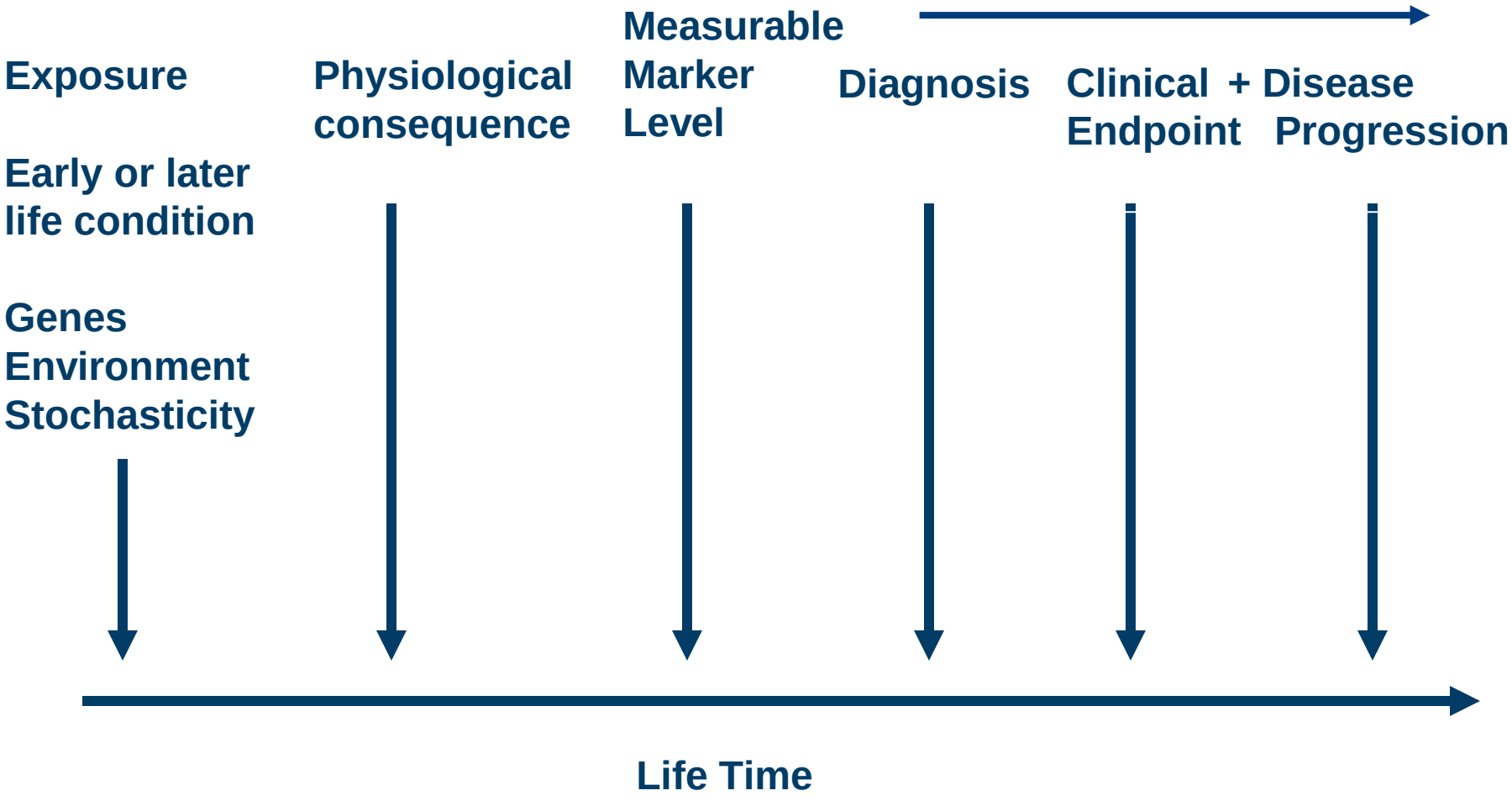
Molecular Epidemiology

- Introduced by Kilbourne (1973), infectious diseases; Schulte and Perera (1993 Principles and Practices)
- Integrates Epidemiology, Medical Sciences and Molecular Biology
- Studies the influence on health of environmental and genetic risk factors measured by (holistic) molecular signatures
- Contributes to
  prediction/prognosis
  monitoring exposure, response to interventions
  etiological understanding (disease mechanisms)

Exposure Events in lifetime perspective



Biomarkers I

- Relation of Exposure /determinant and outcome

- Exposure: environment (early, late, diet, lifestyle, chemicals, geography), host (genetic background, age), health change over time (disease, biological ageing process); outcome = phenotype

- Biomarker (WHO): a substance or biological structure that can be measured in the human body and may influence, explain or predict the incidence or prognosis of outcome of disease

Biomarkers II

or NIH biomarker working group:
a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

Classical Algorithms

Definition Metabolic syndrome

Abdominal obesitas >94 cm men, >80 cm women and ≥ two of the next:

- Fasting glucose >100 mg/dL (5,6 mmol/L) or diabetes

Triglycerides >150 mg/dL (1,7 mmol/L) or treatment

HDL-cholesterol <40 mg/dL for men, < 50 mg/dl for women or treatment

Blood Pressure > 130/85 mmHg SBP or treatment

Definition Framingham risk score (10 years CVD risk)

- Age
- Gender
- Smoking
- Diabetes
- Total cholesterol
- HDL-cholesterol
- Systolic Blood Pressure

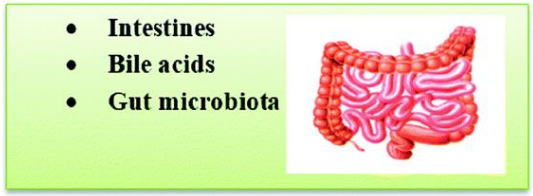
Adding omics data to biomarker research

You have discussed epigenome/transcriptome
Now we will focus on the metabolome.

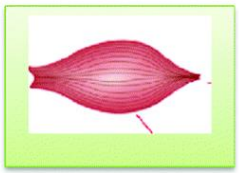
100.000 metabolites in your body
In blood: 4000 metabolites.

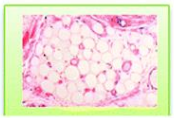
Best platforms measure about 600 in a standardized fashion high throughput

Which tissue functions do the 1H NMR metabolites represent ?




Kidney


Skeletal Muscle


Adipose Tissues

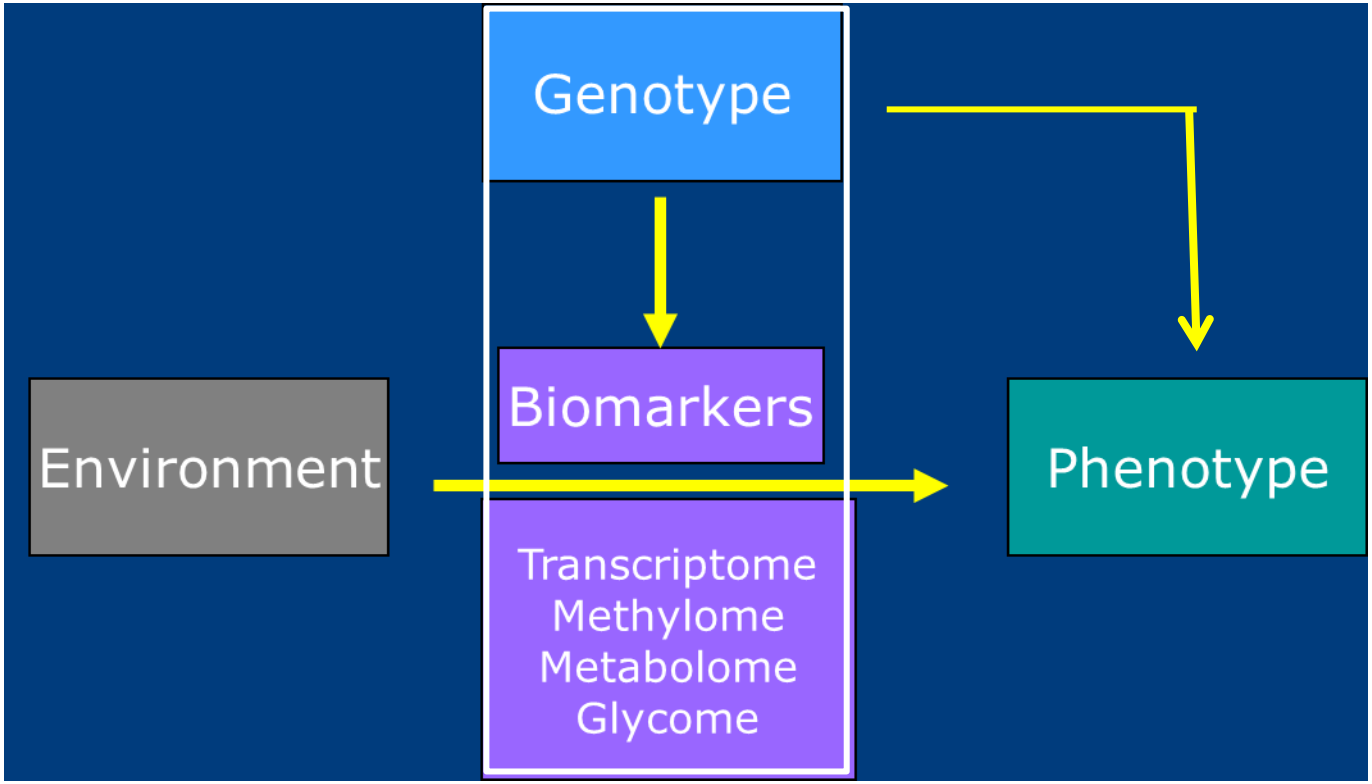

Brain


Heart

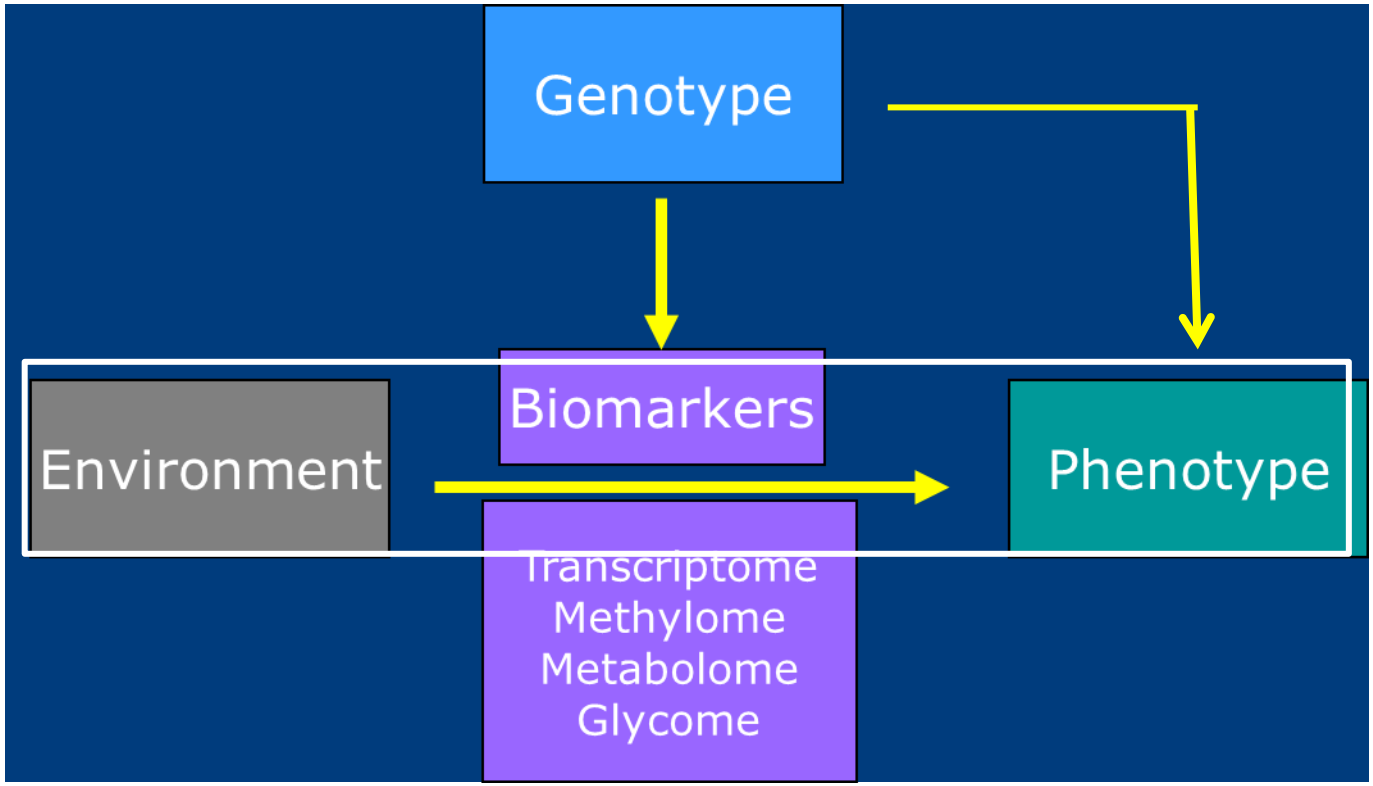

Liver

Betaine
TMAO
Choline
DMG
Glycine
Creatinine
Creatine
1, 5-AG
Phosphotidycholine
Methylamine
Dimethylamine
Phospholipids
Glycogenesis
Carnitines
Serine
Gluconeogenesis
Glucose
Glycogen
Glycogenolysis
Alanine
Glycolysis
Fat Storage
Triglycerides
Pyruvate
Lactate
B-oxidation
Fatty Acids
Acetyl-coA
Branched Chain Amino Acid (BCAA)
Lipogenesis
Acetate
β-hydroxybutyrate
Ketone Bodies
Oxaloacetate
Citrate
Acetoacetate
TCA Cycle
Malate
Isocitrate
Acetone
3-HB
Fumarate
2-Oxoglutarate
Succinate
Succinyl-coA

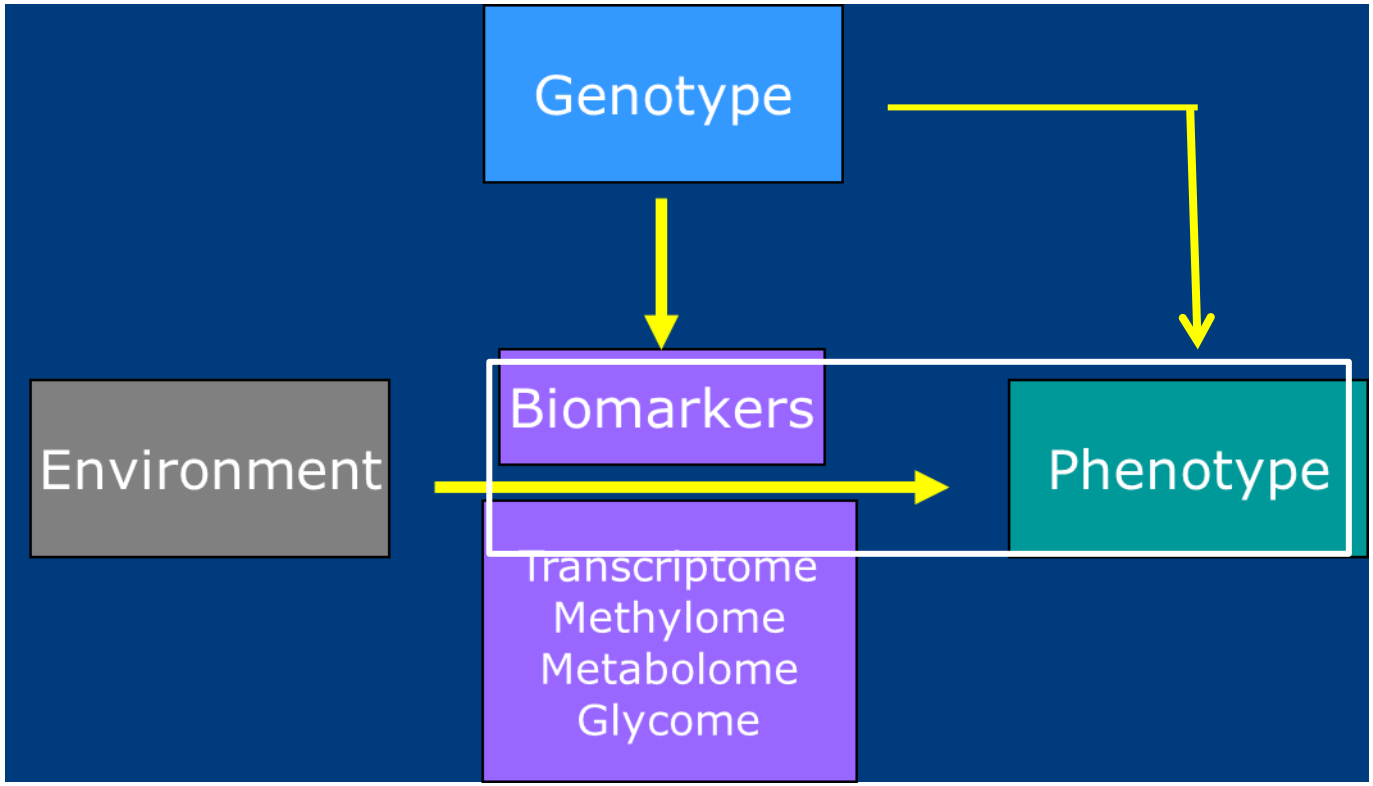
What relations do scientists investigate with molecular profiling data



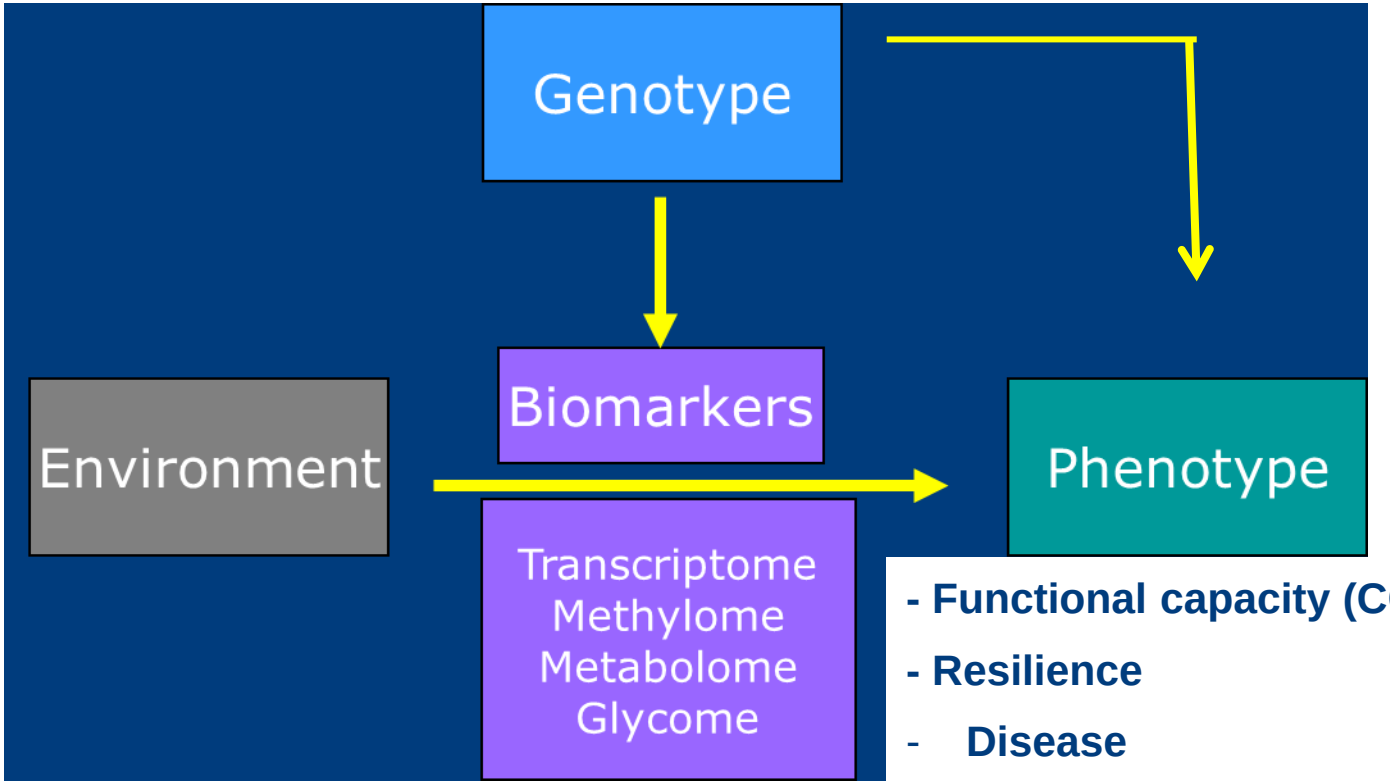
What relations do scientists investigate with molecular profiling data



What relations do scientists investigate with molecular profiling data



Integrated studies in humans Focused on ageing phenotypes



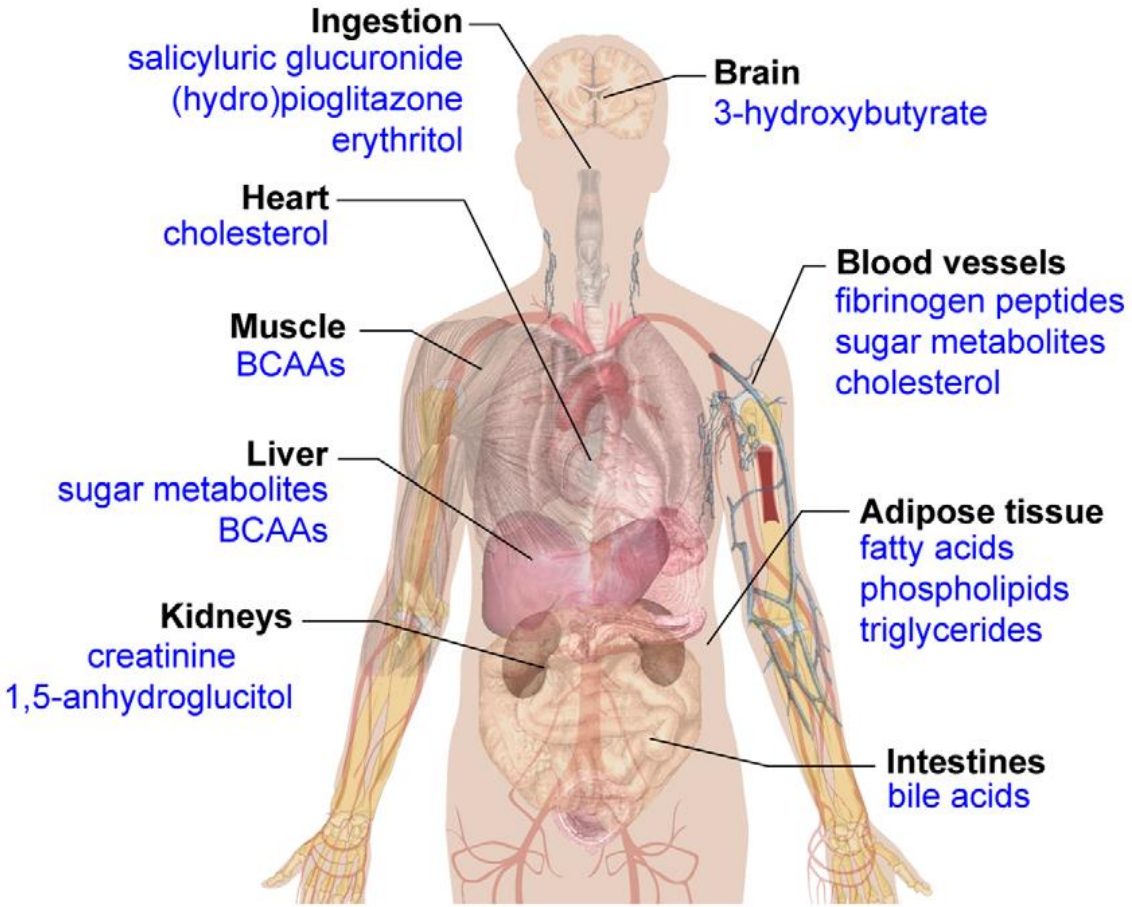
- Functional capacity (CGA)

- Resilience

- **Disease**
- **Mortality, Longevity**

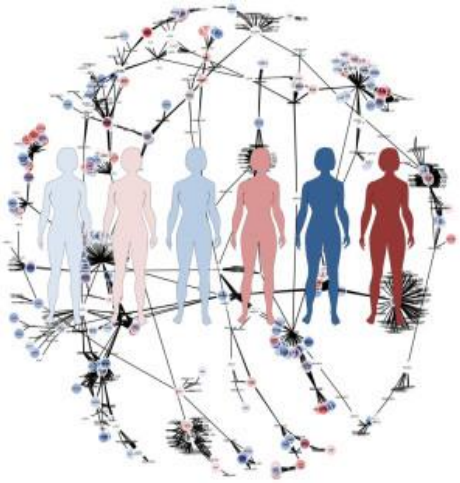
- Biological Age; Surrogate + Endpoints of ageing

Molecular Biomarkers
Type II Diabetes prediction 1H NMR metabolites



Suhre et al. PLoS ONE |November 2010 | Volume 5 | Issue 11

Datasets: BBMRI Biobanking consortium
Multi-level omics data



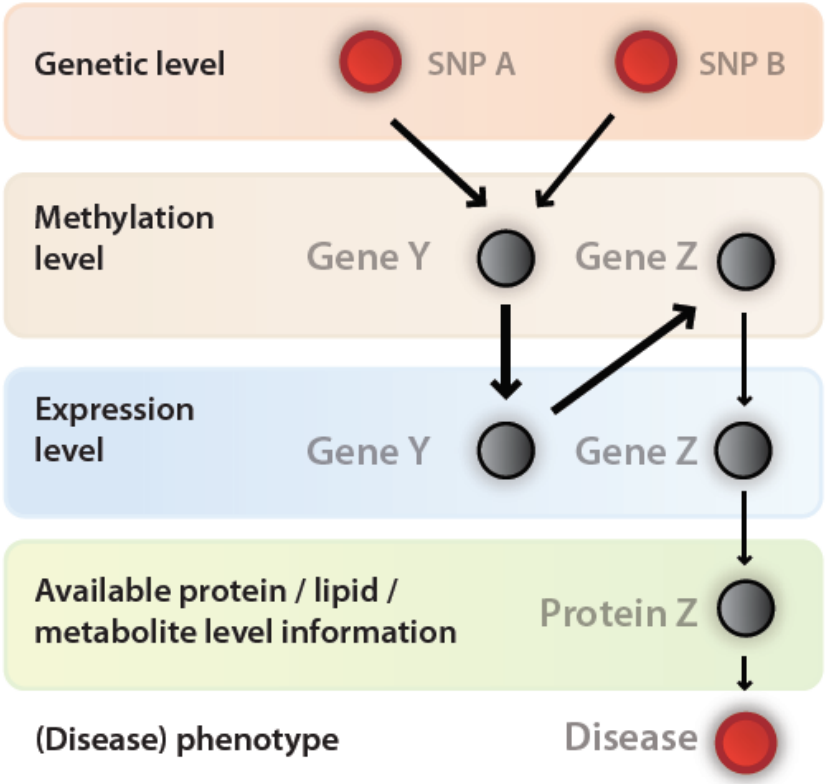
N=100,000 GWAS
N=750 Go.NlL

N=4,000

N=4,000

N=50,000

N>250,000



Go•NL
GENOMEoftheNETHERLANDS

BIOS consortium

BIOS consortium

METABOLITE consortium

Central databases for the research community

1H-NMR metabolomics platform

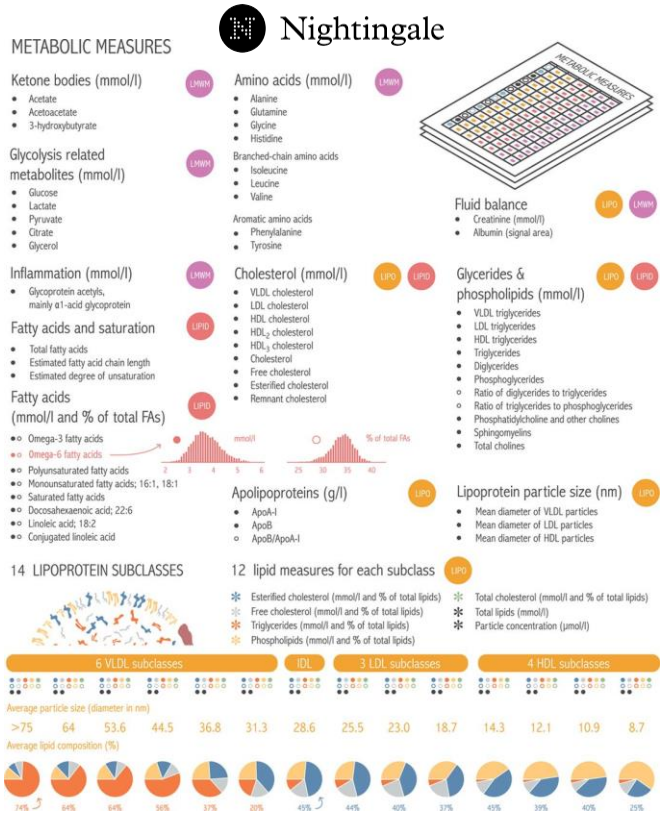
Nightingale

- NMR-based quantification:
  - Standardized
  - High-throughput
  - Quantitative (mmol/l or g/l)

- 226 metabolic biomarkers

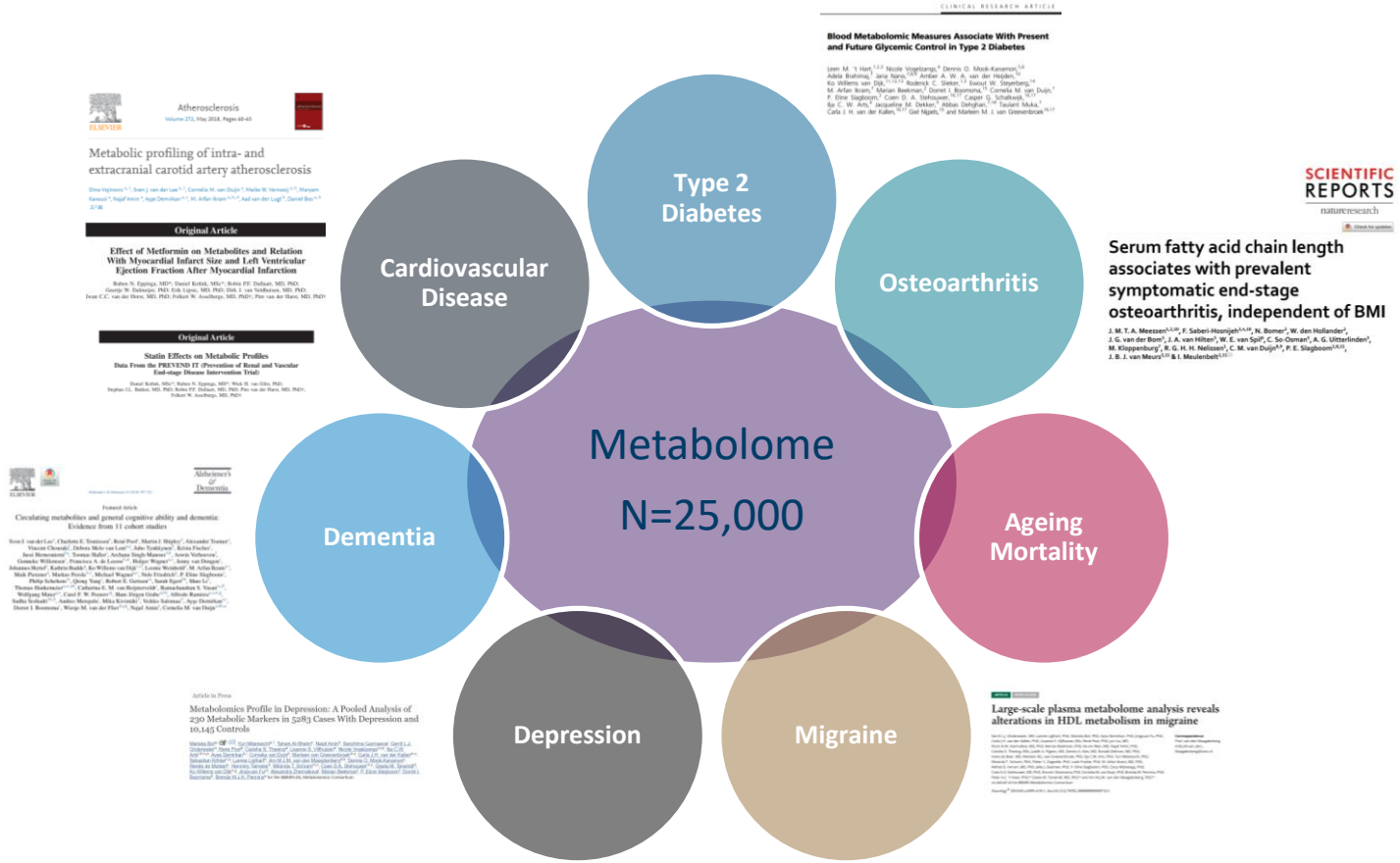
Lipids/FA/(apo)lipoproteins/aminoacid /glycolysis products

- Widely-used in epidemiological studies.
  - Diabetes (type 1 & 2)
  - Cardiovascular disease
  - Neurological diseases



1H-NMR metabolome in 25.000 Dutch Caucasians
22 Prospective Patient and Cohort studies for 7 Diseases
BBMRI-metabolomics consortium

www.bbmri.nl/omics-metabolomics



Steps to prove the molecular markers make a novel predictor

Generate a predictor : factor identification and model development :

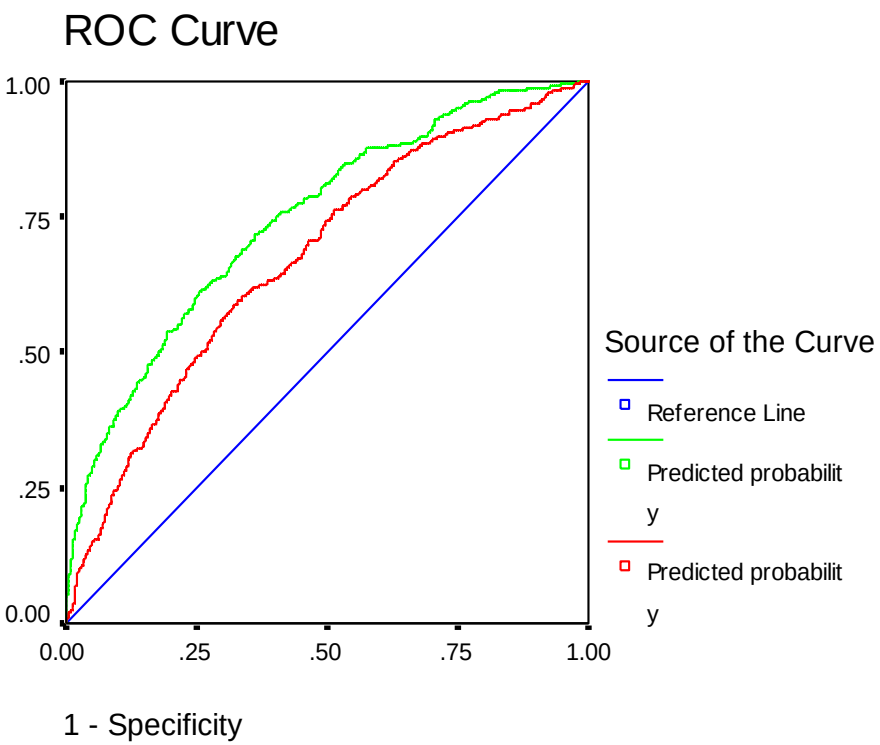
1. **Exploration of associations between metabolites and diverse endpoints.**
2. **Cross sectional → Prospective/longitudinal follow-up studies**
3. **Univariate (single metabolites) , multivariate**
4. **Replication in independent studies**
5. **Meta-analysis in multiple studies, create predictors (for example of mortality risk) and compare to existing predictors**

Prediction of disease risk
Using omics variables; how to understand the literature

Classification Table (Counts)

True Condition		Predicted Condition: Positive	Predicted Condition: Negative	Total
	Positive	True Positive (A)	False Negative (C)	A + C
	Negative	False Positive (B)	True Negative (D)	B + D
	Total	A + B	C + D	A + B + C + D

Receiver Operator Characteristic (ROC) curves to compare novel and traditional predictors (example 10 y CVD risk)



True positive rate

True negative rate

Blue: 50%-50%

→ AUC=0.5

Red: model with Age , Diabetes, Smoking

→ AUC=0.67

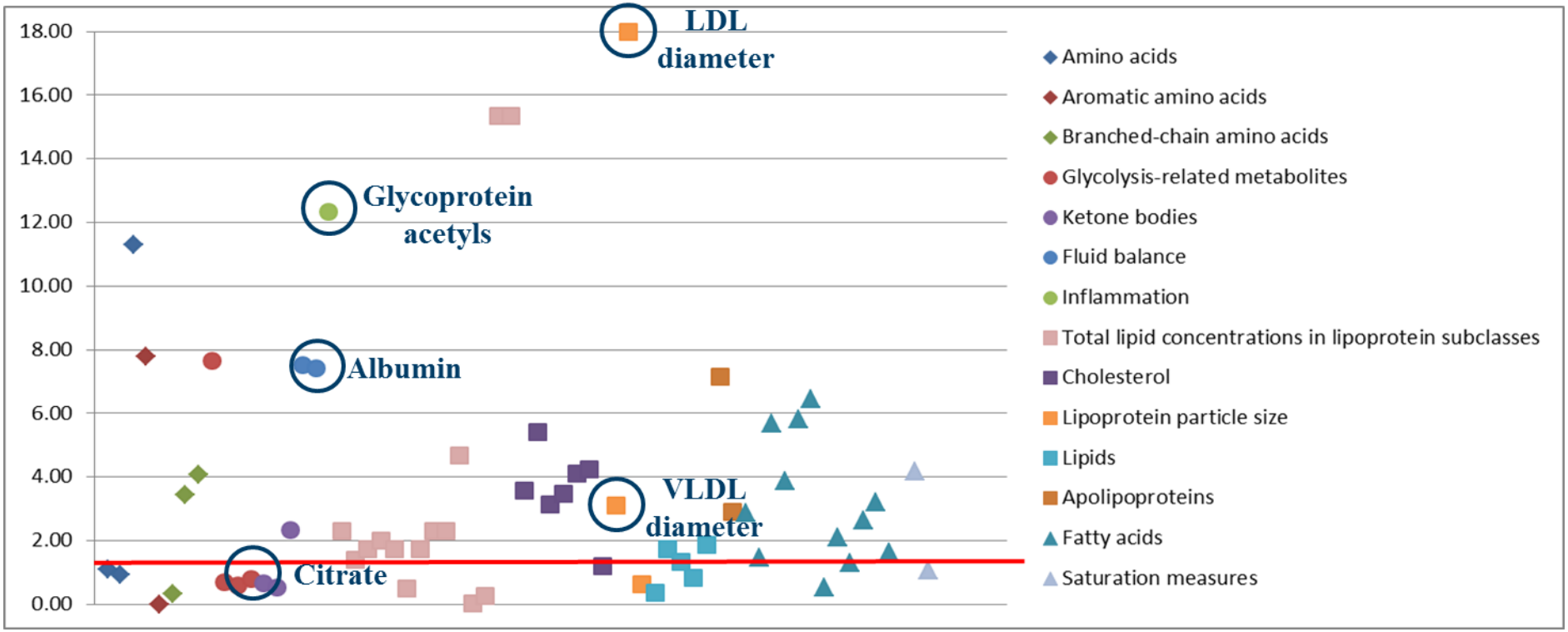
Green: model with Age, Diabetes, Smoking HDLcholesterol and systolic blood pressure

→ AUC=0.75

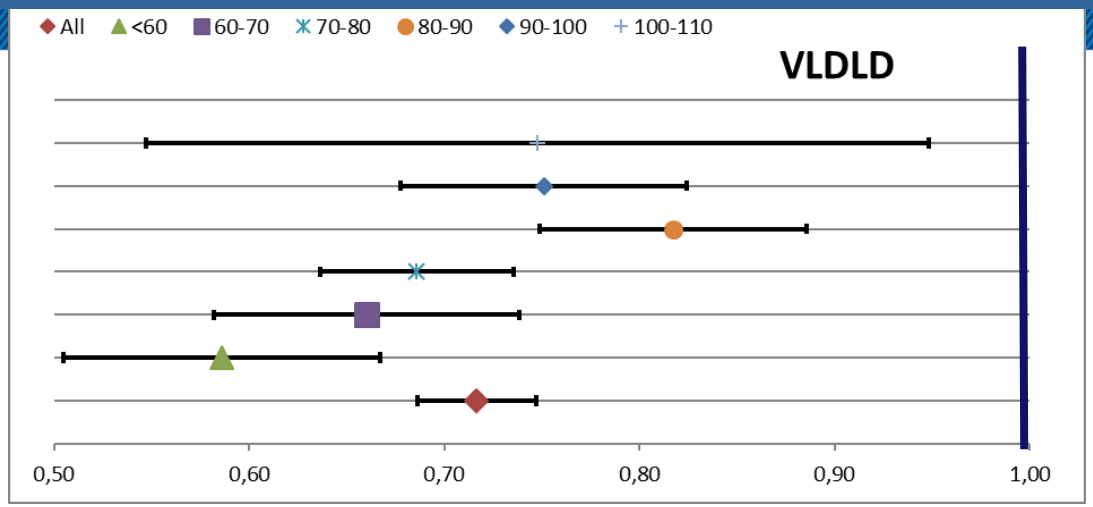
MORTALITY: 1H NMR based analysis
44.000 subjects and 5.500 deaths

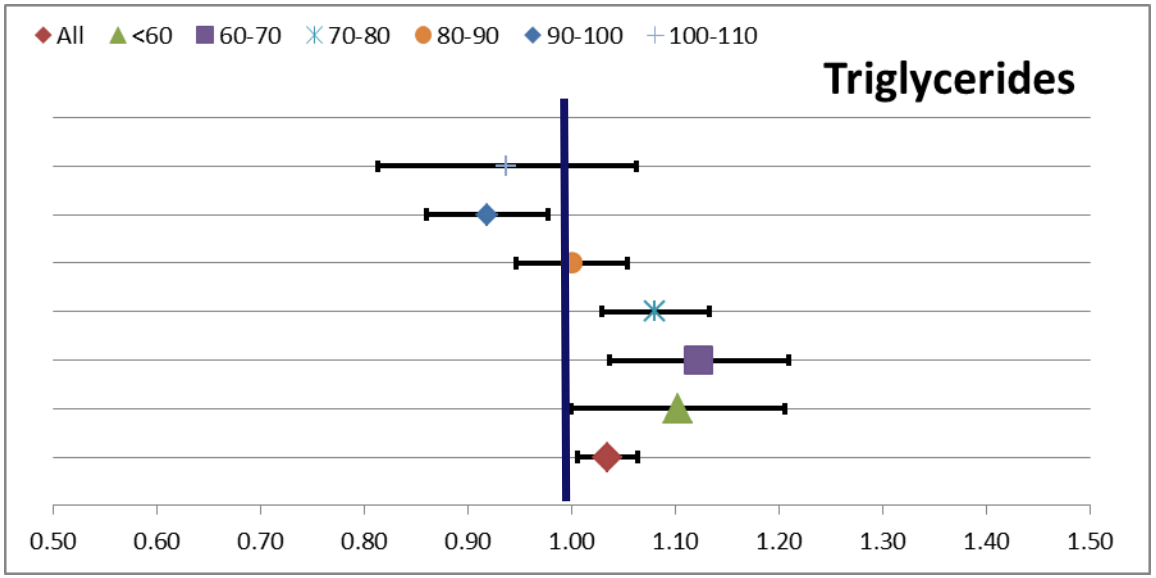
Study	N	Males (%)	Deaths
AlphaOmega	568	428 (75.4%)	157
ALSPAC	4,351	0 (0%)	17
EGCUT	10,988	4,106 (37.4%)	912
ERF	680	307 (45.1%)	107
FINRISK97	7,603	3,778 (49.7%)	1,213
FINRISK07	4,816	2,256 (46.8%)	190
KORA	1,790	871 (48.7%)	123
LLS nonagenarians	843	326 (38.7%)	823
LLS offspring + partners	2,241	999 (44.6%)	191
PROSPER	5,329	2,583 (48.5%)	467
Rotterdam Study	2,963	1,241 (41.9%)	1,254
TwinsUK	1,996	0 (0%)	58
	44,168		5,512

1H NMR-Based Metabolites associating to Mortality



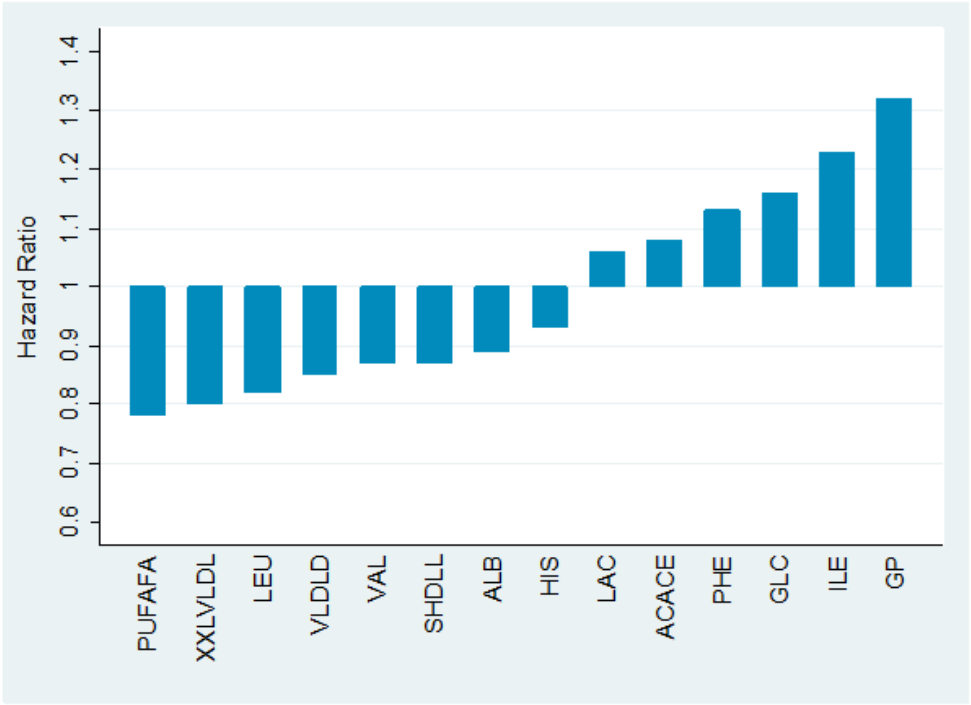
Mortality 1H NMR : Analysis of age strata: two examples
Association of metabolites with mortality depends on age.



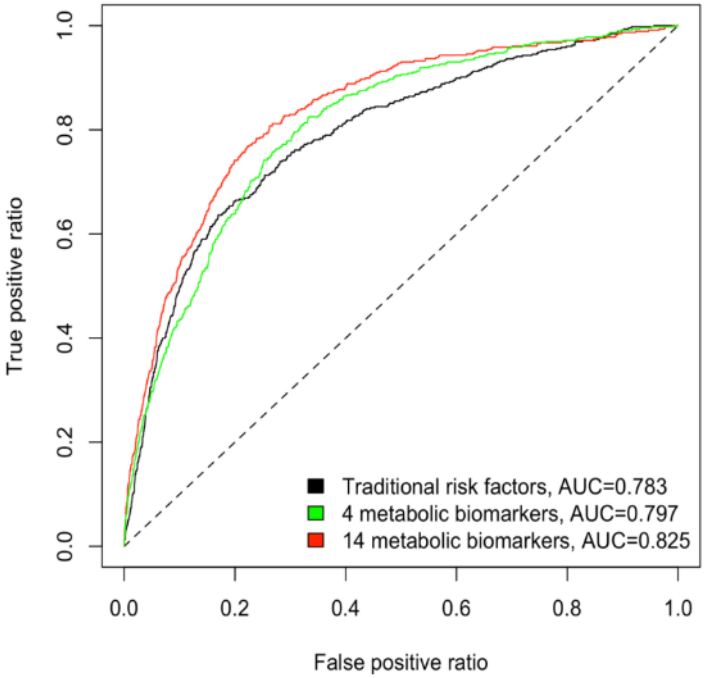


14 metabolites independently associate with mortality
A score of 14 metabolites predicts mortality (cancer/inf disease/cvd) better than conventional variables. Deelen et al., Nat Comm 2019

Independent effects of 14 metabolites on mortality

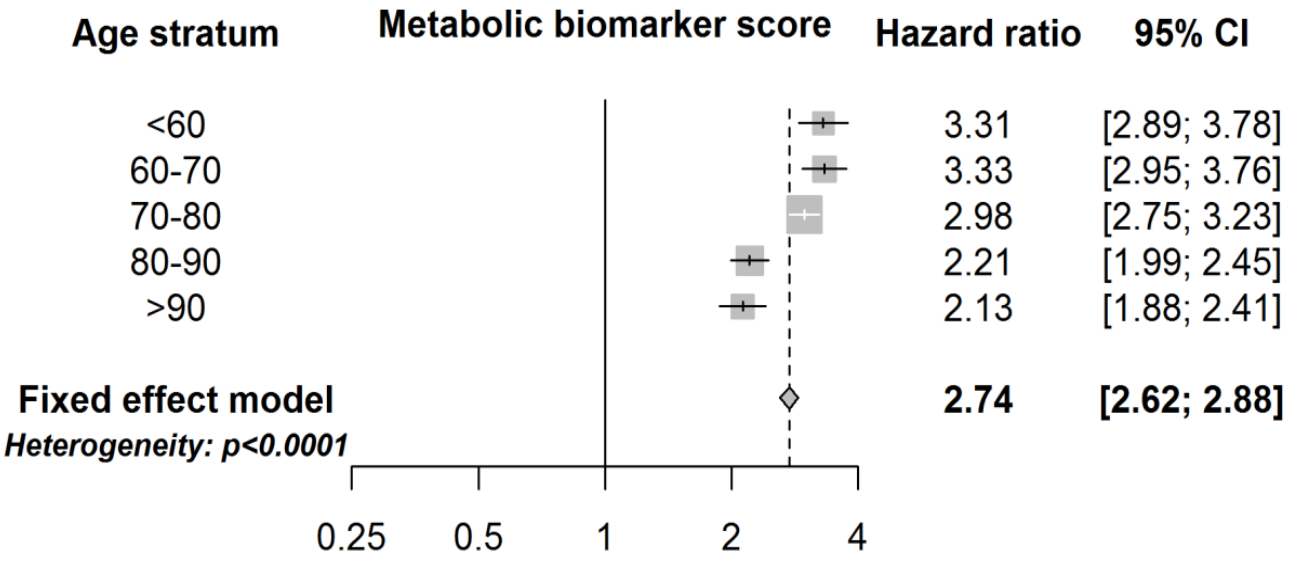


5 years mortality

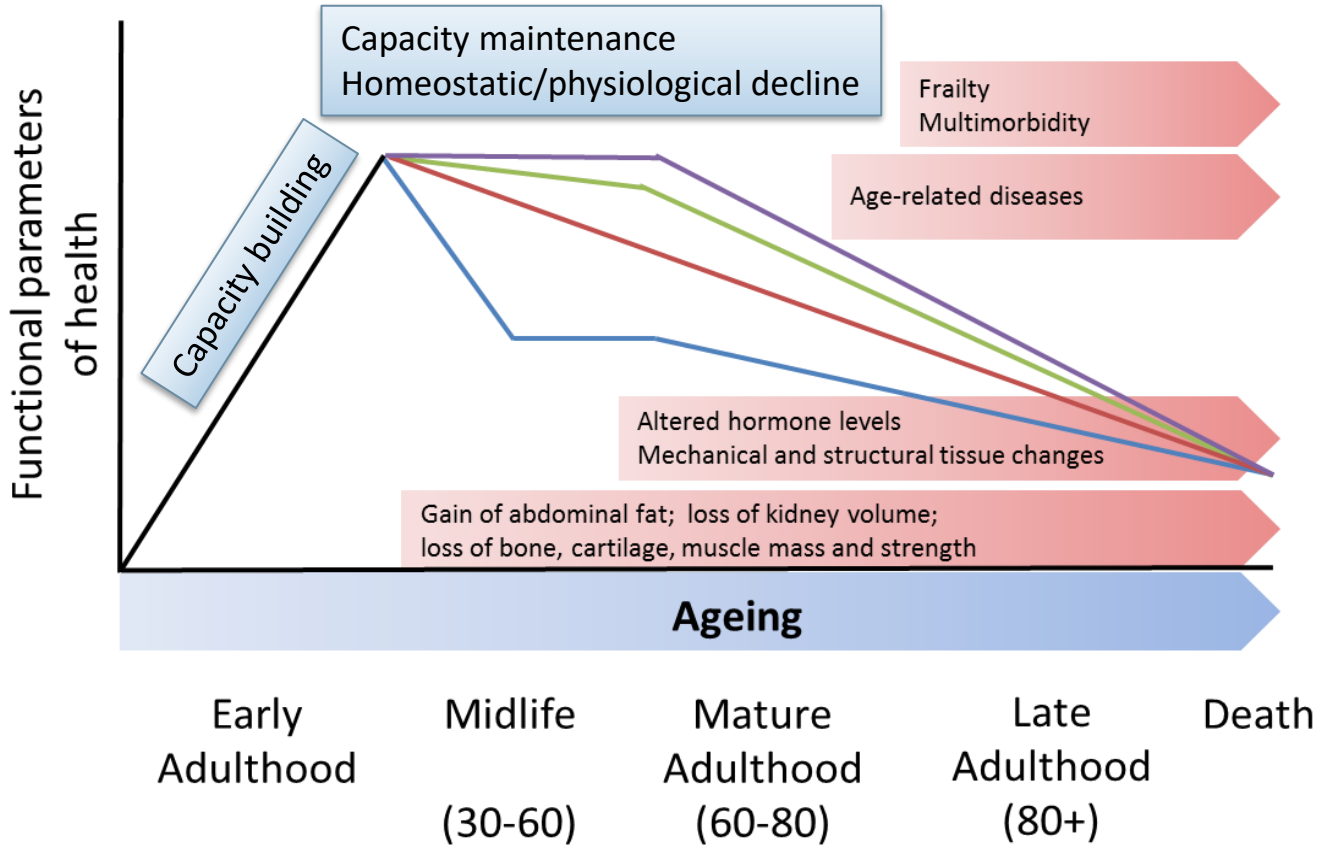


Lipoprotein particle sizes (VLDL, HDL), branched chain/aromatic aminoacids, Keton bodies, inflammation, glucose/lactate, PUFA, fluid balance

The effect of the biomarkers on all-cause mortality is consistent across studies and age strata



Ageing is a personal process
Capacity building, maintenance, and decline



Facing up to the global challenges of ageing
Nature 5**61**, pages45–56 (2018)

Biological Age (BA) versus Chronological Age (CA)

Alex Comfort 1969 Lancet. 1969;2:1411–1414.

Test-battery to measure ageing-rate in man.

Clinical variables as read out of multiple organ systems

(skeleton, body composition, liver , kidney, lung , heart; immune and metabolic health)

height , weight , BMI, waist circumference , systolic /diastolic blood pressure ,

fasting blood sugar, total cholesterol, (HDL-C and LDL-C) , triglyceride, hemoglobin (Hgb),

serum creatinine, glomerular filtration rate (eGFR),

aspartate amin¬otransferase (AST), alanine aminotransferase (ALT),

gamma glutamyl transpeptidase (r-GTP), urine protein,

forced expiratory volume at 1 second (FEV1),

C-reactive protein (CRP), and cytomegalovirus (CMV) optical density.

Now: also molecular variables. Telomere repeat length, DNA methylation etc.

Biological Age Predictors WHY

- to measure an individual's overall health status
- predict the risk of death , surrogate endpoint
- predict the risk of age-related disease incidence
- evaluate the effect of a health care program
- evaluate the effect of lifestyle/management interventions
- to use as phenotype in etiological studies
- cross over to animal studies

Biological age – markers
Clinical variables (clocks) tracking age

Output multiple organ systems. Belsky et al., PNAS 2015 .

1000 subjects 38 year old. NHANES Biological Age index: 28-61 years

Longitudinal measures at 26, 32 and 38 years: personal rates of physiological decline

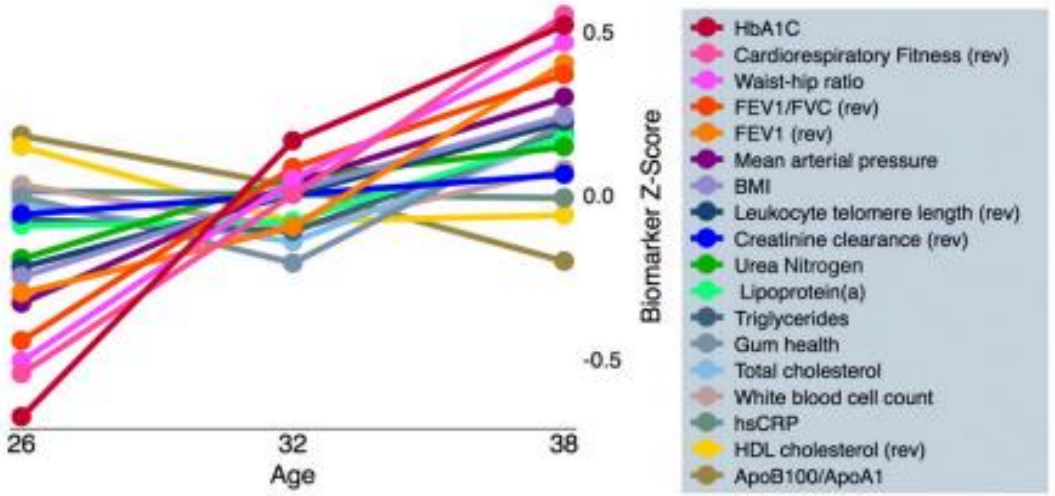
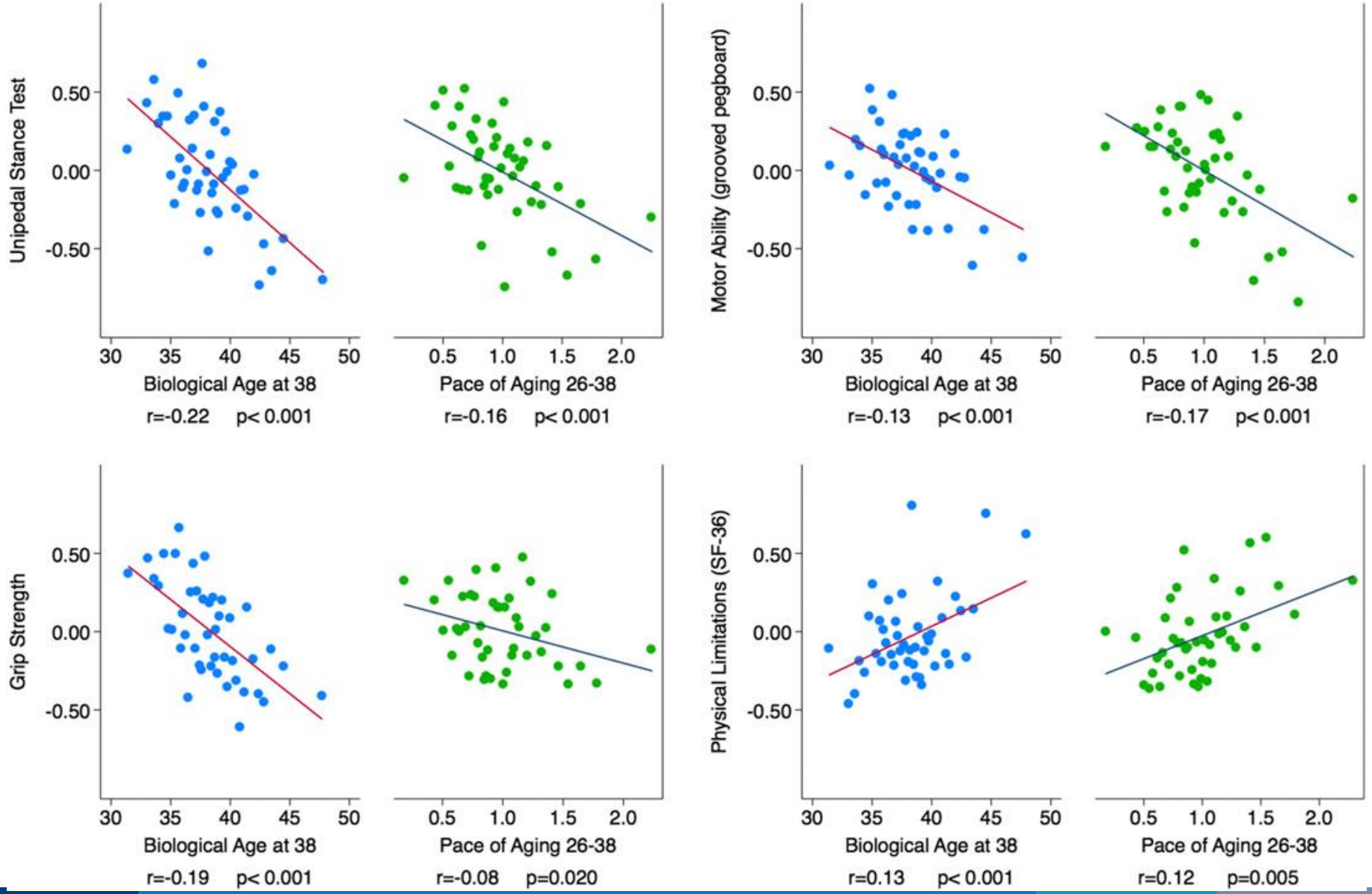
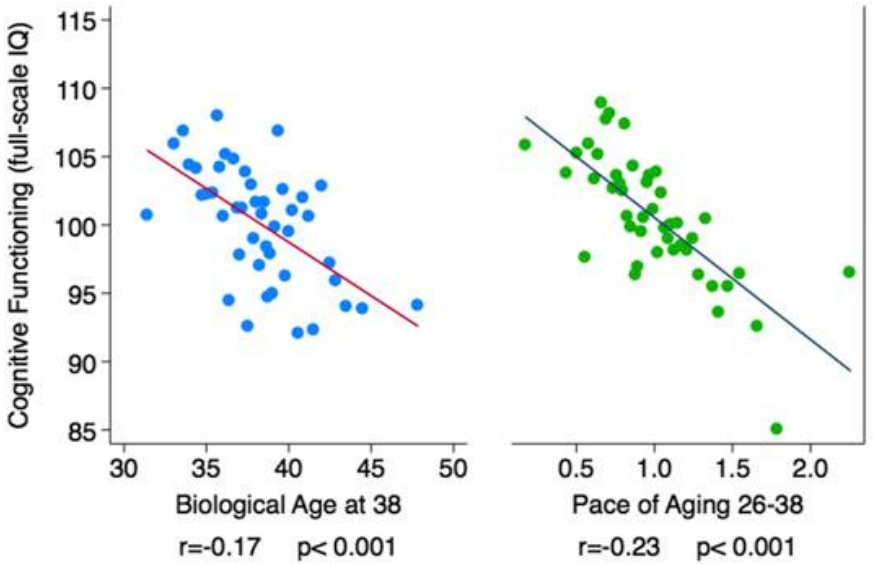


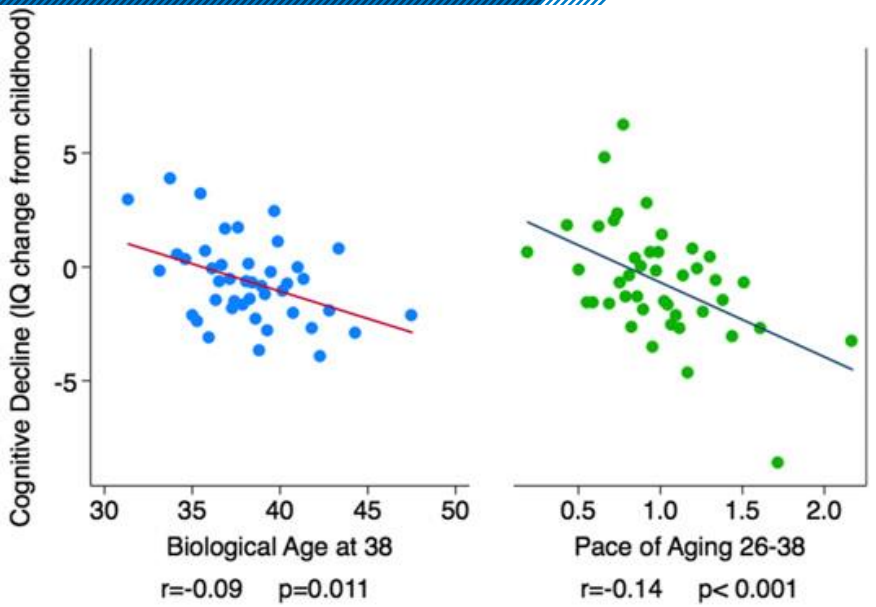
Fig. 3. Healthy adults exhibit biological aging of multiple organ systems over 12 y of follow-up. Biomarker values were standardized to have mean = 0 and SD = 1 across the 12 y of follow-up (Z scores). Z scores were coded so that higher values corresponded to older levels of the biomarkers; i.e., Z scores for cardiorespiratory fitness, lung function (FEV_1 and FEV_1/FVC), leukocyte telomere length, creatinine clearance, and HDL cholesterol, which decline with age, were reverse coded so that higher Z scores correspond to lower levels.

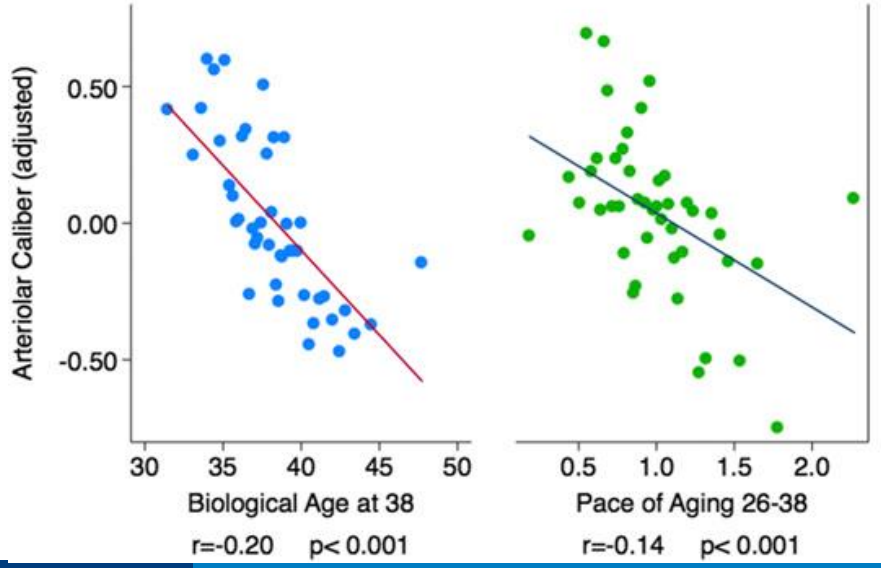
Physical capability

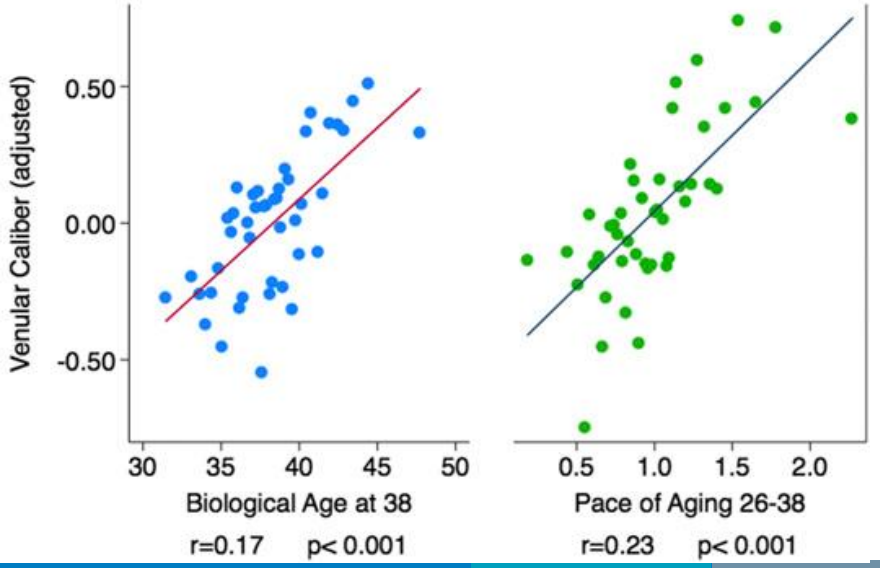


Cognition


Cognitive Functioning (full-scale IQ)
115
110
105
100
95
90
85
30
35
40
45
50
Biological Age at 38
r=-0.17 p< 0.001
0.5
1.0
1.5
2.0
Pace of Aging 26-38
r=-0.23 p< 0.001


Cognitive Decline (IQ change from childhood)
5
0
-5
30
35
40
45
50
Biological Age at 38
r=-0.09 p=0.011
0.5
1.0
1.5
2.0
Pace of Aging 26-38
r=-0.14 p< 0.001

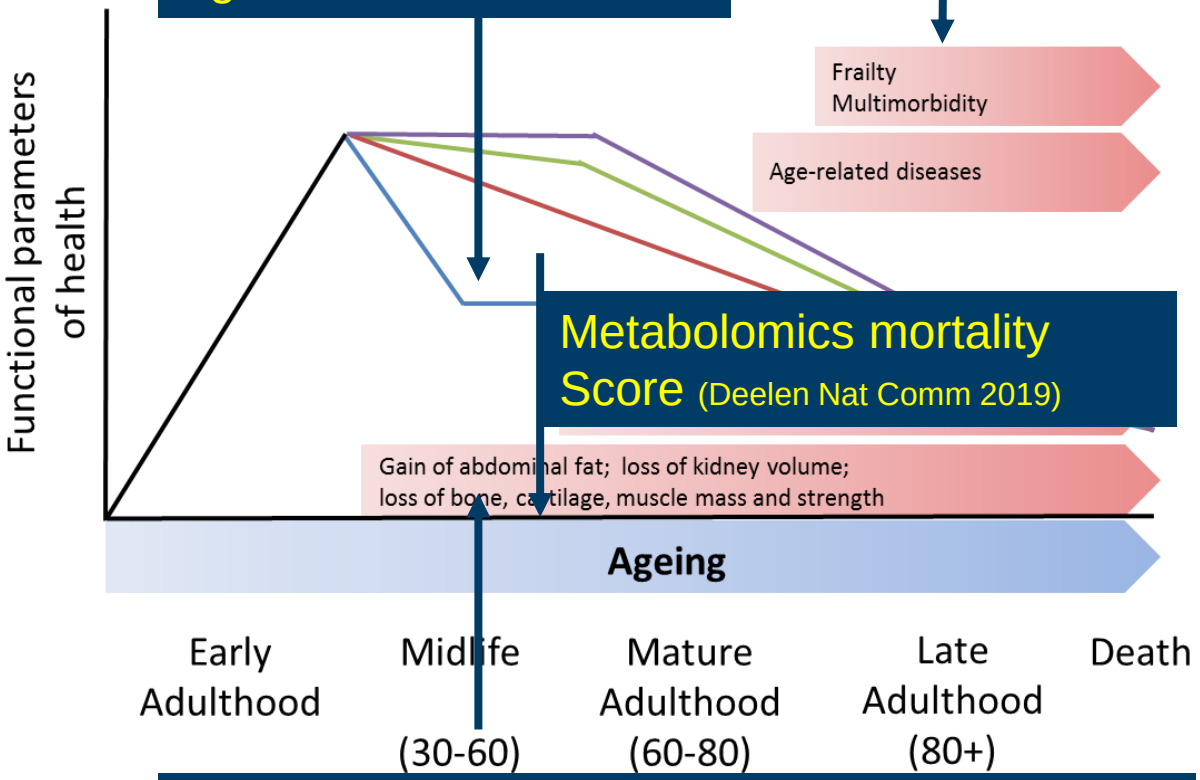

Arteriolar Caliber (adjusted)
0.50
0.00
-0.50
30
35
40
45
50
Biological Age at 38
r=-0.20 p< 0.001
0.5
1.0
1.5
2.0
Pace of Aging 26-38
r=-0.14 p< 0.001


Venular Caliber (adjusted)
0.50
0.00
-0.50
30
35
40
45
50
Biological Age at 38
r=0.17 p< 0.001
0.5
1.0
1.5
2.0
Pace of Aging 26-38
r=0.23 p< 0.001

Multivariate combinations of biomarkers

Frailty: an accumulation of deficits
Mitnitski AB, Mogilner AJ, Rockwood K.
Sci World J. 2001
Now: electronic health records
for hospital based frailty scores

NHANES Biological Age
Levine and Crimmins
Am J Hum Biol. 2014
Age >30



Metabolomics mortality Score (Deelen Nat Comm 2019)

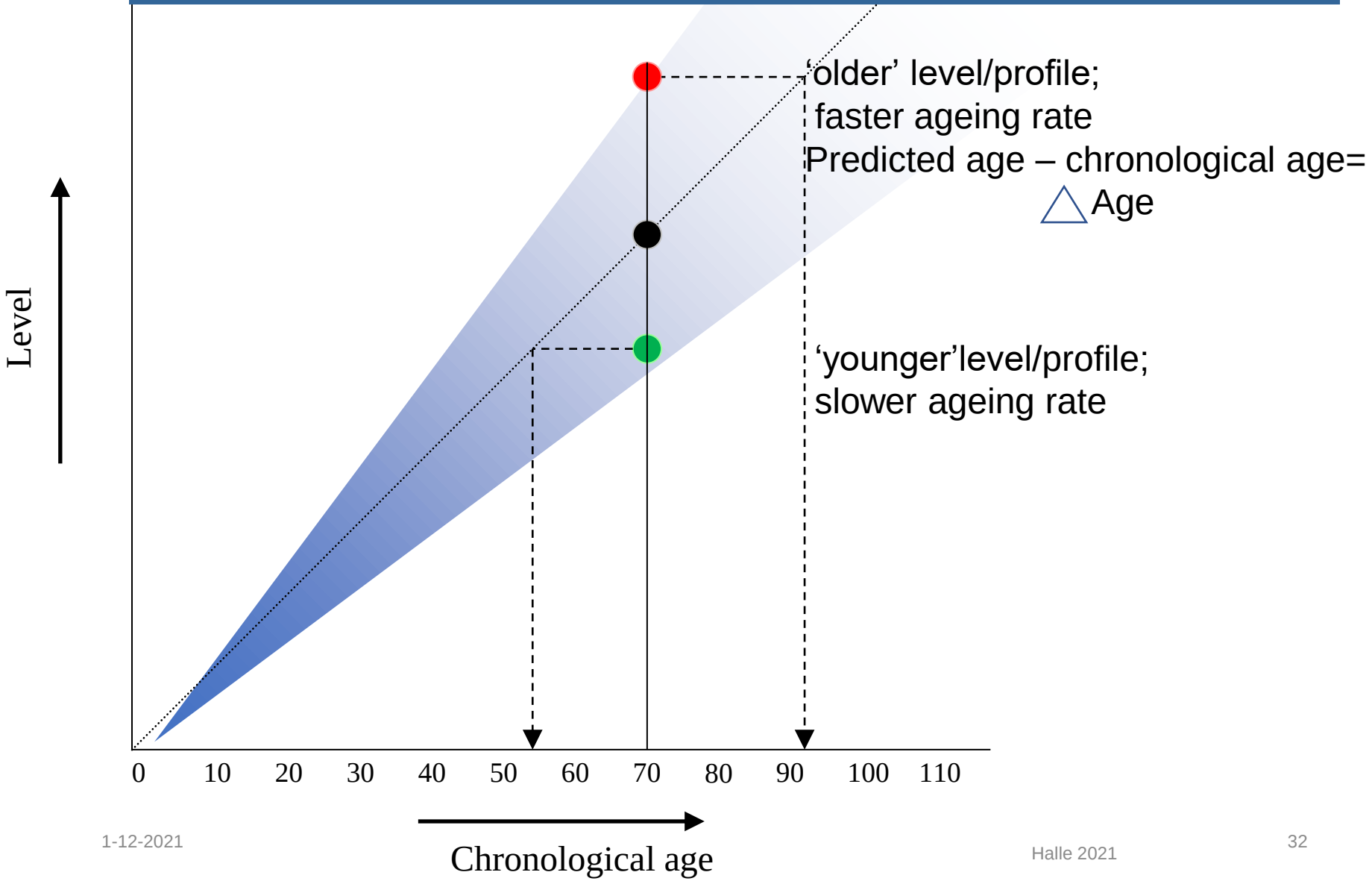
Pace of Aging Belsky et al., PNAS 2015
Age 26-38, multiple organ systems
Gum health; also telomeres

Remark

Others have tried to make predictors of biological age on the basis of the relation of molecular patterns and chronological age (mostly in cross sectional studies). This was done for DNA methylation, transcriptome and for metabolome.

Biological Age Prediction based on chronological age
Molecular data on cross-sectional blood levels

Horvath et al., DNAmAge 2013 ; Deelen et al., 2013

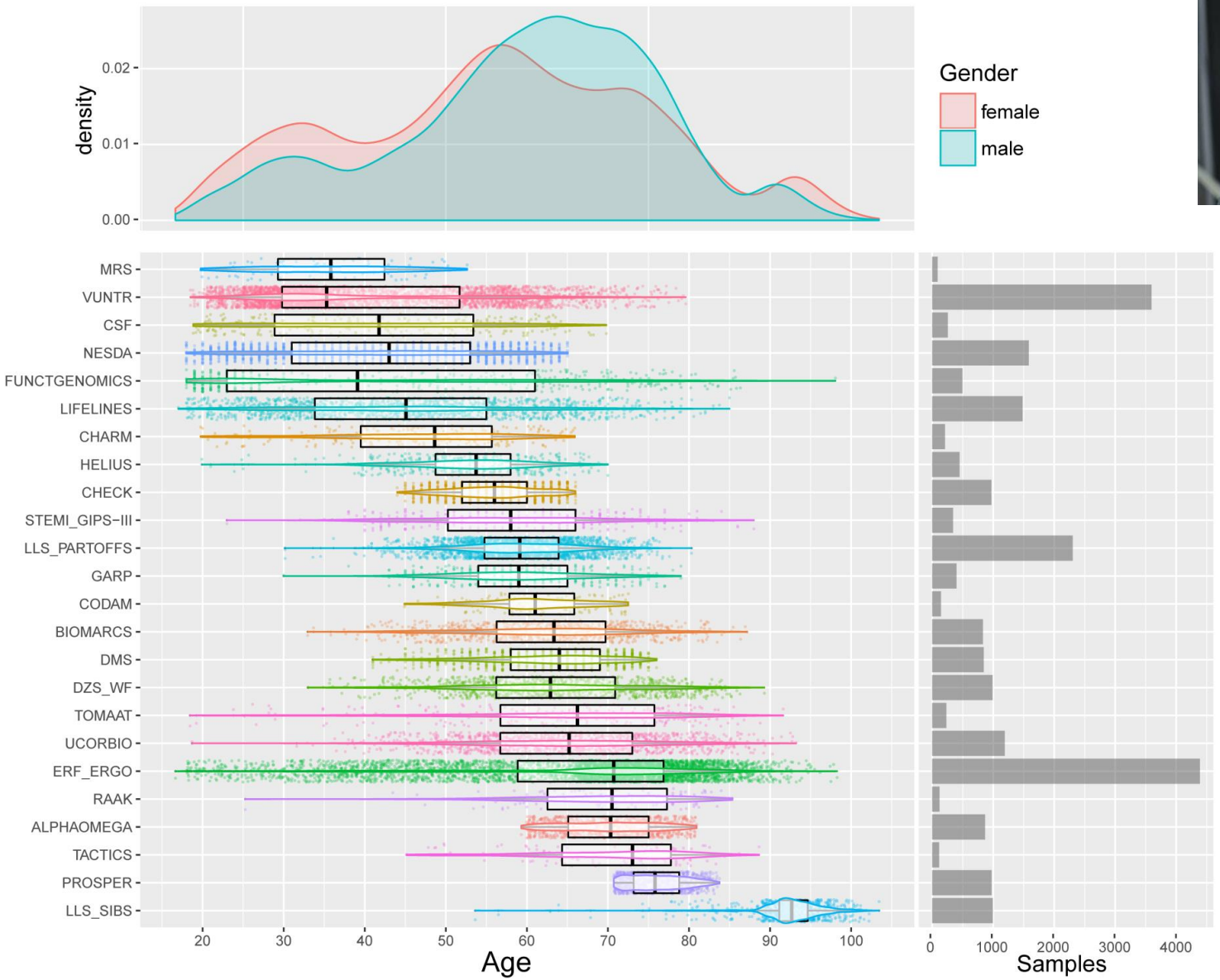


BBMRI Study Population after Quality Control

BBMRI: 25 453 samples from 26 biobanks

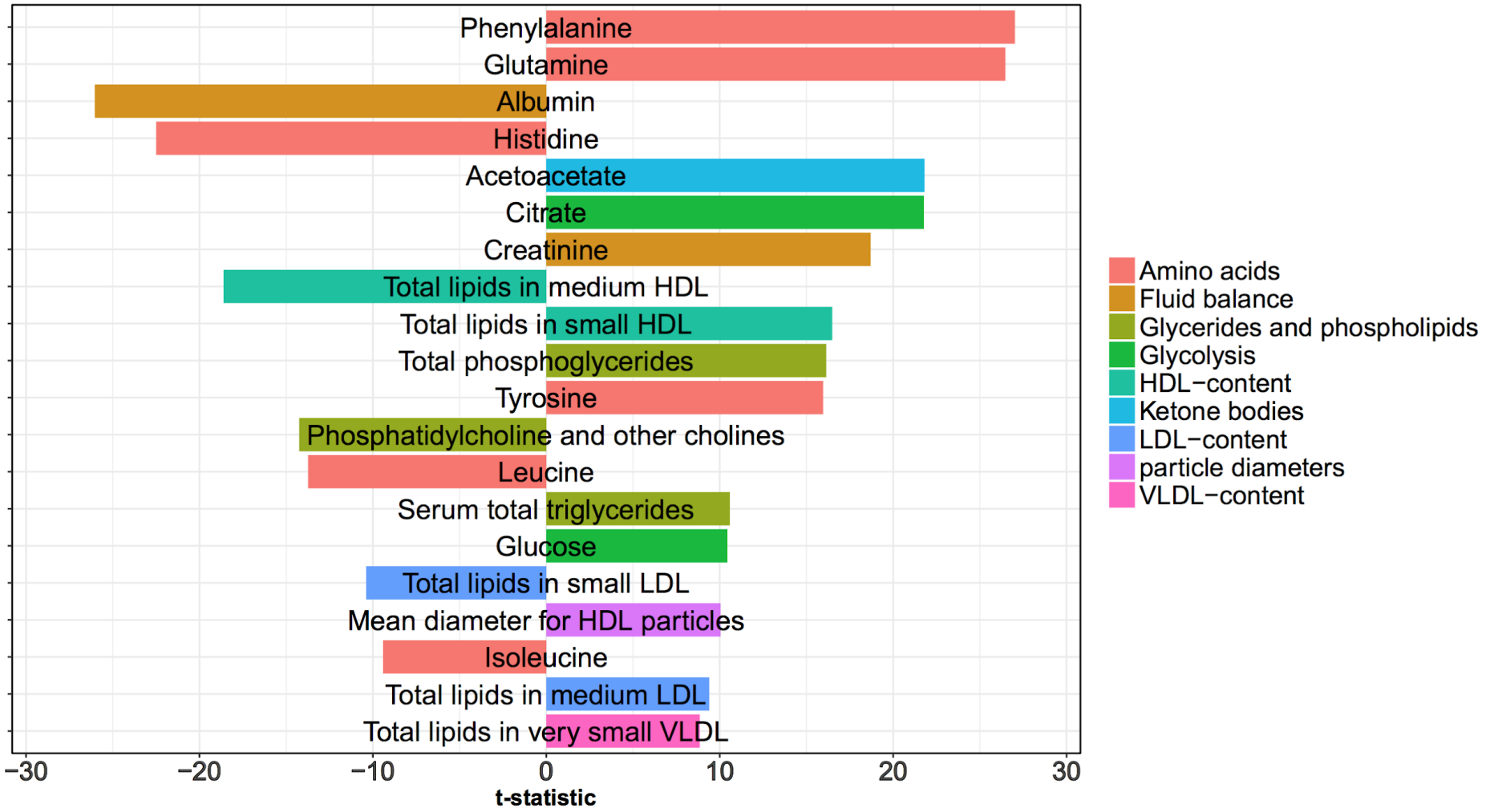
E.B. van den Akker et al., Circ Genom Precis Med. 2020; 13

Erik van den Akker



Training the age-predictor

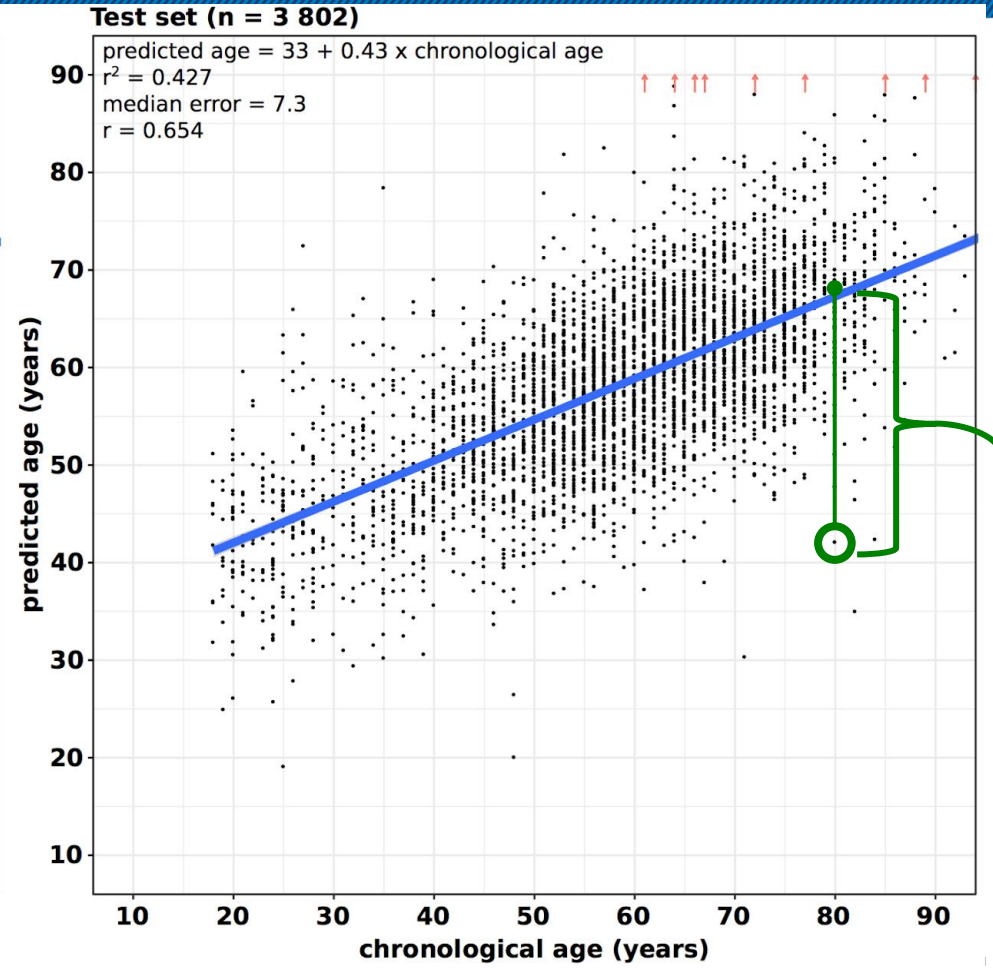
Contributing metabolites



before modelling!

Construction of the metaboAge Score

The age-independent part of the age-predictor



Δage = predicted age - chronological age

Regress out chronological age:
metaboAge = resid(lm(Δage ~ chron. age))

ΔmetaboAge associates to incident mortality
LLS : 811 90+ individuals; PROSER 6000 70+ individuals

van den Akker et al. *Circ Genom Precis Med* 2020

C Associations of incident cardio-metabolic disease with ΔmetaboAge in PROSPER

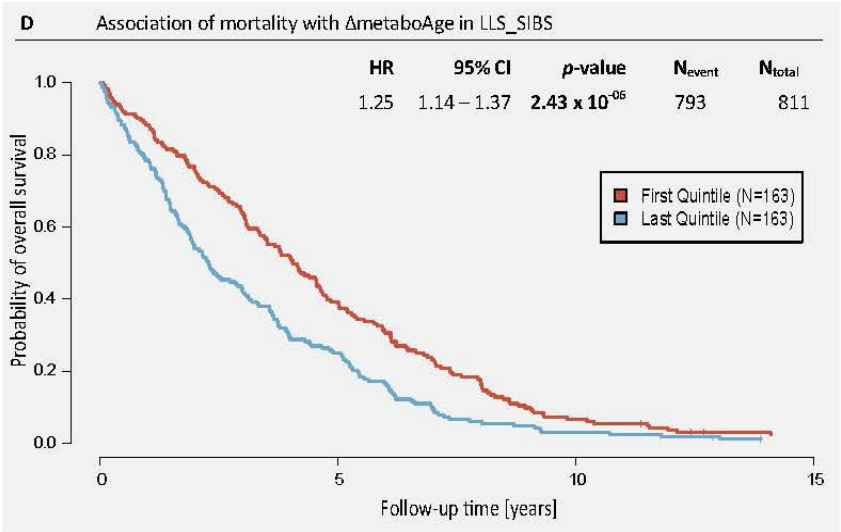
Phenotype	HR	95% CI	*p*-value	N_{case}	$N_{control}$
Coronary events	1.25	1.11 – 1.40	**2.64×10^{-04}**	569	4,851
Cardiovascular events	1.20	1.08 – 1.33	**4.86×10^{-04}**	865	4,555
Vascular mortality	1.57	1.31 – 1.88	**8.56×10^{-07}**	237	5,183
All-cause mortality	1.42	1.25 – 1.61	**9.14×10^{-08}**	477	4,943
Heart failure hospitalisation	1.68	1.37 – 2.06	**5.42×10^{-07}**	189	5,324

Mortality in PROSPER

Mortality in LLS : HR = 1.25 p = 2.4 x 10 -6

With every year older metaboAge:
2-4% increased mortality risk
Comparable to DNAmAge

Independent of BMI, smoking, diabetes and hypertension and medication



Remark

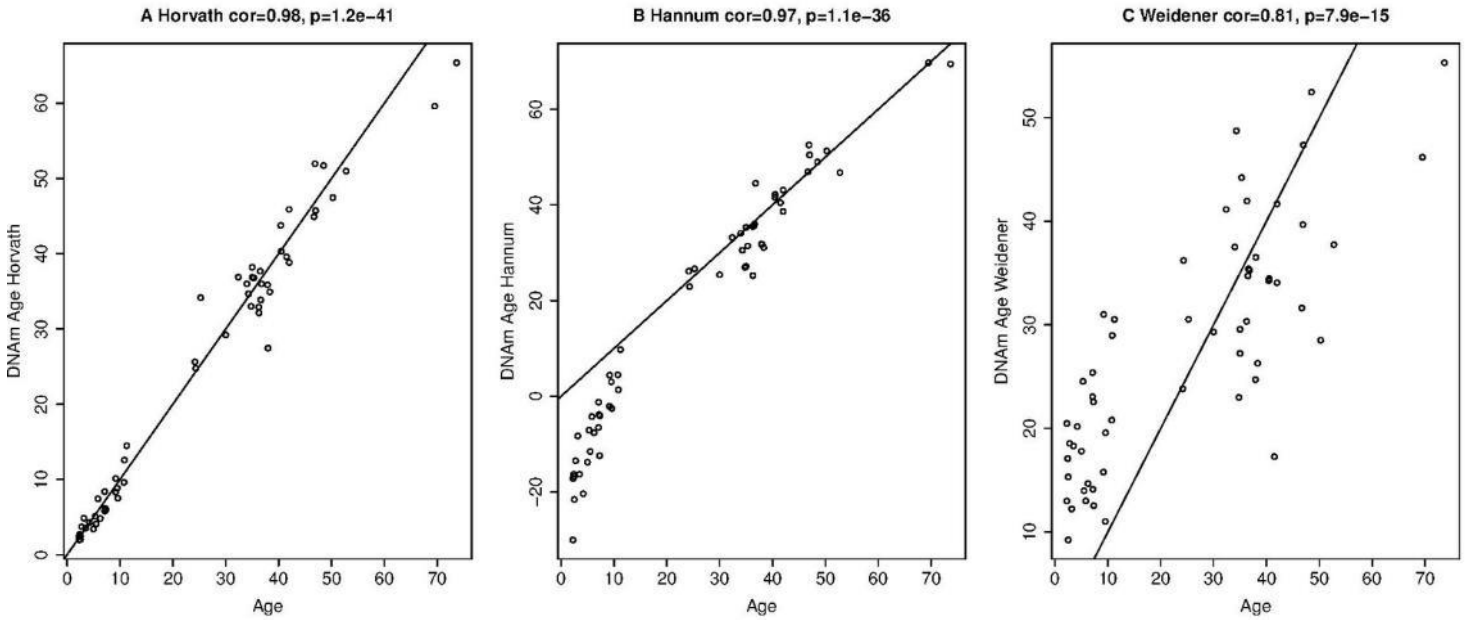
You must prove that a predictor predicts a relevant outcome: if the predictor indicates biological age than it should associate with and predict onset of disease and mortality, for example.

Regularities with chronological age. DNA methylation (Hannum, Horvath)

DNAmage predicts:

Mortality
Cancer risk
Obesity
Gestational age
Brain tissue ageing

HR 1.02-1.04 Effect size for mortality:



Remark

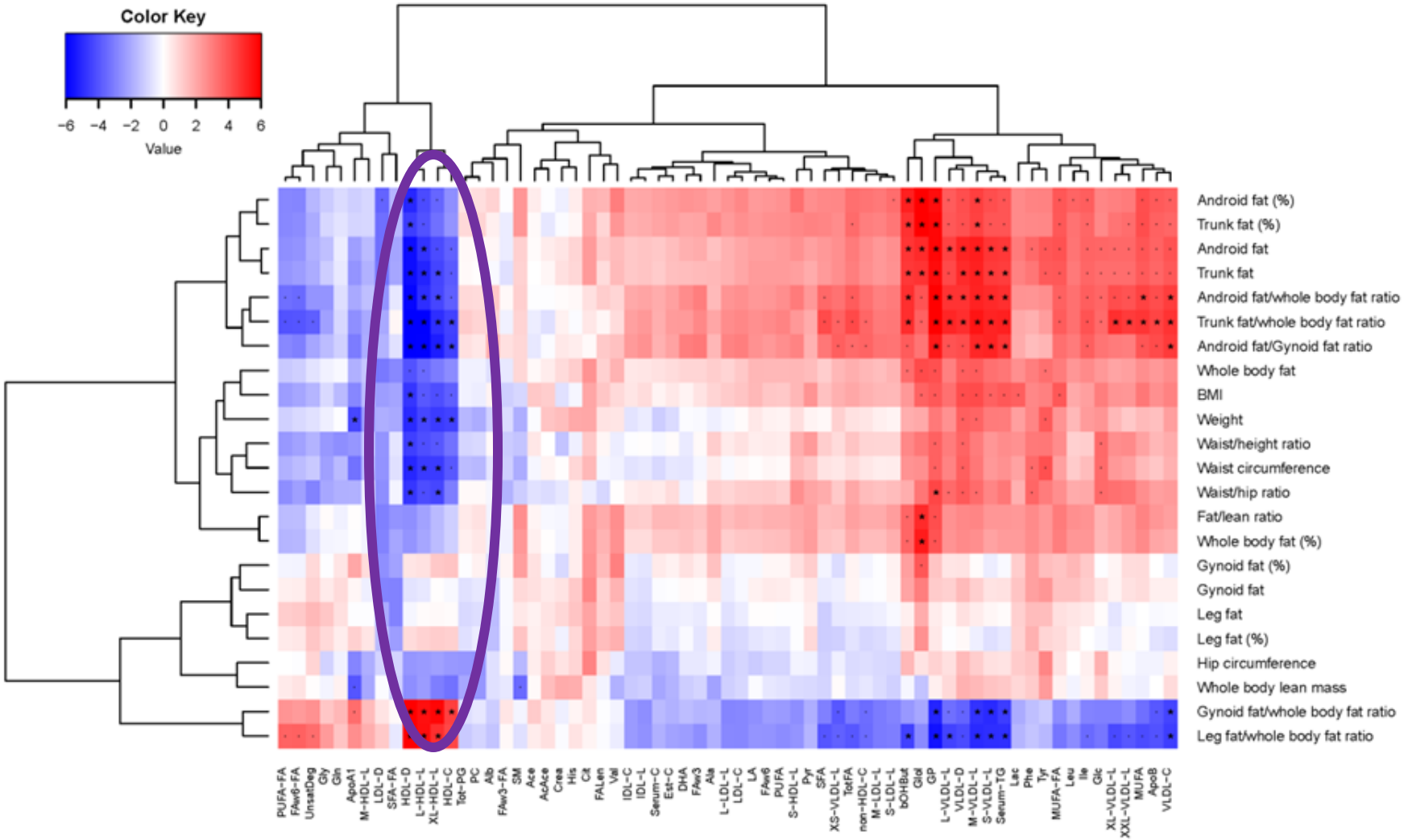
Two attempts are being made to make predictors from the metabolome.

1. Based on chronological age
2. Based on mortality

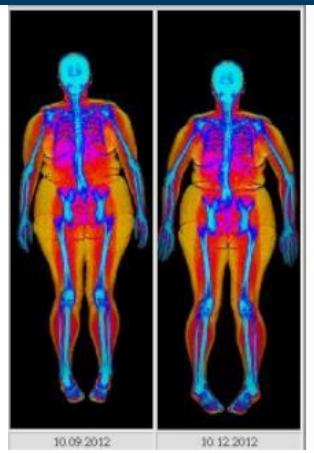
Molecular Epidemiology

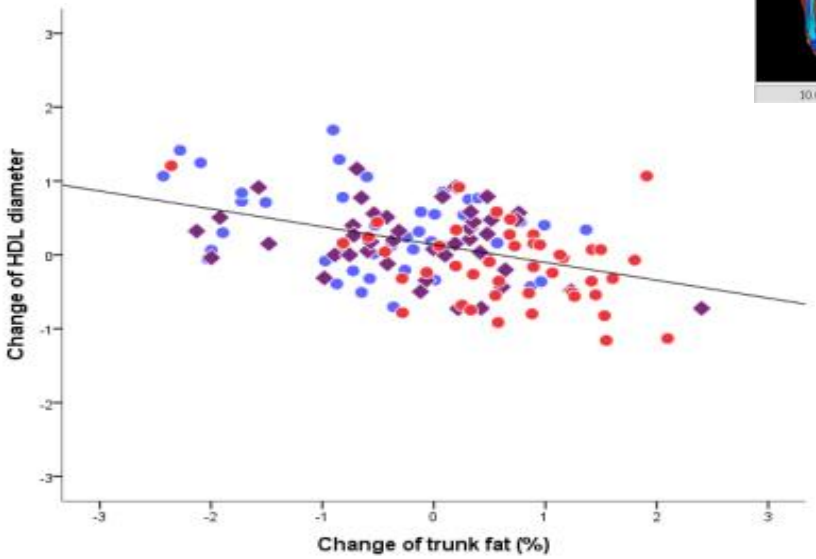
- Introduced by Kilbourne (1973), infectious diseases; Schulte and Perera (1993 Principles and Practices)
- Integrates Epidemiology, Medical Sciences and Molecular Biology
- Studies the influence on health of environmental and genetic risk factors measured by (holistic) molecular signatures
- Contributes to
  prediction/prognosis
  monitoring exposure, response to interventions
  etiological understanding (disease mechanisms)

Which metaboliotes associate at baseline with DEXA scan variables of fat distribution. Heat map
Which metabolites monitor response to intervention



Measure 1H NMR metabolome In intervention studies
Generate omics biomarkers for biological age prediction
And generate Health biomarkers indicating a response to a lifestyle intervention





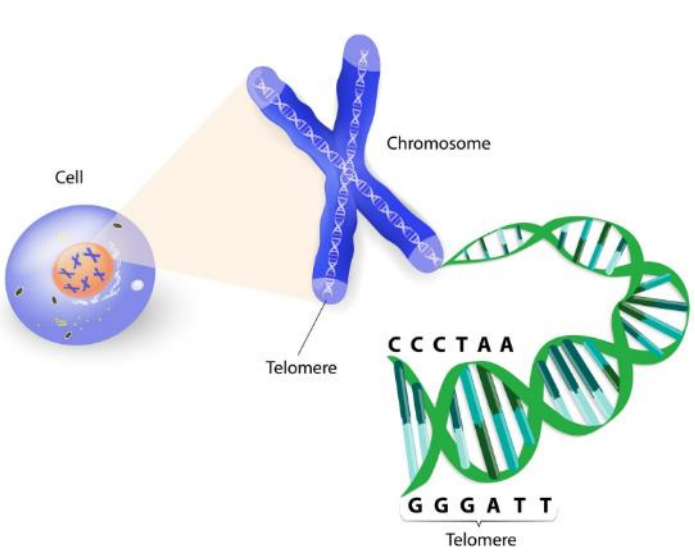
Example : indicate a change in body composition on a DEXA scan by metabolomics markers (HDL diameter)

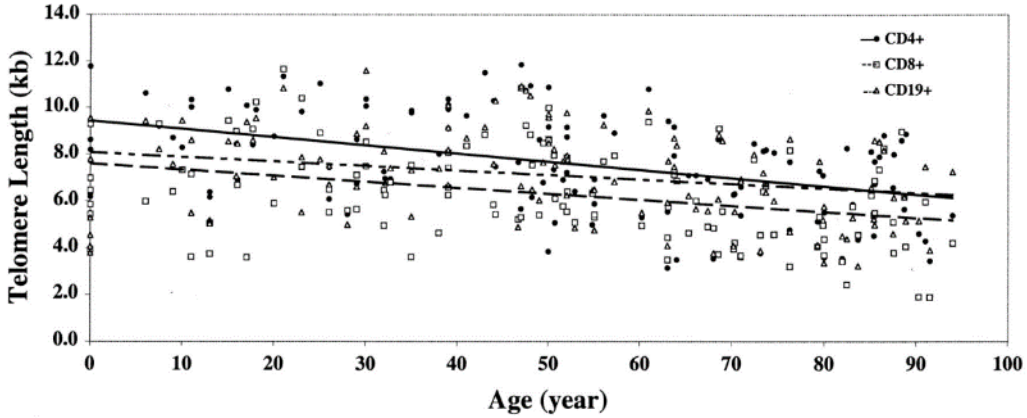
Exercise: discussion of Marioni paper

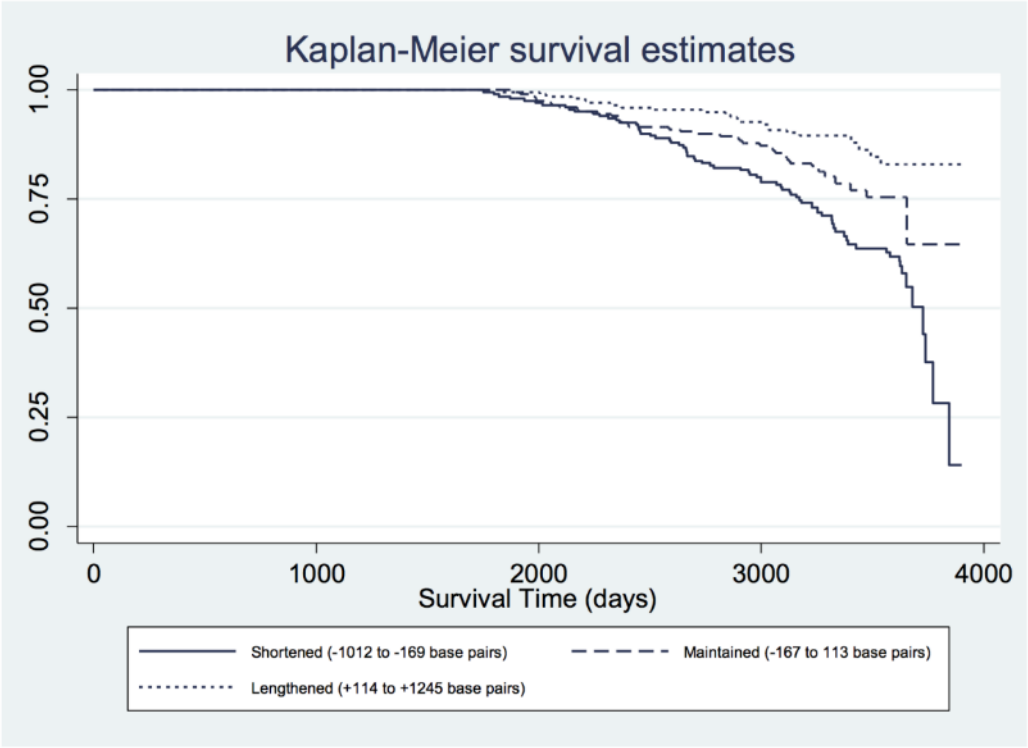
Biomarkers of biological age:

DNA methylation
Telomeres

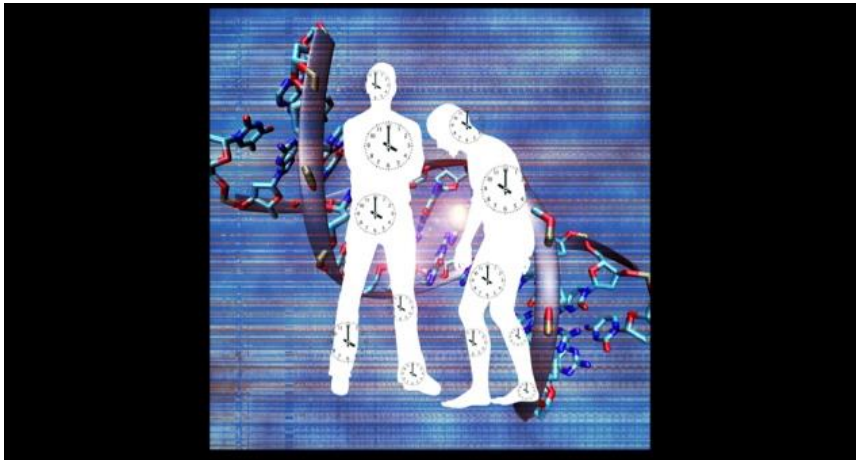
Telomere shortening, age and mortality prediction

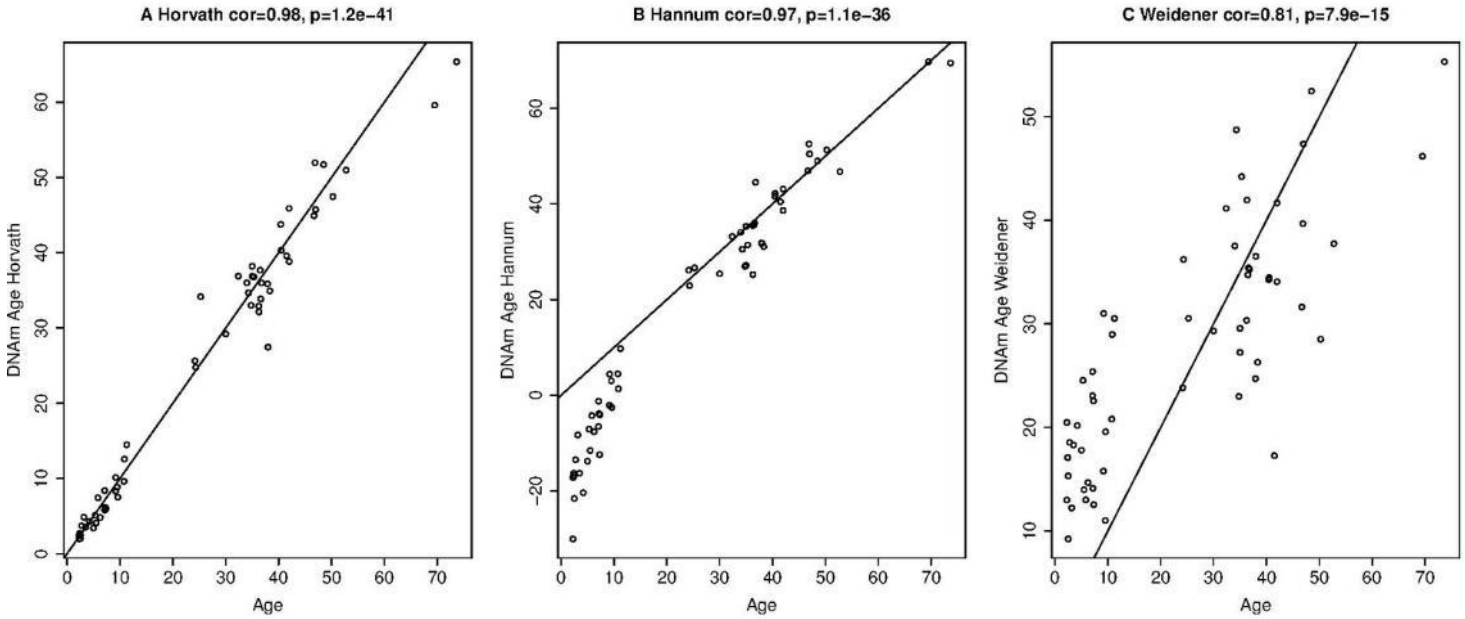






Regularities with chronological age. DNA methylation (Hannum, Horvath)





Questions for the Discussion on Biological Age Predictors. Marioni paper

1. What is the main aim of the paper, main research question and subquestions.
   Q1, 2 etc.

2. Why, do authors investigate this, what is the issue or the problem?

3. What new research strategy would solve the problem

4. What is the study design (which can be variable for answering the different questions). What is measured in which study population. Is ther anything remarkable about the study populations ? Usually Table 1

5. What answers were found to the questions
   Q 1, 2 etc.

6. What are the main conclusions and the main discussion points the authors bring up.

7. What do you think of the paper ? Was it clear, was it presented in a logical way, what would you have done differently.