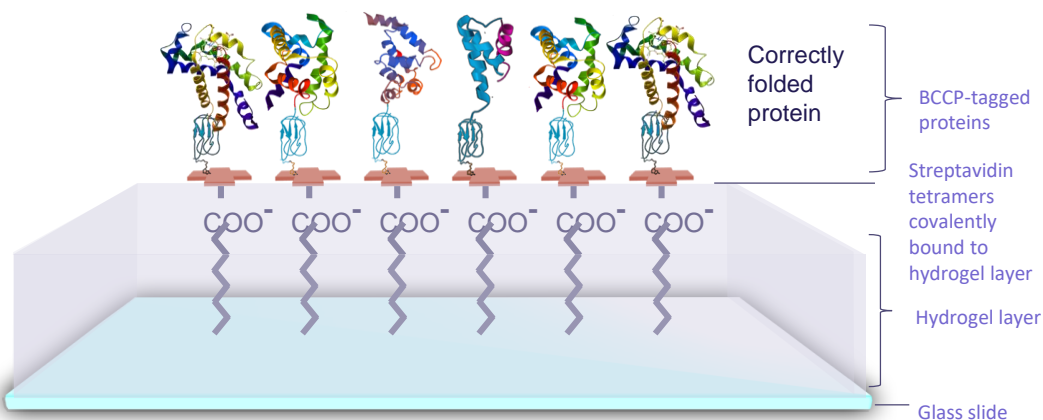


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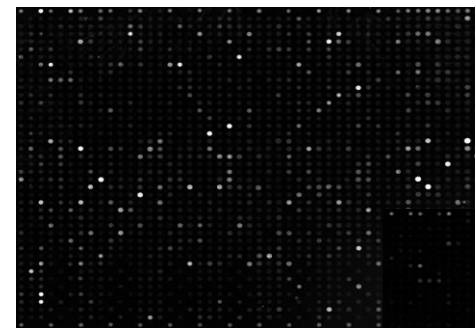
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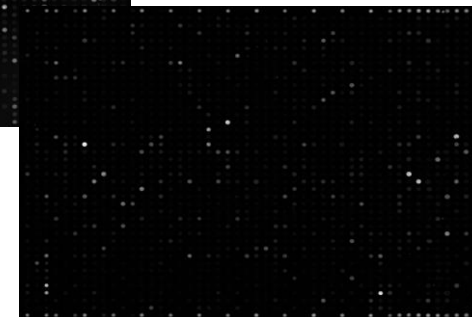


Discover diagnostic autoantibody biomarkers

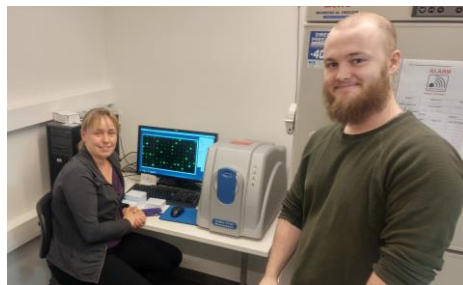


Autoimmune disorder
Anti-CCP pos

Autoimmune disorder
Anti-CCP neg



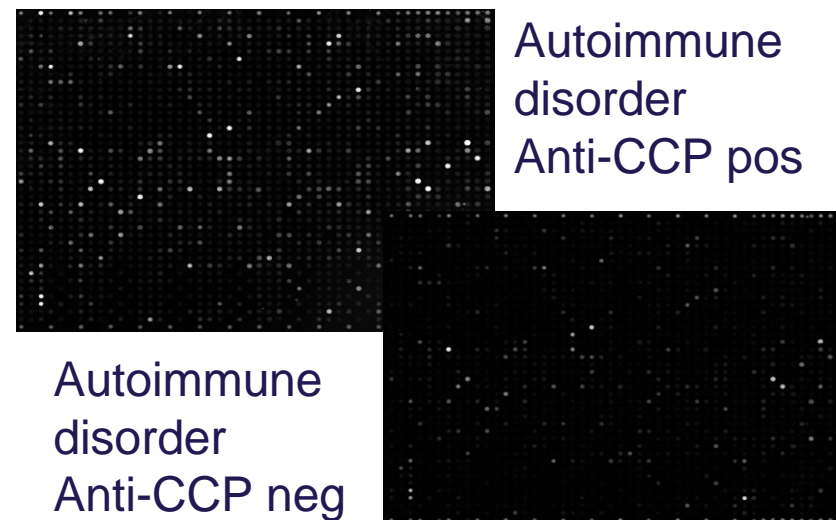
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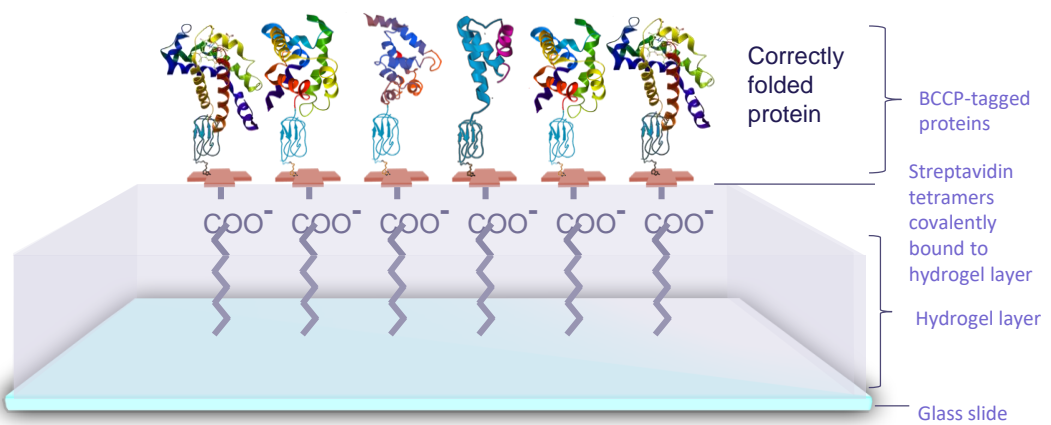
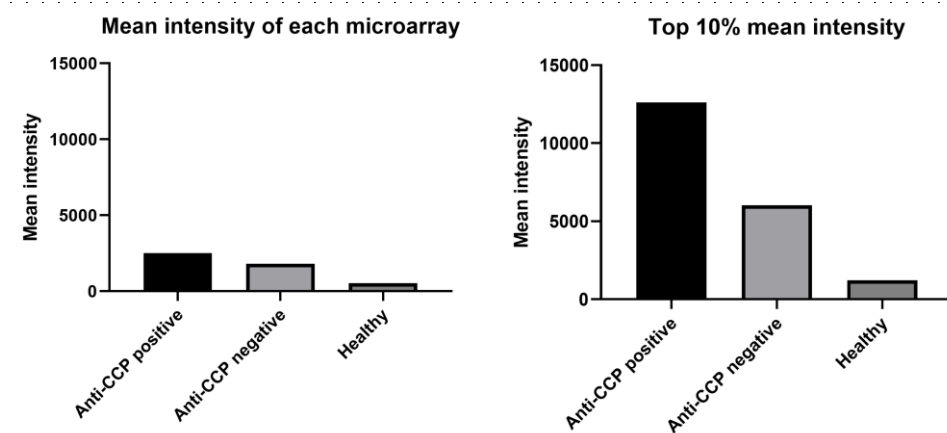
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Discover diagnostic autoantibody biomarkers



Low noise of array technology enabling



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Glucocorticoid Responsive mechanisms in plasma from Polymyalgia Rheumatica patients



Michael Kruse Meyer



Polymyalgia rheumatica

Areas of pain

Polymyalgia rheumatica is a relatively common inflammatory rheumatic disease.

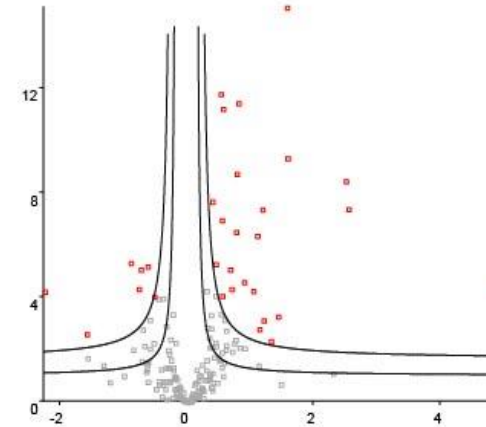
There are no validated international guidelines available for the diagnosis and treatment of PMR; however, diagnostic and classification criteria are currently being developed.

A quantitative proteome study design to compare with DMARD naïve RA patients and matched controls

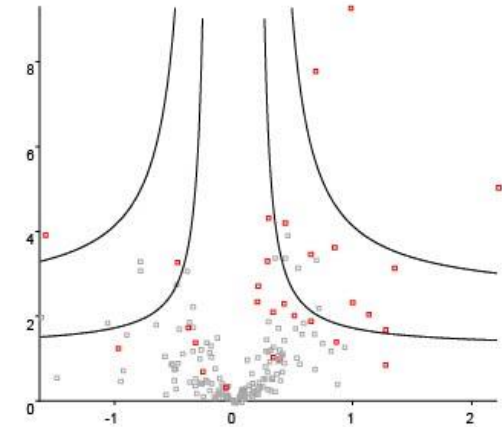


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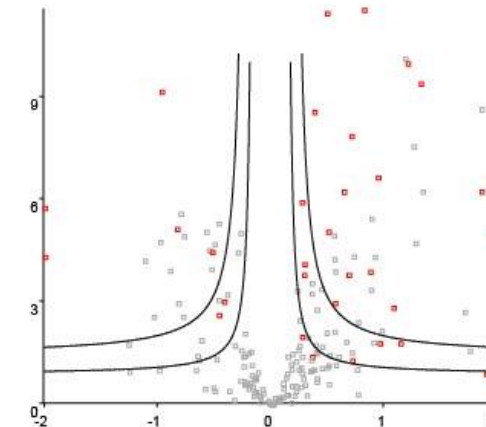
PMR before treatment



PMR after treatment



Naïve RA patients



- 9 patients included in the study;
- Serum samples before versus after 3 months treatment

Glucocorticoid Responsive mechanisms in plasma from Polymyalgia Rheumatica patients



Michael Kruse Meyer



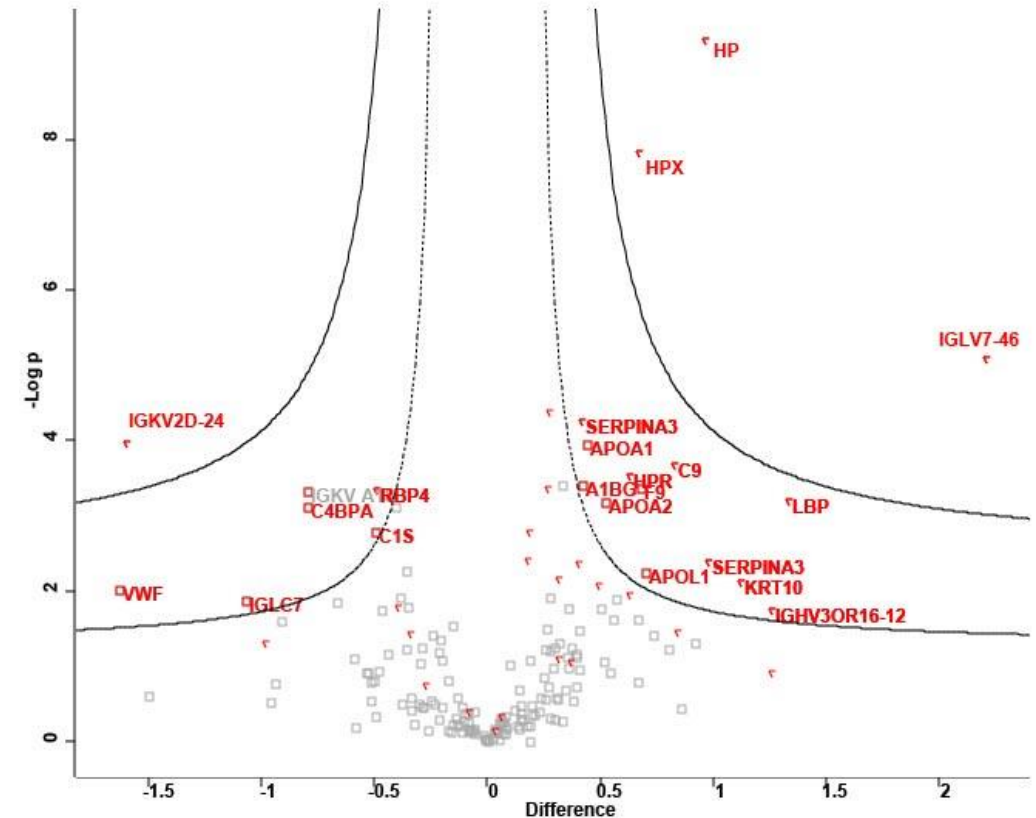
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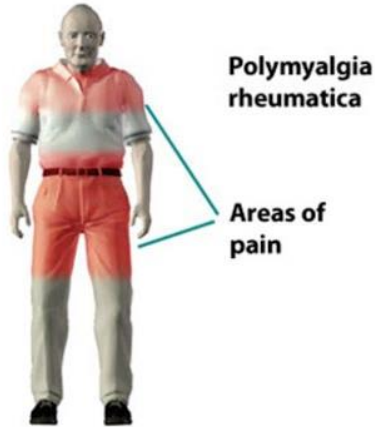
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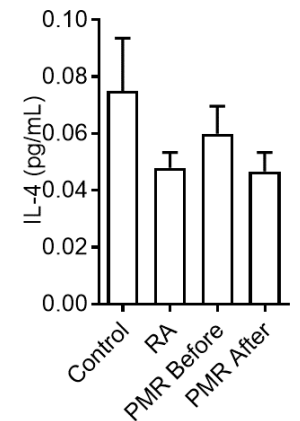
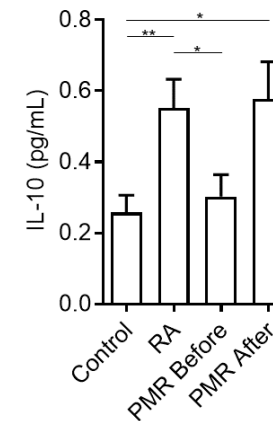
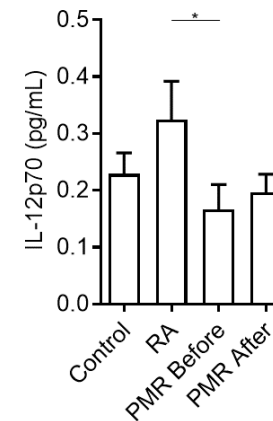
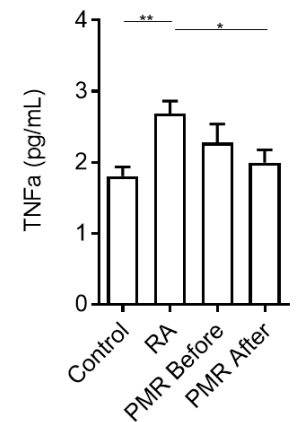
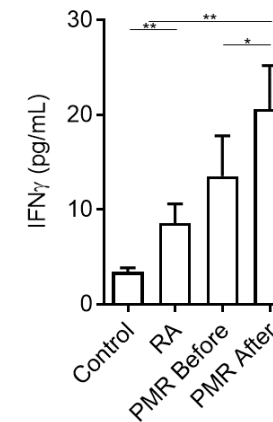
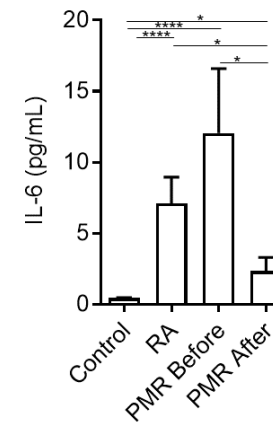
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- Tue Bjerg Bennike
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- Joakim Bastrup
- Ditte Kristensen
- Kenneth Kastanjegaard
- Michael Kruse Meyer

Sangild Lab

- Tik Muk
- Ping ping Jiang
- Ninh Duc Nguyen



Other Important People for the presented work

- Malene Møller Jørgensen
- Rikke Bæk
- Rikke Gry Nielsen (vet)
- Jonathan Blackburn - Sengenics



Danish National Hospital

- Claus H. Nielsen
- Dres Damgaard

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Svend Andersen Fonden



DET FRIE
FORSKNINGSRÅD
DANISH COUNCIL
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LUNDBECK FOUNDATION



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OBELSKE
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