Normal human variation – We don't know remotely enough about the nature and origins of normal variation in "biomarkers" and virtually every important parameter for early disease detection.

Pre-clinical "occult" natural history of cancer – we don't know what we need to detect to make a difference.

Rigorous comprehensive description of source of samples – Sloppy standards. Can't trust results without this.

Sample divisibility – trade-offs between use and preservation of samples.

Sample handling and processing is an important source of extrinsic/confounding variation, but…

Most of the variation, especially the variation that can introduce unrecognized bias, is due to factors that act **before** sample collection – “Biology” (much larger parameter space, harder or impossible to enforce control).

Illustrative example….

Diagnostic Markers for Early Detection of Ovarian Cancer

Irene Visintin,[1] Ziding Feng,[2] Gary Longton,[2] David C. Ward,[3] Ayesha B. Alvero,[1] Yinglei Lai,[4] Jeannette Tenthorey,[1] Aliza Leiser,[1] Ruben Flores-Saaib,[5] Herbert Yu,[6] Masoud Azori,[1] Thomas Rutherford,[1] Peter E. Schwartz,[1] and Gil Mor[1]

Abstract **Purpose:** Early detection would significantly decrease the mortality rate of ovarian cancer. In this study, we characterize and validate the combination of six serum biomarkers that discriminate between disease-free and ovarian cancer patients with high efficiency.

Conclusions: We describe the first blood biomarker test with a sensitivity of 95.3% and a specificity of 99.4% for the detection of ovarian cancer. This novel multiplex platform has the potential for efficient screening in patients who are at high risk for ovarian cancer.

Diagnostic Markers for Early Detection of Ovarian Cancer

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Sample collection

Ten mL of peripheral blood was drawn from subjects using standardized phlebotomy procedures (11). Within 2 to 4 hours of collection, samples were processed using guidelines set by the National Cancer Institute Inter-Group Specimen Banking Committee and stored at -80°C in the Tissue/Sera Bank of the Discovery to Cure program.

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Abstract **Purpose:** Early detection would significantly decrease the mortality rate of ovarian cancer. In this study, we characterize and validate the combination of six serum biomarkers that discriminate between disease-free and ovarian cancer patients with high efficiency.

Ovarian cancer group. The disease group (n = 156) includes women with newly diagnosed ovarian cancer (pelvic mass). All samples were collected previous to diagnosis at the gynecologic oncology clinic. Of

Control group. The healthy control group (n = 362) included age-matched healthy individuals who came for a regular gynecologic examination. These individuals did not have a diagnosis of any type of cancer, were not genetically predisposed to develop ovarian cancer, and were disease free at least 6 months after sample collection. A total of

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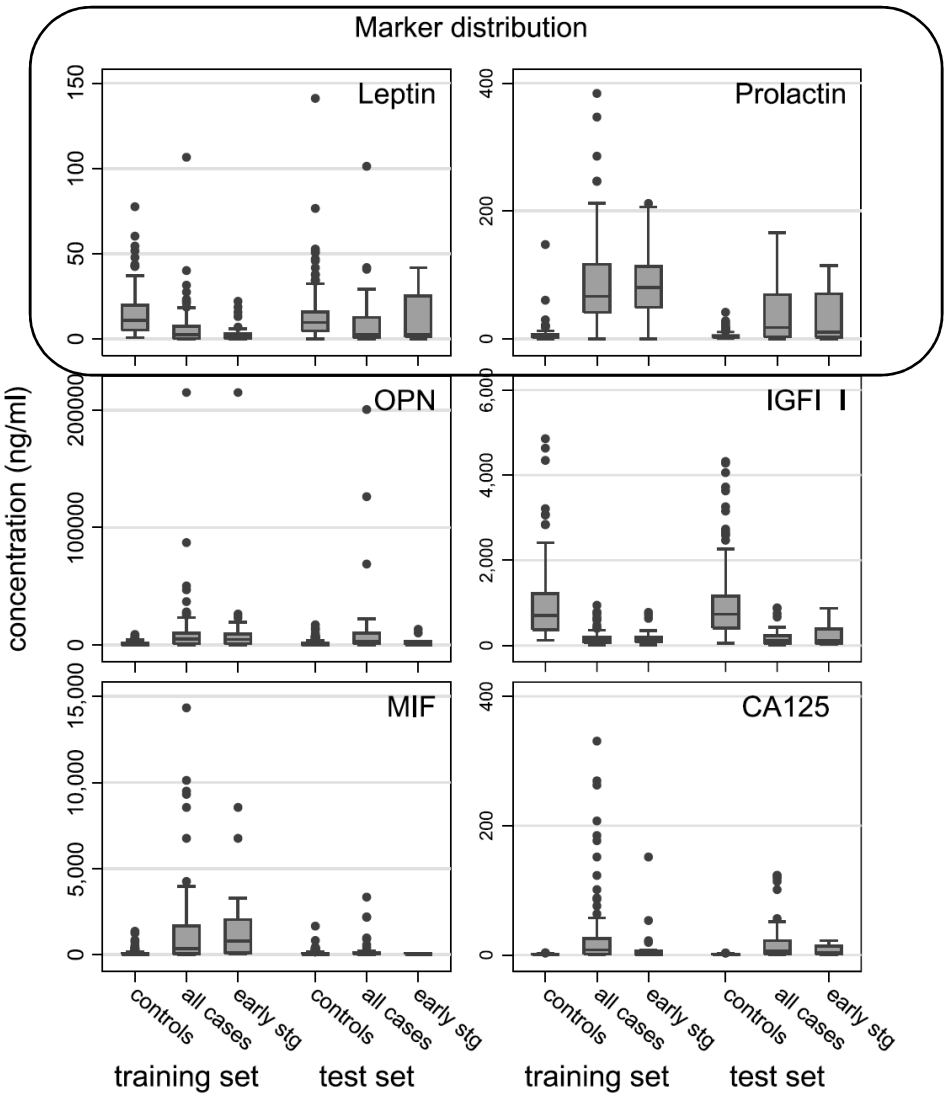

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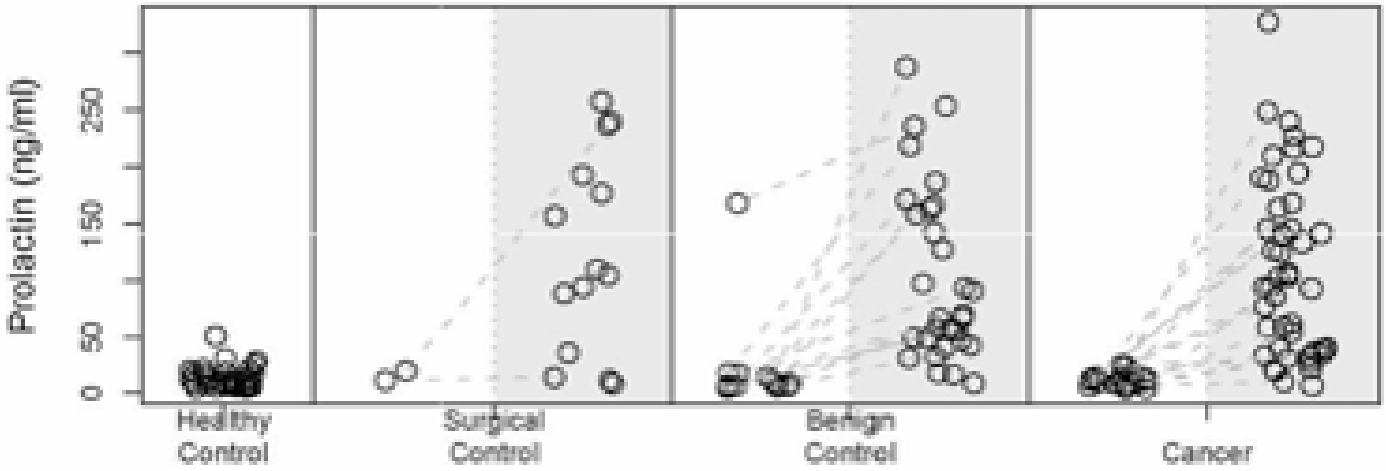
PLOS one

Effects of Blood Collection Conditions on Ovarian Cancer Serum Markers

Jason D. Thorpe[1*], Xiaobo Duan[2], Robin Forrest[1], Kimberly Lowe[1], Lauren Brown[1], Elliot Segal[2], Brad Nelson[3], Garnet L. Anderson[4], Martin McIntosh[1], Nicole Urban[1]

1 Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America, 2 Onco Detectors International LLC, Bethesda, Maryland, United States of America, 3 BC Cancer Agency, Trev and Joyce Deeley Research Centre, Victoria, British Columbia, Canada, 4 Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America



Prolactin	Blood Collection Conditions	At Clinic	(Reference)		
		In Surgery	93.23	8.82	<0.005
	Case/Control Group	Healthy Control	(Reference)		
		Surgical Control	15.23	20.28	0.45
		Benign Control	0.45	11.49	0.97
		Ovarian Cancer	2.37	5.97	0.69

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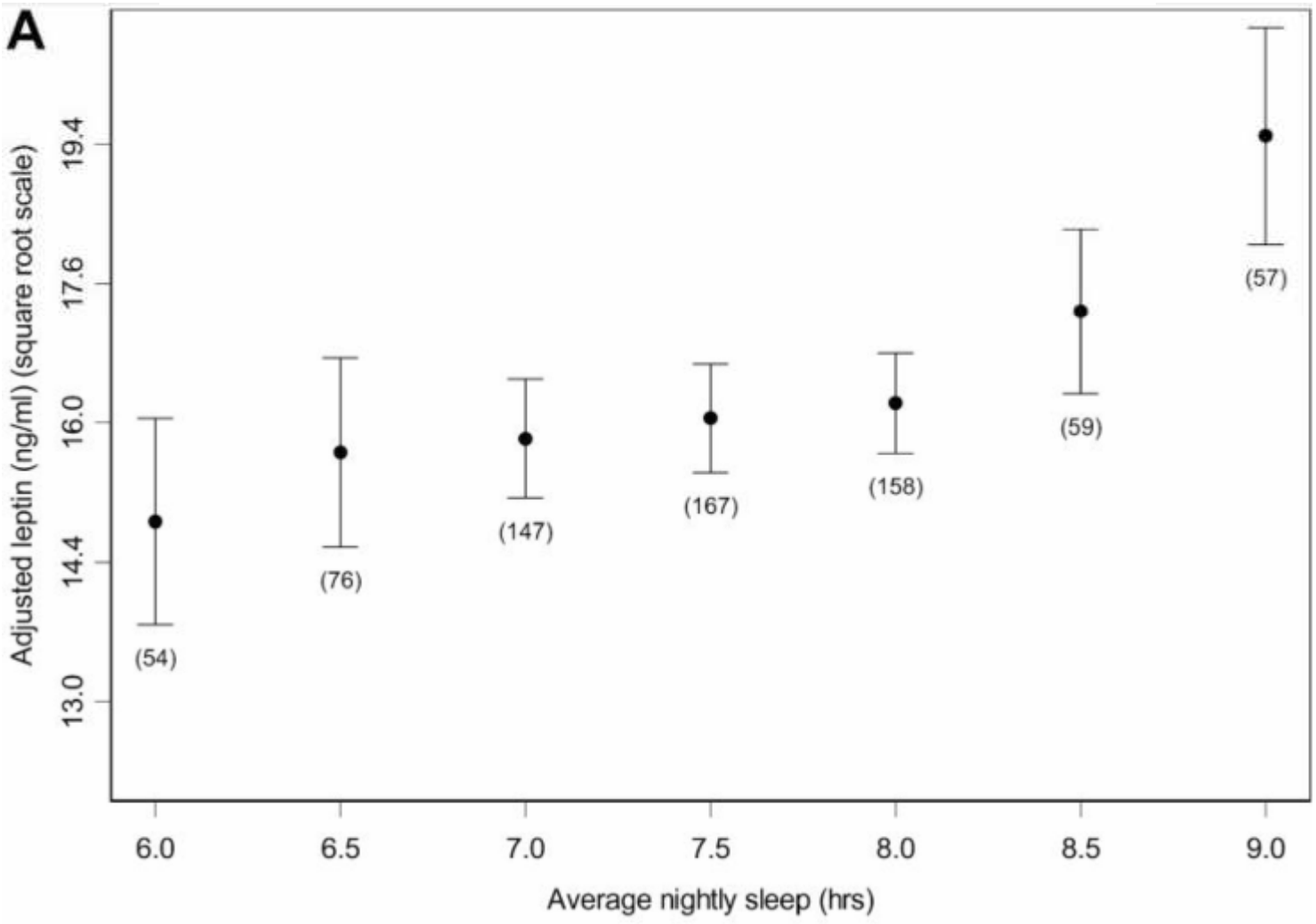
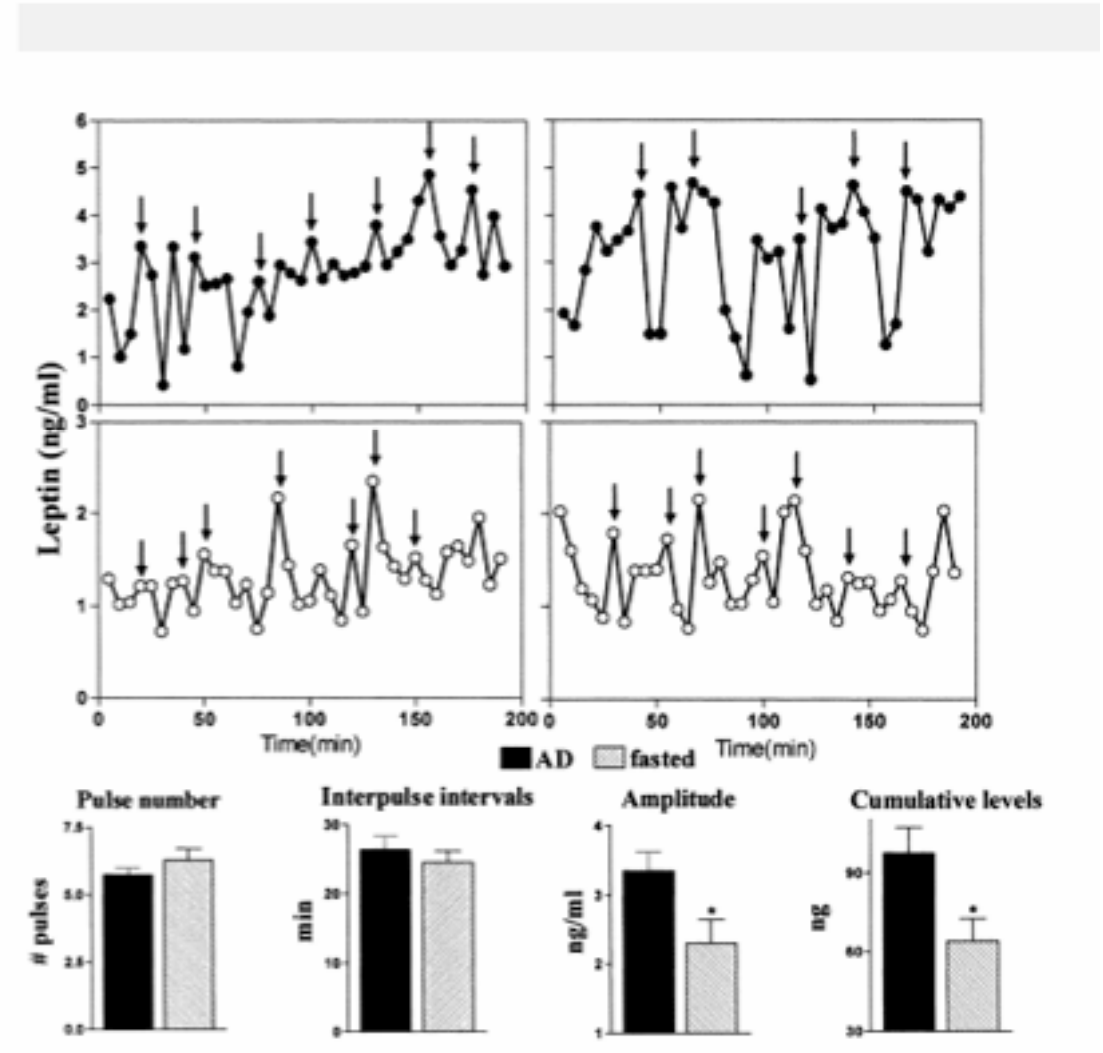
Short Sleep Duration Is Associated with Reduced Leptin, Elevated Ghrelin, and Increased Body Mass Index

Shahrad Taheri[1☺], Ling Lin[1], Diane Austin[2], Terry Young[2], Emmanuel Mignot[1*]

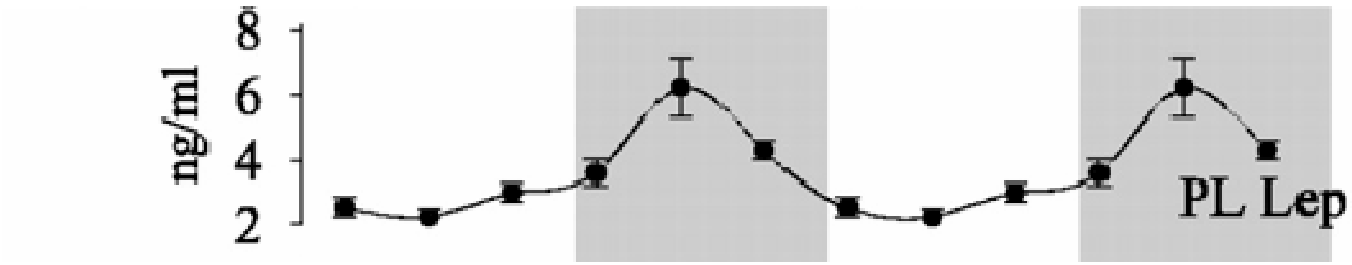
1 Howard Hughes Medical Institute, Stanford University, Palo Alto, California, United States of America, 2 Department of Population Health Sciences, University of Wisconsin, Madison, Wisconsin, United States of America

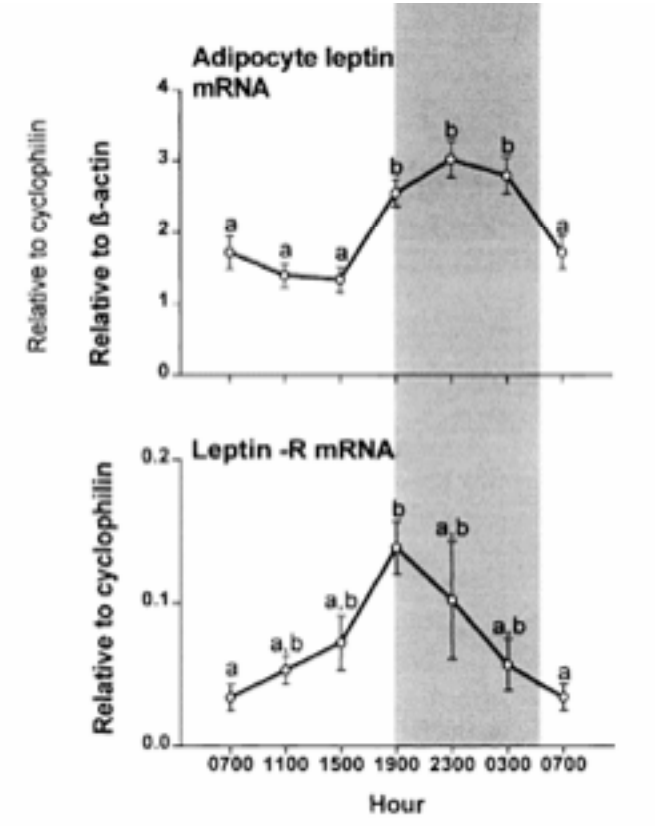


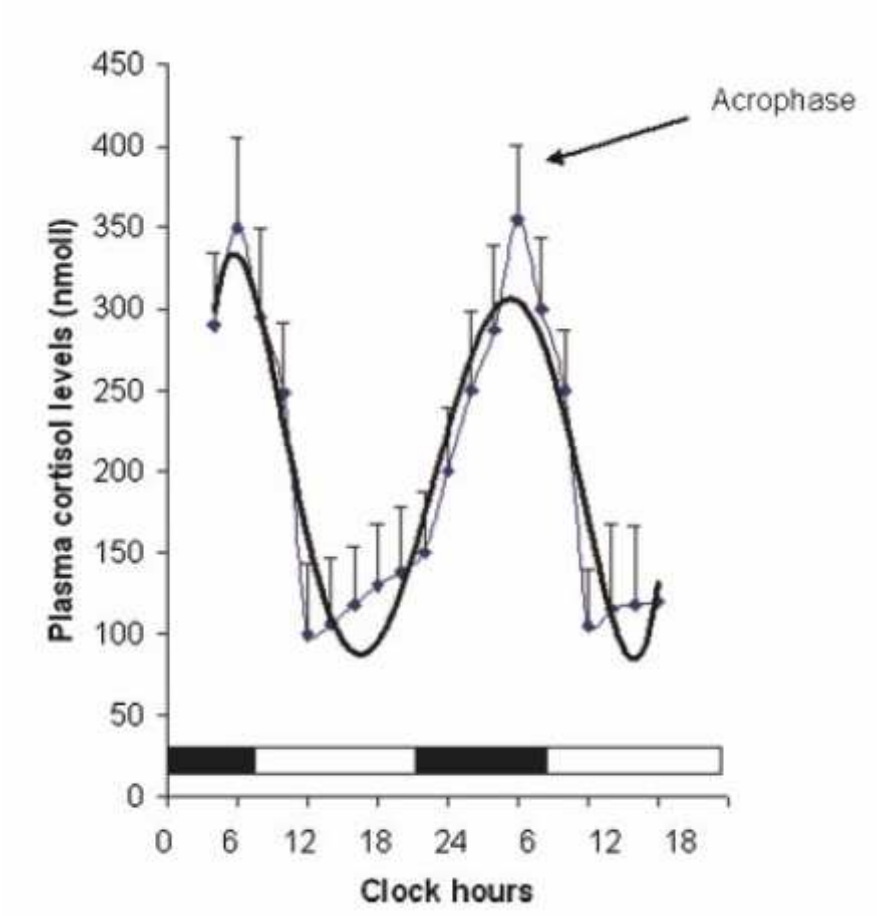


Fasting/eating dependent, ultradian variation in serum leptin

Circadian variation in plasma leptin and peripheral expression

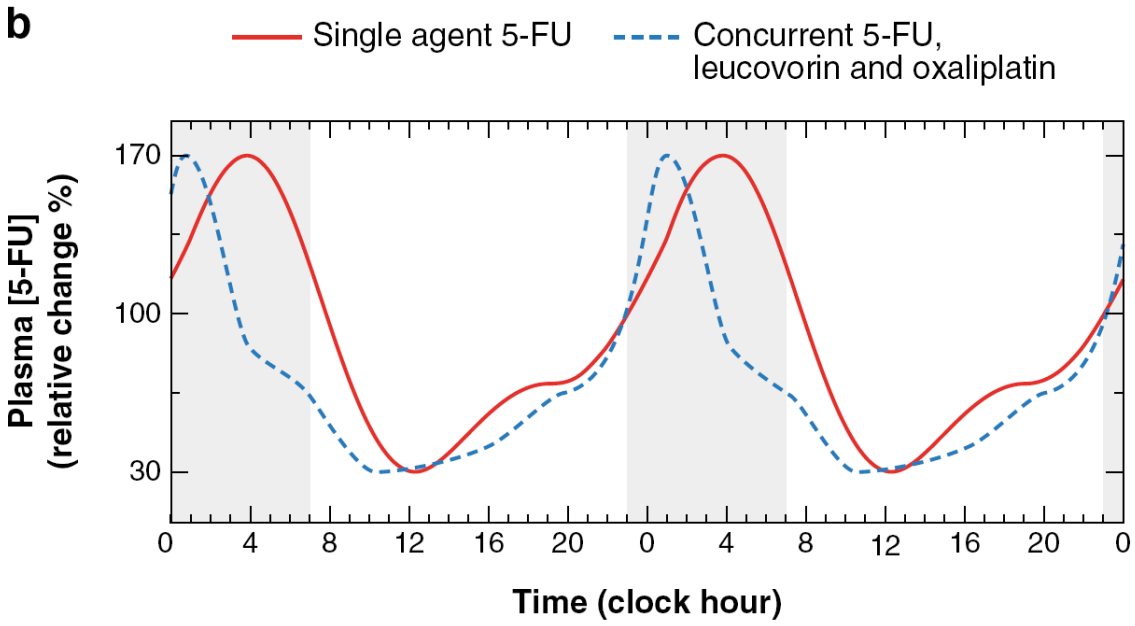


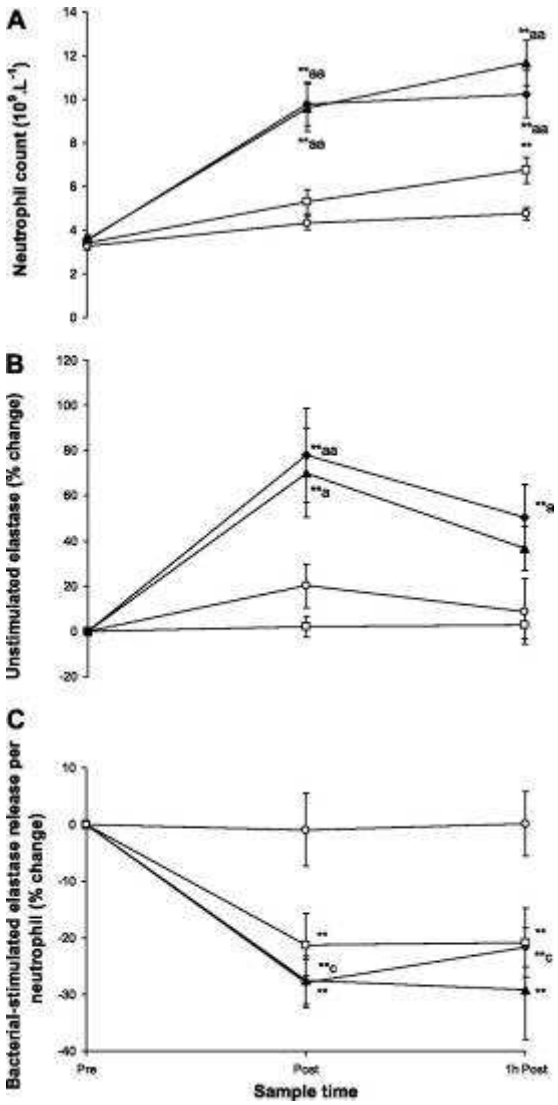




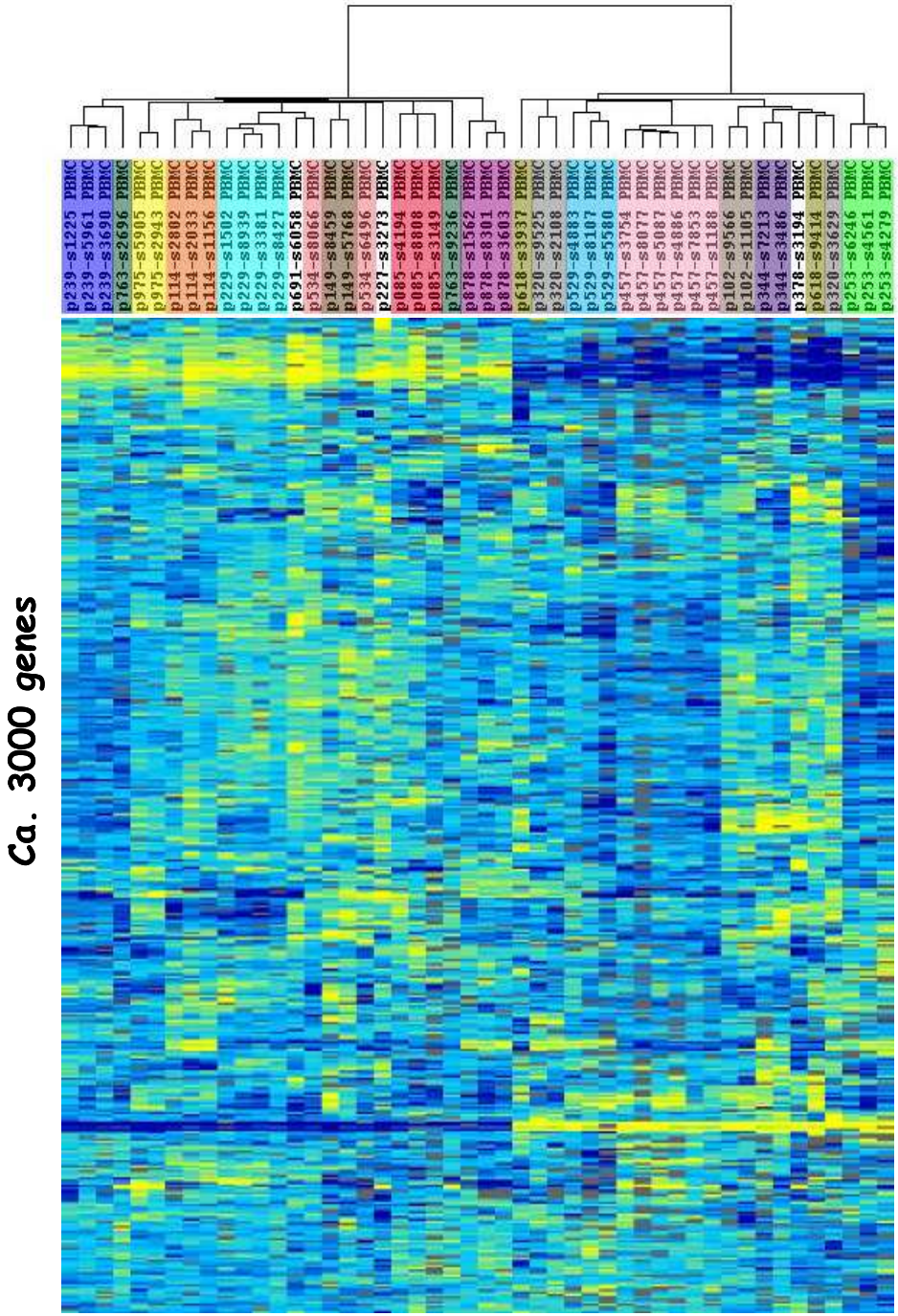
Circadian variation in serum cortisol

Circadian variation in 5FU metabolism



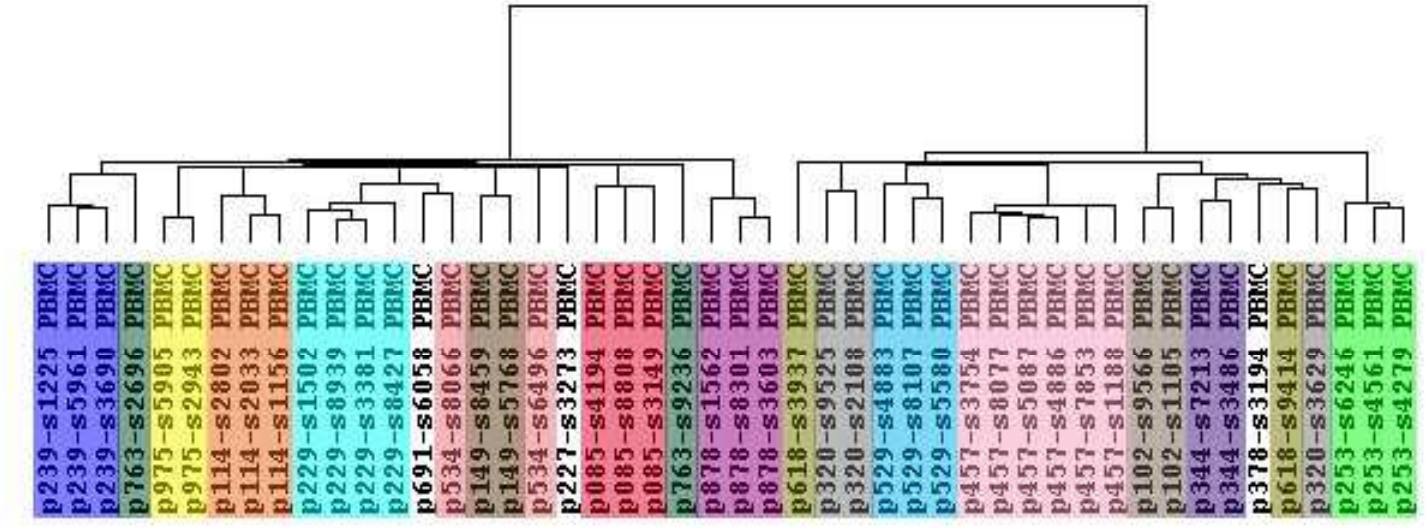


Neutrophil counts and exercise



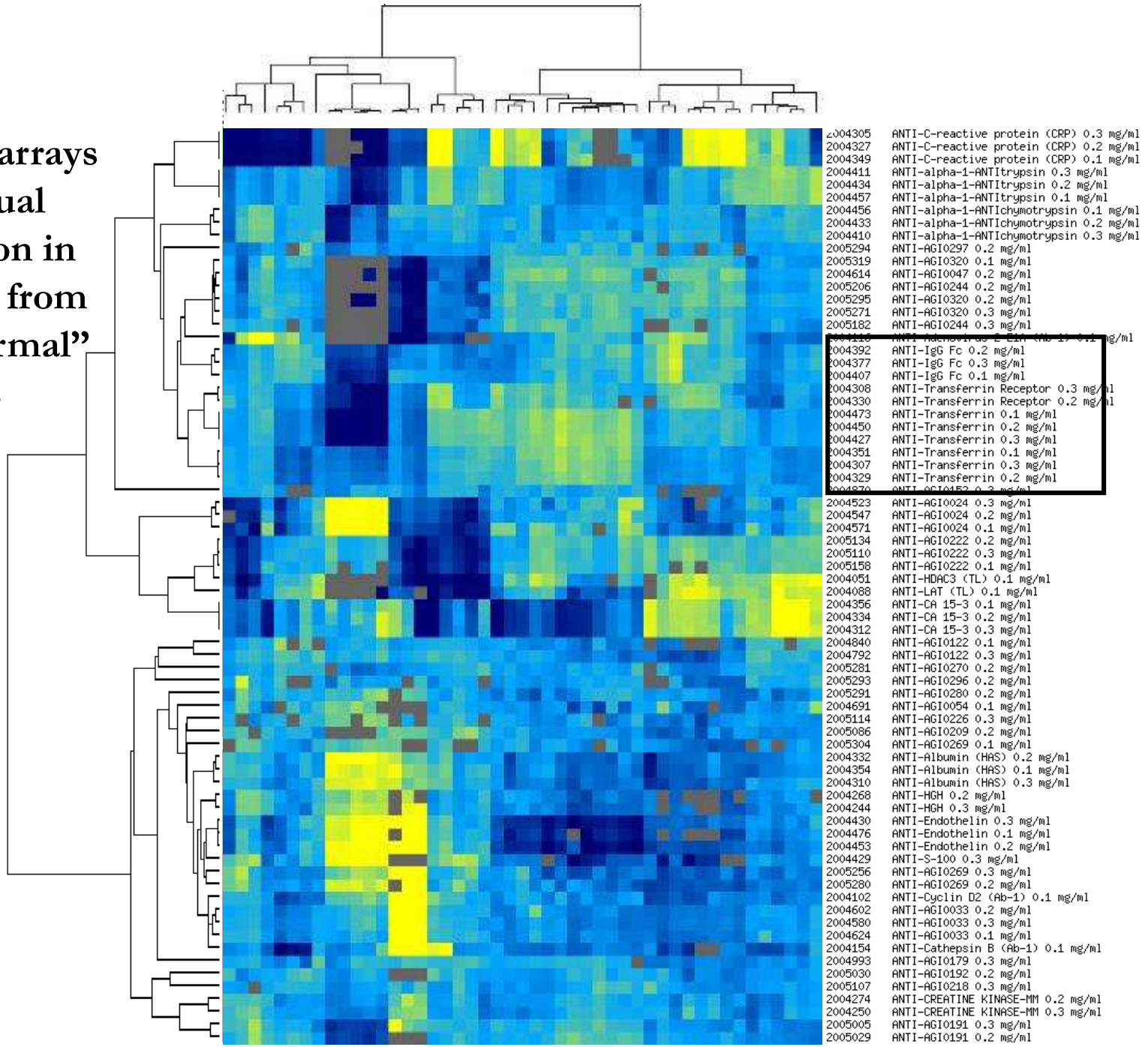
Multidimensional variation in gene expression patterns observed in peripheral blood samples from "normal" volunteers

Addie Whitney
Kate Rubins
Jen Boldrick
Max Diehn
Ash Alizadeh
David Relman

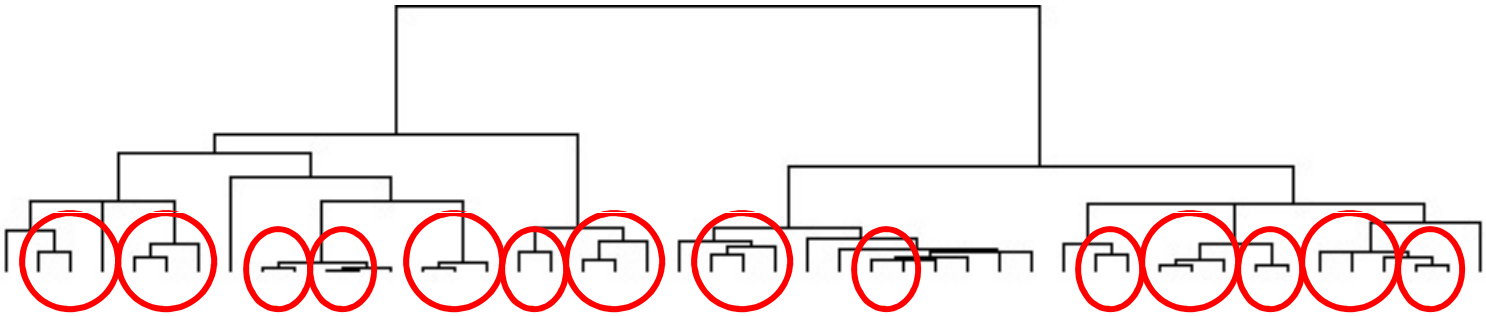


Distinctive, individual-specific features of gene expression patterns in peripheral blood cells persist over at least weeks

Antibody microarrays inter-individual protein variation in serum samples from "apparently normal" volunteers



Most replicate serum samples cluster together as nearest neighbors



Important potential confounding factors for any molecular marker include:

Interindividual genetic variation
Intercurrent illness or physiological stress
Psychological stress
Sleep
Nutrition
Medication
Time of day
?

We lack a basic interpretative framework for molecular (and anatomic/histological) variation.

For any observation that might be interpreted as evidence of disease, what are all the factors that can influence it. (Differential diagnosis of molecular/anatomic/histological variation – in quantitative, probablistic terms).

Disease is defined as a distinct deviation from the range of normal variation and detection and diagnosis of disease implicitly depends on knowing the scope and boundaries of "normal" variation. **Knowing what "normal" can look like is the foundation of medical diagnosis.**

But….

We know astonishingly little about the "normal human"

A **miniscule** fraction of molecular studies (eg. gene expression) have been devoted to defining what normal cells, tissues and fluids can look like, and how the variation relates to genetic, environmental and physiological factors.

We need a systematic characterization of normal human phenotypic variation

Anatomy
Histology
Expression patterns
Molecular profiles of cells, tissues, fluids
Microbial flora

-links to genotype and environmental factors.

Biospecimens

We need to launch a deliberate thoughtful attempt to collect tissues, fluids and associated data from the "general population".

On a **very large** scale – need to map out (rigorously and quantitatively) the gamut of variation.

Needs to be large scale because a lot of what appears to be pathological deviation may be outer limits of normal – but we don't know. Knowing the rare non-pathological variants is critical for screening and early detection of low-prevalence disease (most cancers) and diagnostic interpretation of apparent anomalies.

Autopsies!

Systematic, concerted data collection, not small series and case reports. High resolution imaging, meticulous histopathology.

How prevalent are occult neoplasms or other pathological variations in the population?

Autopsies of “normal” people are a tall order.

?Transplant donors

?Medical examiners

Not easy – but necessary! Need to be resourceful

Pre-clinical natural history of cancer

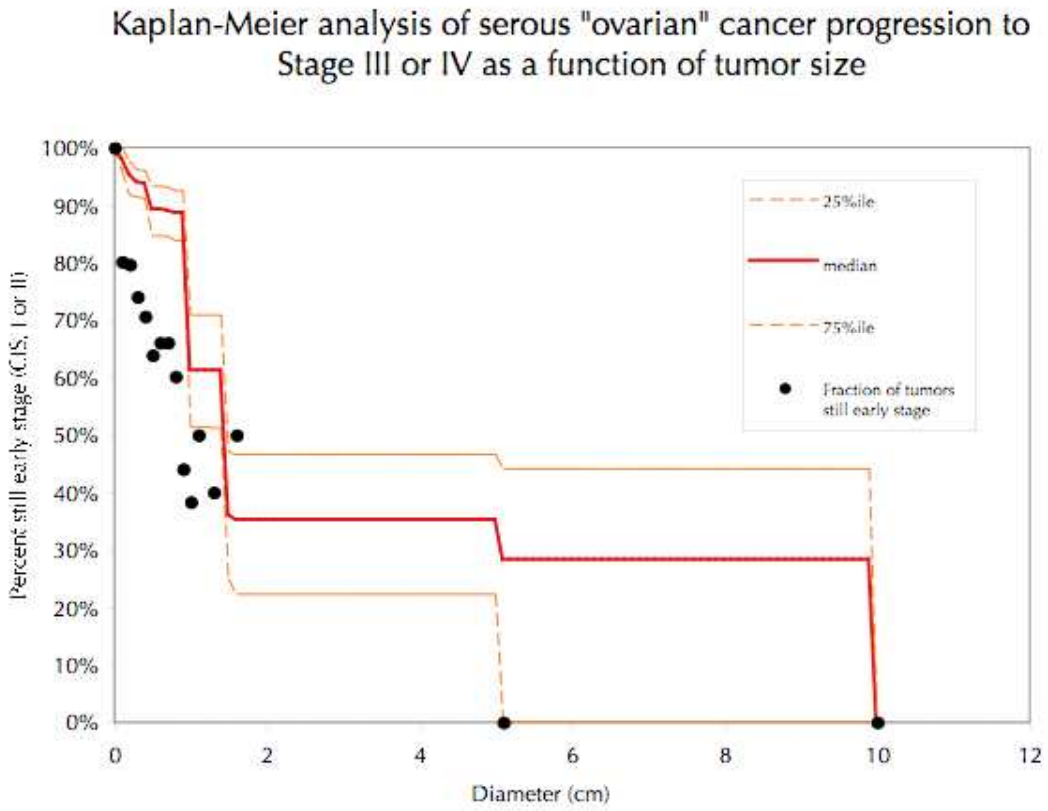
Critical for rational early detection – Duh! ‘

We need to know what we need to detect to make a difference!

Very difficult challenge, needs to be a priority.

Prevalent assumptions are commonly unfounded and very likely wrong.

Example: Serous ovarian cancers progress to advanced stage at a size 1000 times smaller than the median clinical early stage ovarian cancer



Palmer and Brown, unpublished

Balancing use and preservation of precious biospecimens.

Sample divisibility independent of prior aliquoting design

(eg., sliceable, resealable tubes of frozen fluids).

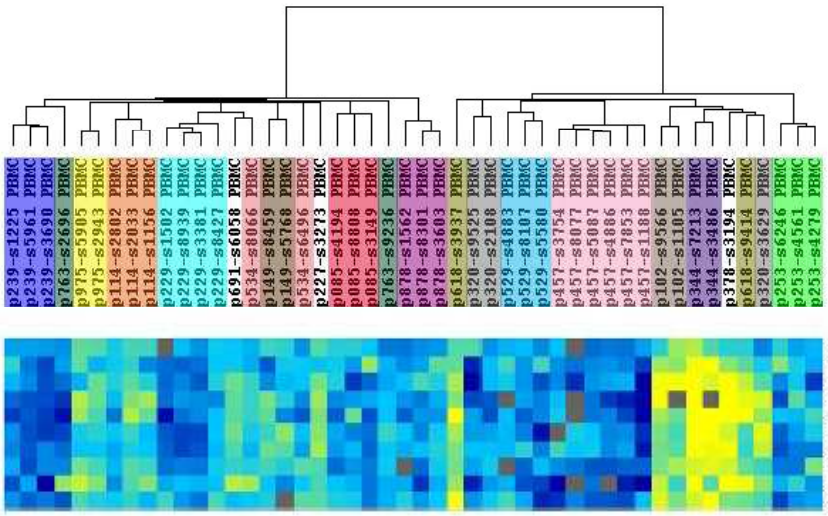
Research article

Open Access

Early detection of breast cancer based on gene-expression patterns in peripheral blood cells

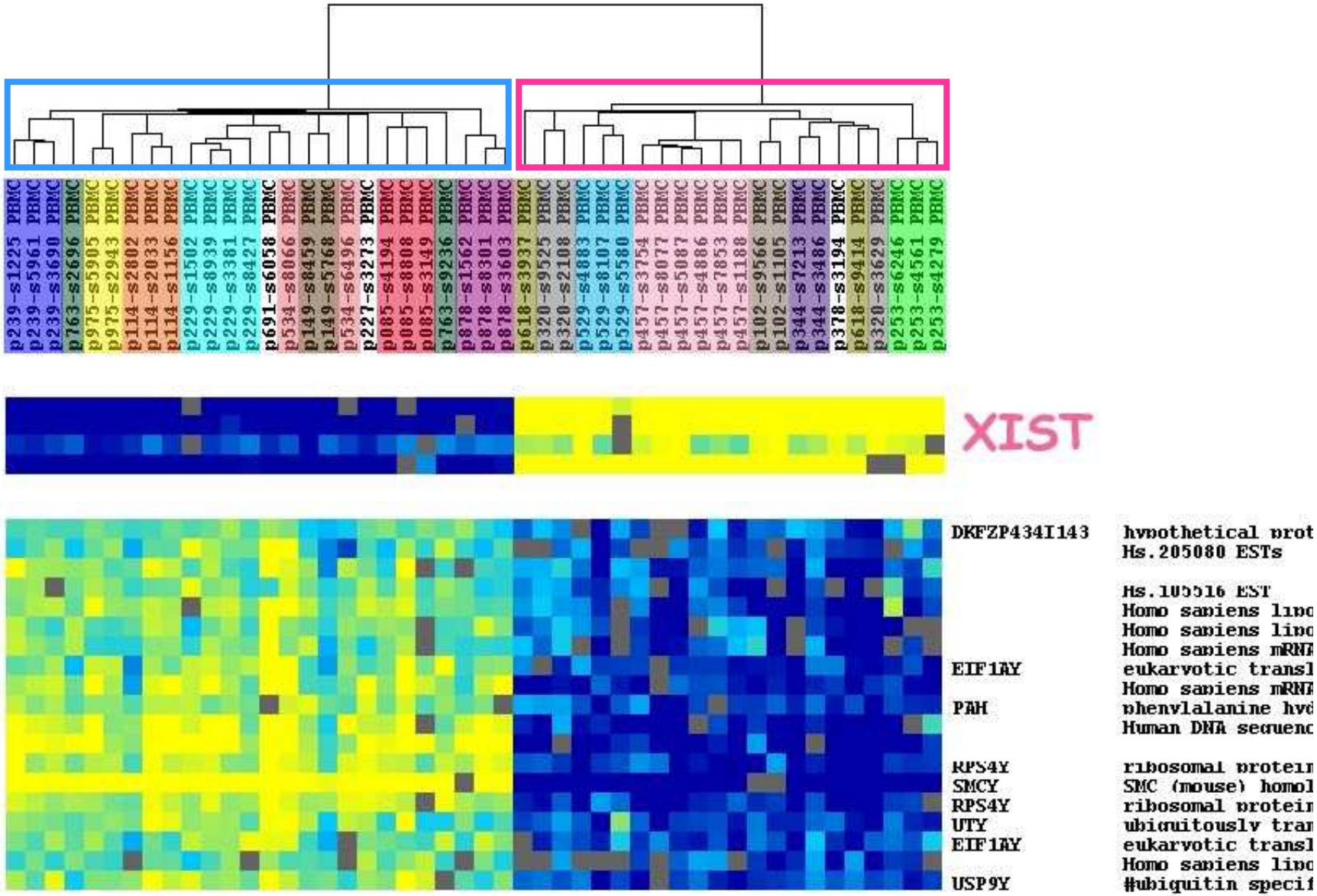
Praveen Sharma[1], Narinder S Sahni[1], Robert Tibshirani[2], Per Skaane[3], Petter Urdal[4], Hege Berghagen[1], Marianne Jensen[1], Lena Kristiansen[1], Cecilie Moen[1], Pradeep Sharma[1], Alia Zaka[1], Jarle Arnes[5], Torill Sauer[6], Lars A Akslen[5], Ellen Schlichting[7], Anne-Lise Børresen-Dale[8] and Anders Lönneborg[1]

[1]DiaGenic ASA, Oslo, Norway



"Interferon-induced"

Sex-specific



American Journal of Epidemiology
Vol. 154, No. 6
Printed in U.S.A.

Epidemiology of the Insulin-like Growth Factor System in Three Ethnic Groups

J. K. Cruickshank,[1] A. H. Heald,[2] S. Anderson,[1] J. E. Cade,[1] J. Sampayo,[2] L. K. Riste,[1] A. Greenhalgh,[1] W. Taylor,[3] W. Fraser,[3] A. White,[2] and J. M. Gibson[2]

IGF-II showed a strong inverse association with African-Caribbean ($\beta = -0.264$, $p = 0.001$) and Pakistani ($\beta = -0.240$, $p < 0.0001$) ethnicity compared with European ethnicity