UNIVERSITY of GREENWICH

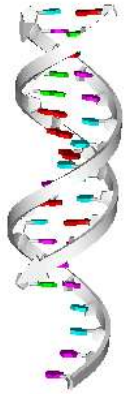
Pharmacometabonomics: an Important New Paradigm for Personalised or Precision Medicine

Jeremy Everett

September 2014

Royal Institution, London

review of –omics world…

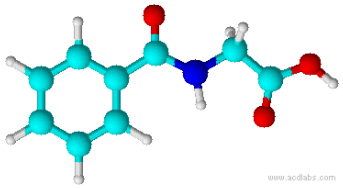


genomics

study of genomes; complete set of genes encoded by an organism

proteomics

study of proteomes; profile of proteins expressed and modified by an organism



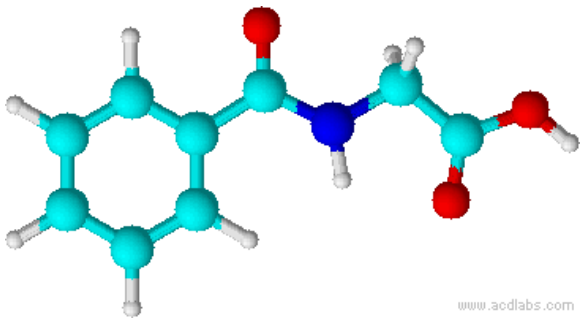
metabonomics

study of ?

metabonomics is defined as:

"*The study of the metabolic response of organisms to disease, environmental change or genetic modification*"

metabonomics, a science complementary to genomics and proteomics



Lindon, Nicholson, Holmes and Everett, Concept Magn. Reson, (2000)

how do we measure metabonomic data?

- NMR spectroscopy or mass spectrometry
- human or animal biofluids
  - urine, plasma, csf, bile, saliva, milk
- human or animal tissues
  - use special techniques such as solid state NMR

why is metabonomics important?

- metabonomics provides important window on actual metabolic response of an organism and its symbiotic partners in a systems biology (in vivo) approach

- genomics or transcriptomics demonstrate what could happen in an organism: not necessarily what will happen

- in particular metabonomics provides a window on both genetic and environmental factors
  - diet, disease, drugs, microbiome

human evolution…

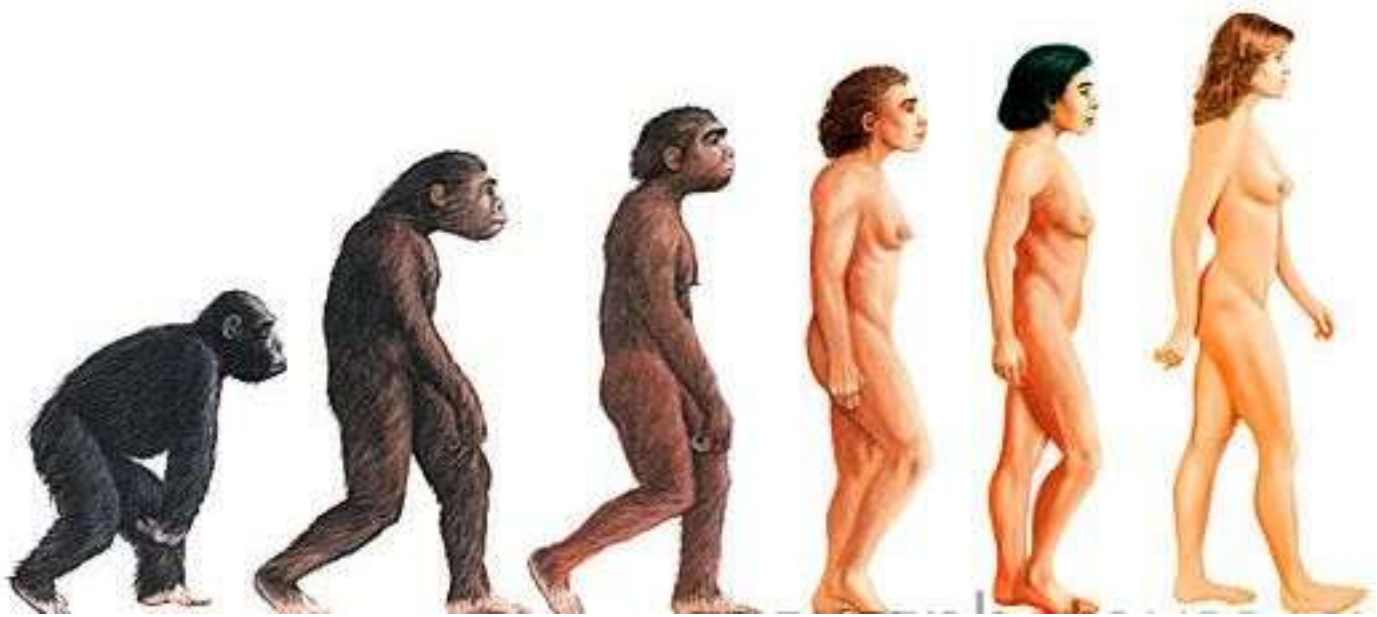
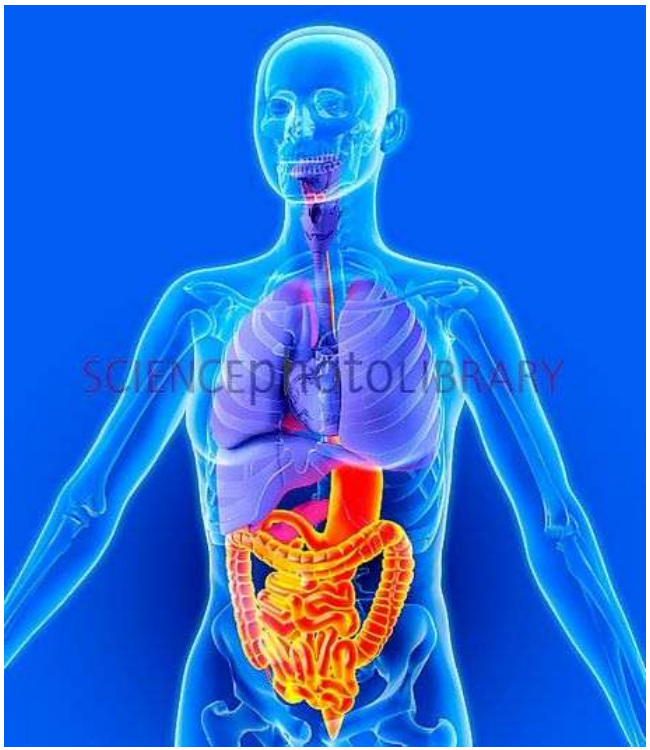
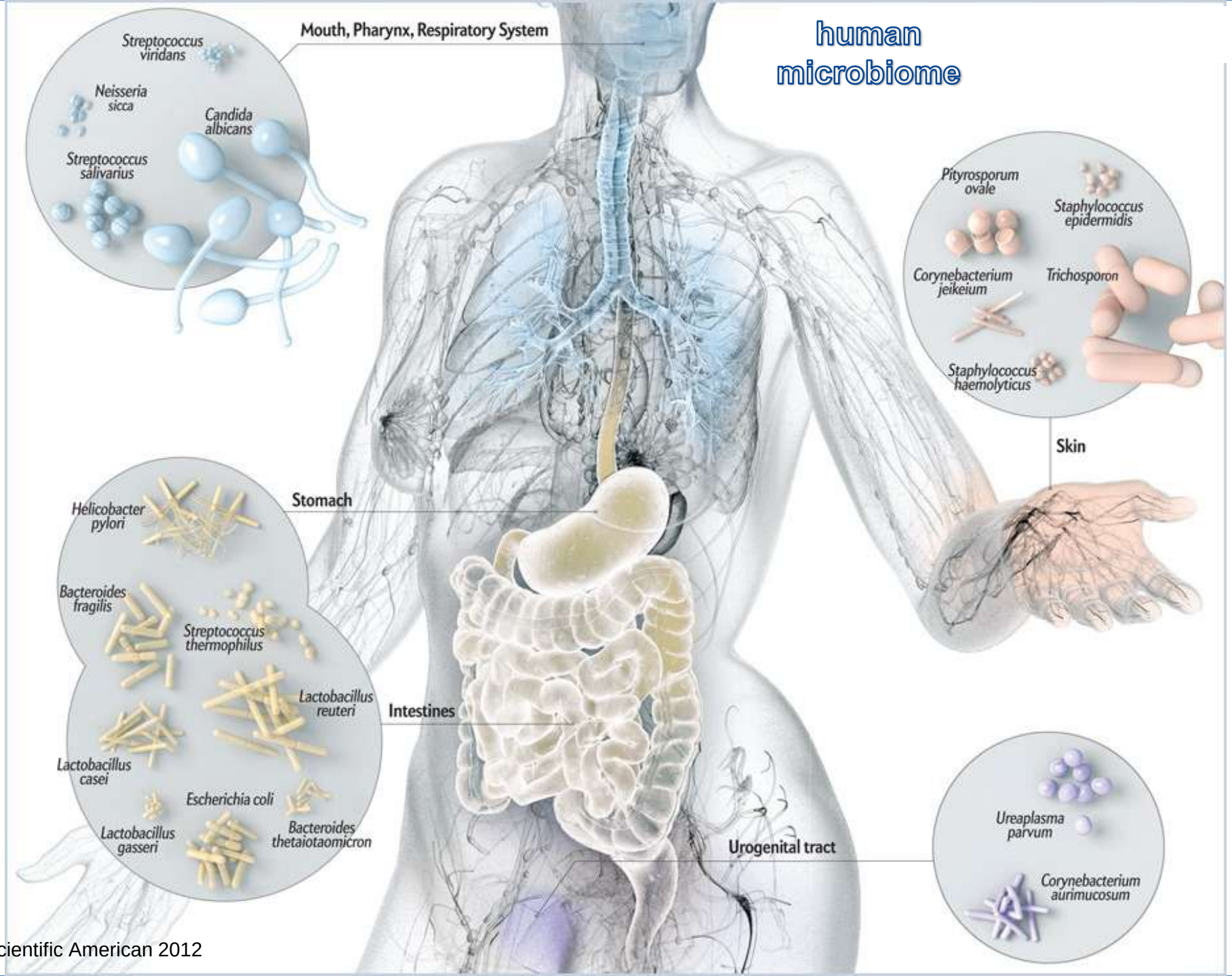


image: www.kelionesirpramogos.lt

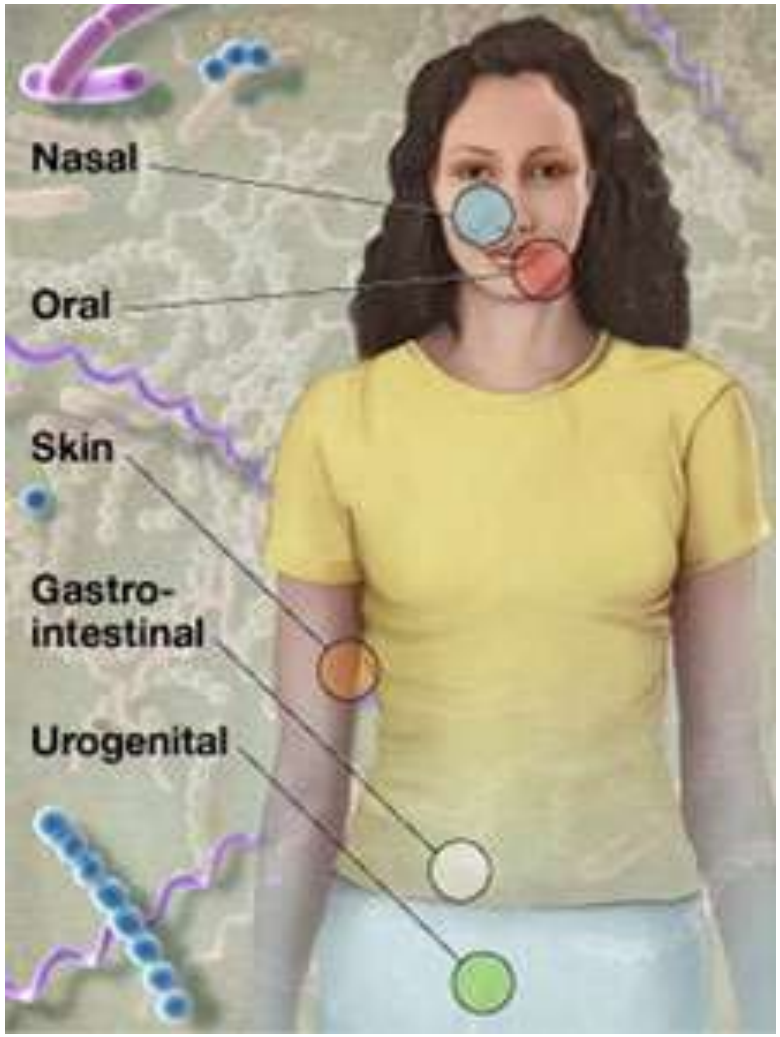
our microbiome!

- the collection of microorganisms living in and on our bodies
  - bacteria, fungi, viruses
- each part of our body surface and orifice has its own micro-environment and unique collection of bacteria, viruses and fungi, especially our gut
- the microbiome has significant and complex interactions with our genome and plays a significant role in metabolism and in disease





Scientific American 2012



- human microbiome project aiming to sample and analyse microbes from 5 major sites in humans
- follow-on from human genome project : total budget of $115 million over 5 years: 2008 to 2013

of human cells in humans?
of microbes in/on human?
of human genes?
bacterial genes in/on humans?

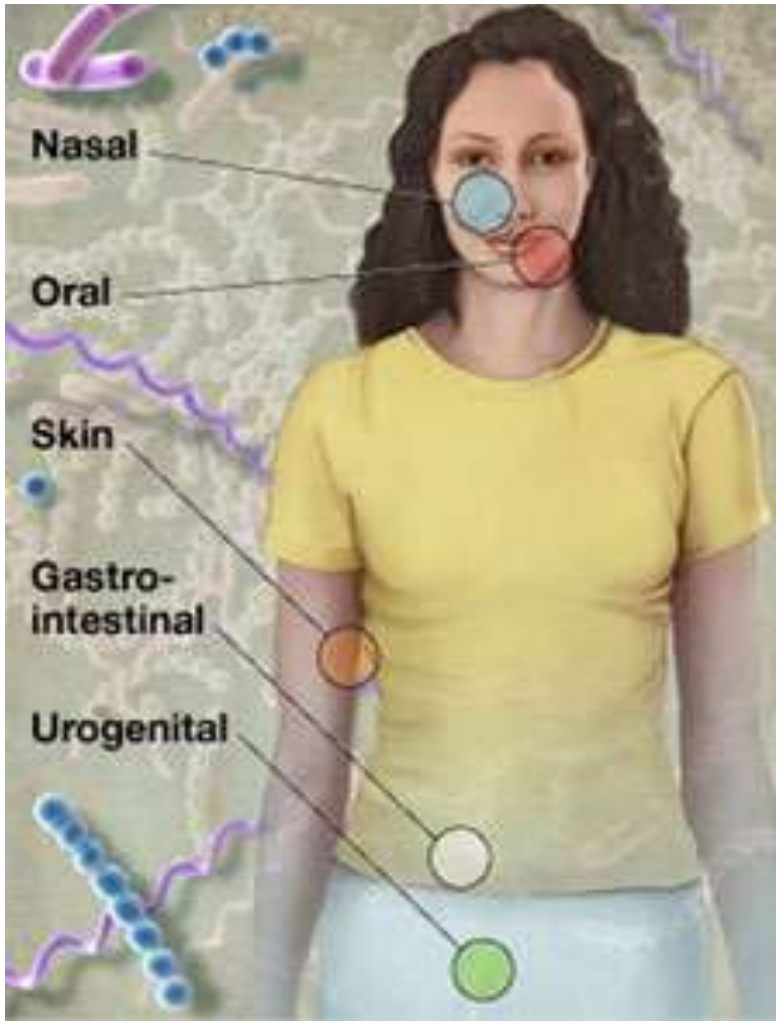
Superscript

MetaHIT

Metagenomics of the Human Intestinal Tract

European research project

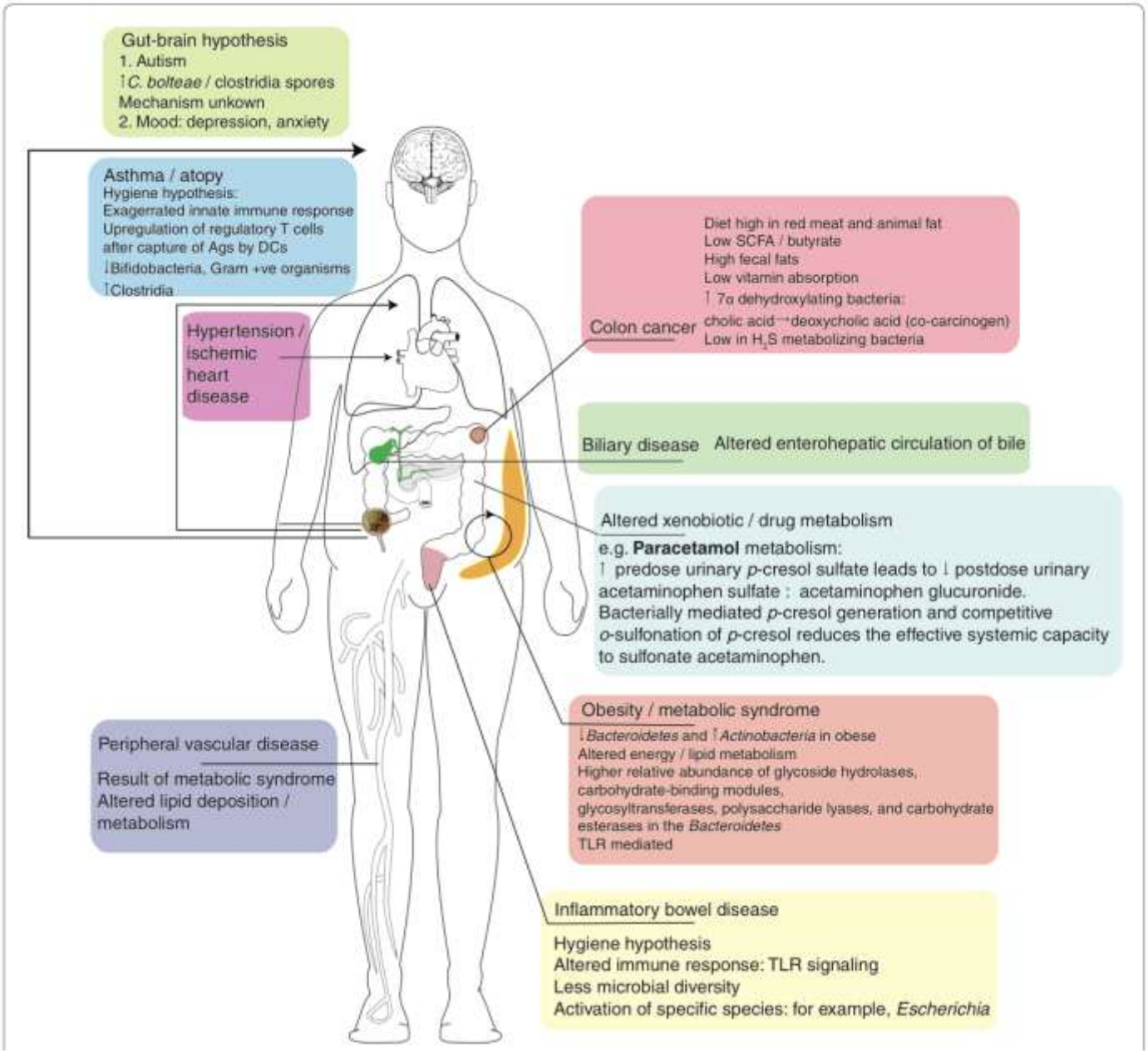
HMP



- human microbiome project aiming to sample and analyse microbes from 5 major sites in humans
- follow-on from human genome project : total budget of $115 million over 5 years: 2008 to 2013

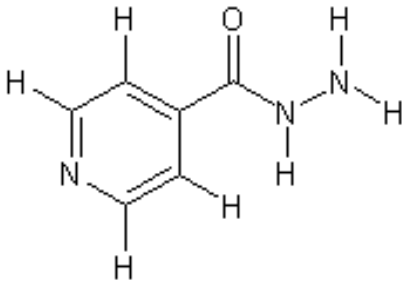
# of human cells in humans?	ca 50 Trillion
# of microbes in/on human?	**ca 500 Trillion**
# of human genes?	23,450
# bacterial genes in/on humans?	**ca 3,000,000**

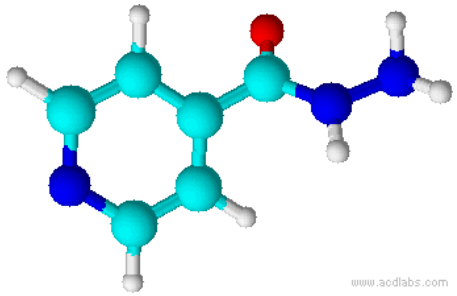
diseases influenced by gut microbial metabolism



James M Kinross, Ara W Darzi and Jeremy K Nicholson, Genome Medicine 2011

metabonomics study of isoniazid

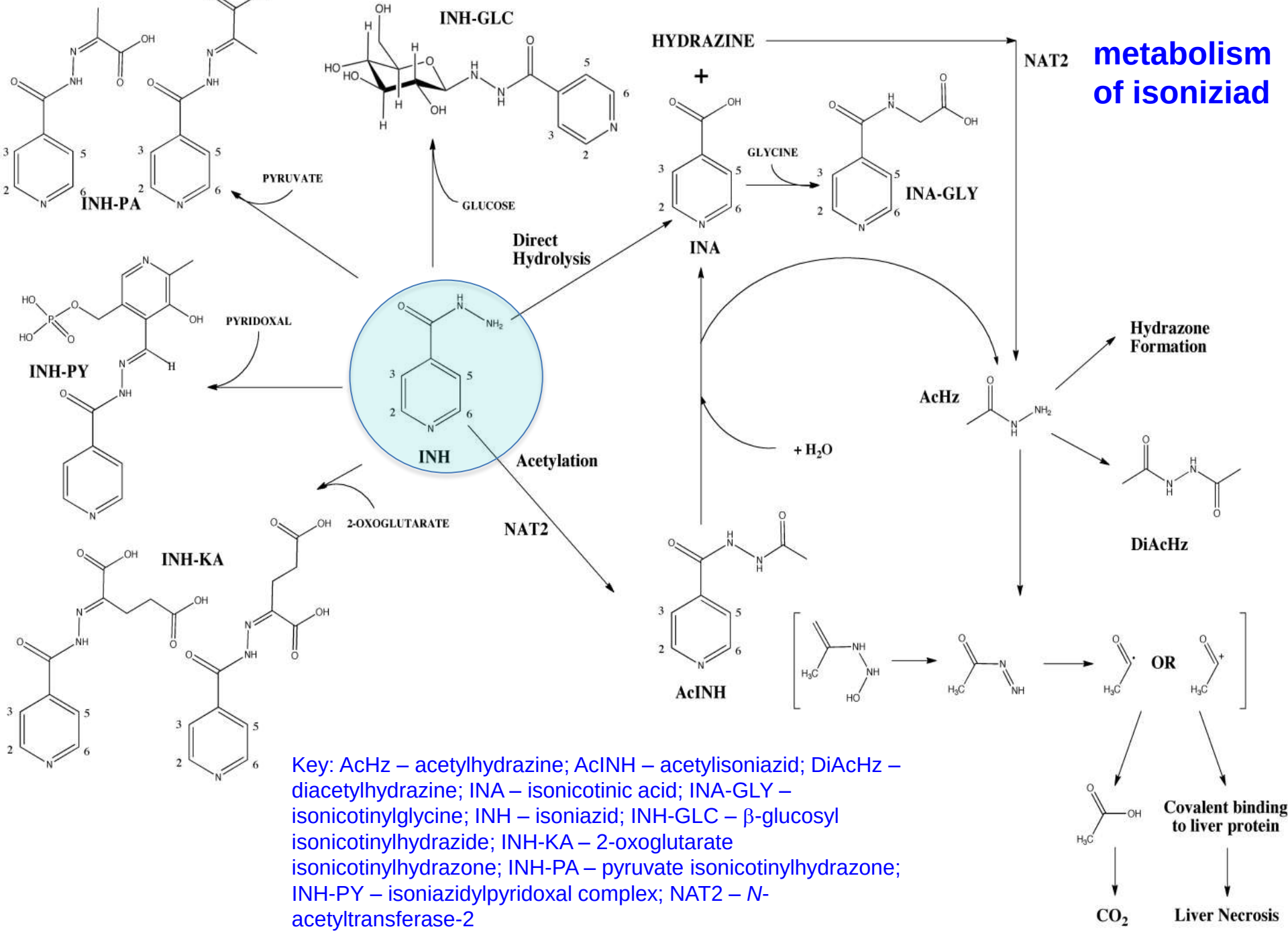




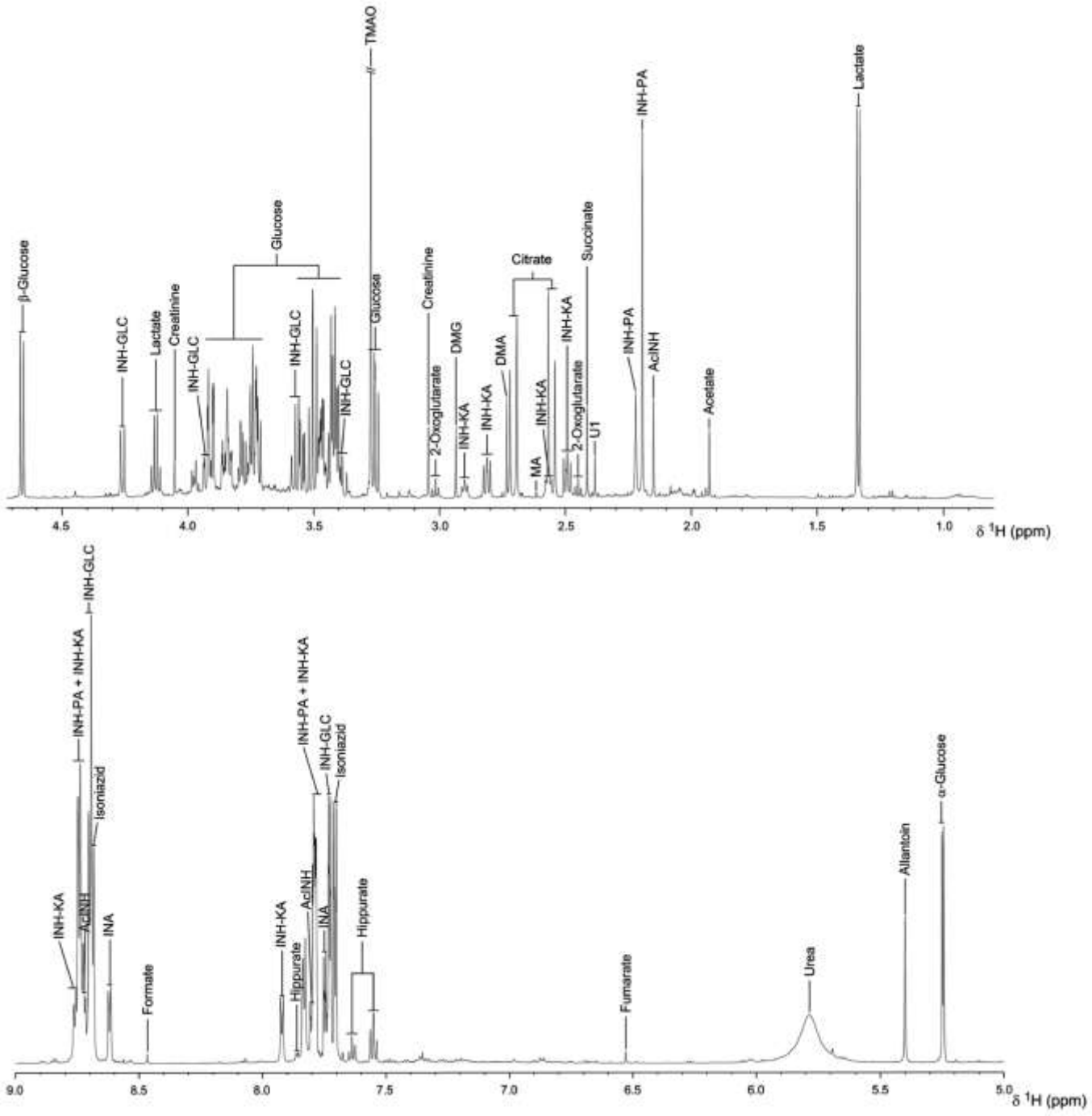
- anti-tuberculosis drug: therapeutic and prophylactic
- significant side-effects
  - rash, hepatotoxicity, peripheral neuropathy
  - CNS effects
- metabonomics study in Sprague-Dawley rats to study inter-individual variability in response (400 mg/kg, high dose; 200 mg/kg low dose and 0.9 % saline, control, n=10)

tuberculosis patient, Port-au-Prince, Haiti
Los Angeles Times

metabolism of isoniziad



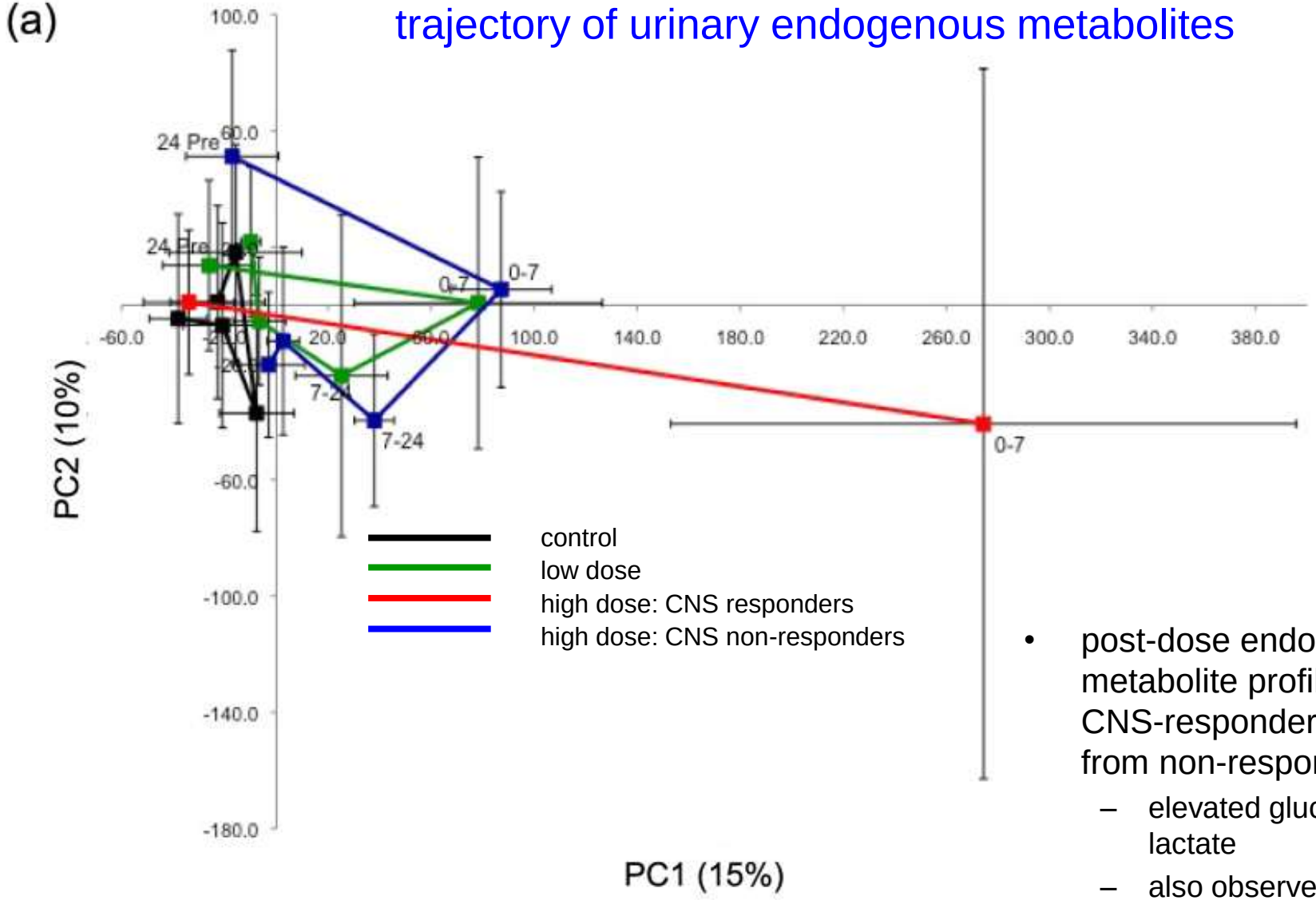
Key: AcHz – acetylhydrazine; AcINH – acetylisoniazid; DiAcHz – diacetylhydrazine; INA – isonicotinic acid; INA-GLY – isonicotinylglycine; INH – isoniazid; INH-GLC – β-glucosyl isonicotinylhydrazide; INH-KA – 2-oxoglutarate isonicotinylhydrazone; INH-PA – pyruvate isonicotinylhydrazone; INH-PY – isoniazidylpyridoxal complex; NAT2 – *N*-acetyltransferase-2



600 MHz 1H NMR spectra of urine from rat 0–7 hrs after dose of 400 mg/kg isoniazid

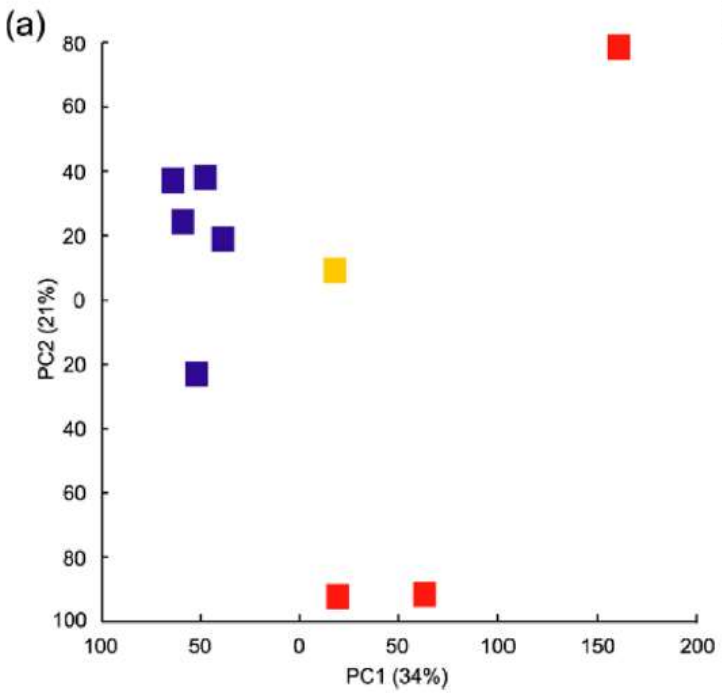
Key: AcINH – Acetylisoniazid; DMA – Dimethylamine; DMG – Dimethylglycine; INA – Isonicotinic Acid; INA-GLY – Isonicotinyl Glycine; INH-GLC – Glucose Isonicotinyl Hydrazide; INH-KA – α-Oxoglutarate Isonicotinyl Hydrazone; INH-PA – Pyruvate Isonicotinyl Hydrazone; MA – Methylamine; TMAO – Trimethylamine-*N*-oxide; U1/2 – Unassigned INH-related metabolites

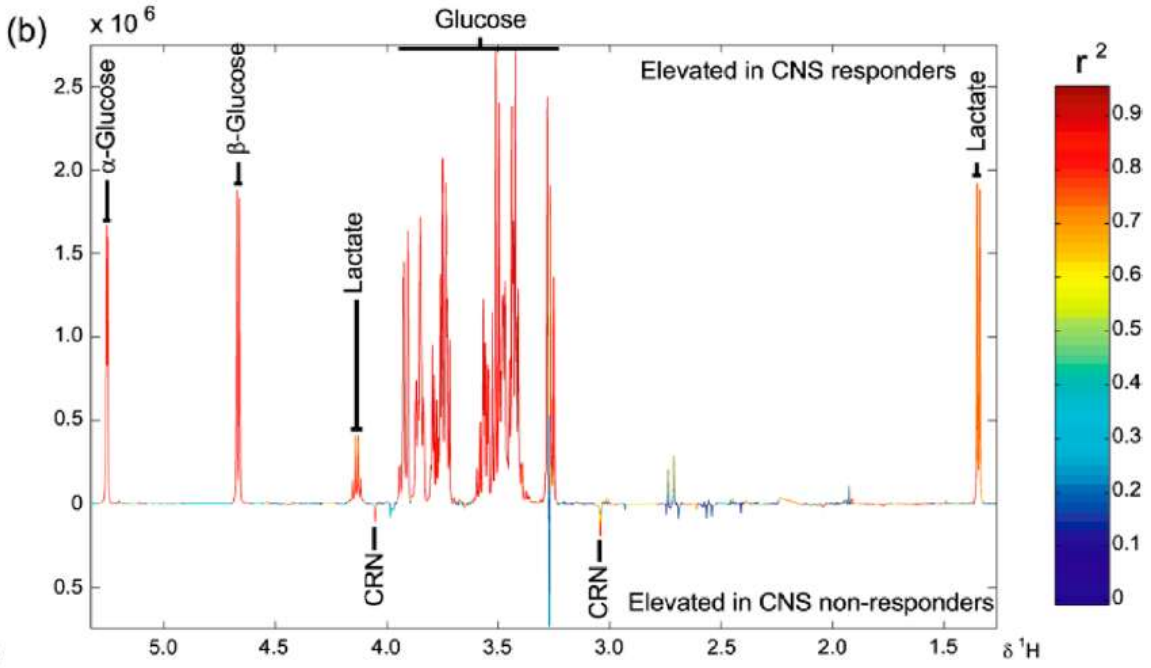
Principle Components Analysis (PCA) of metabolic trajectory of urinary endogenous metabolites



- post-dose endogenous metabolite profile of CNS-responders differs from non-responders
  - elevated glucose and lactate
  - also observed following isoniazid neurotoxicity in man

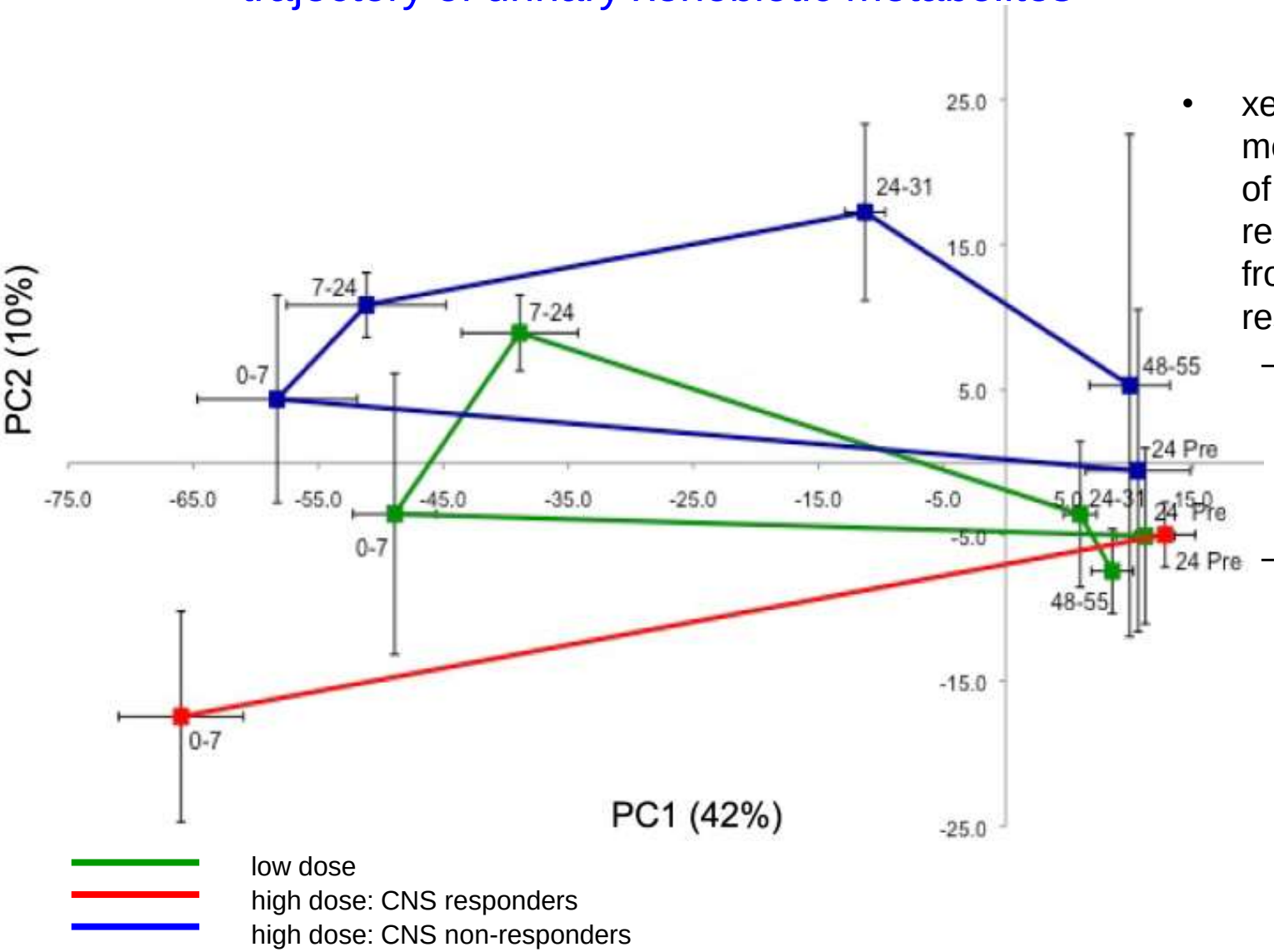
Principle Components Analysis (PCA) of urinary endogenous metabolite data at 0 to 7 hours post-dose for high dose INH





- CNS responder
- CNS non-responder
- CNS non-responder B (high glucose but no elevation in lactate)

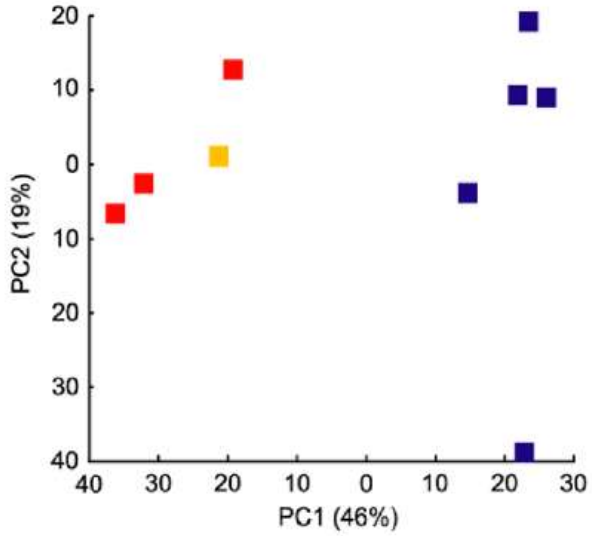
Principle Components Analysis (PCA) of metabolic trajectory of urinary xenobiotic metabolites



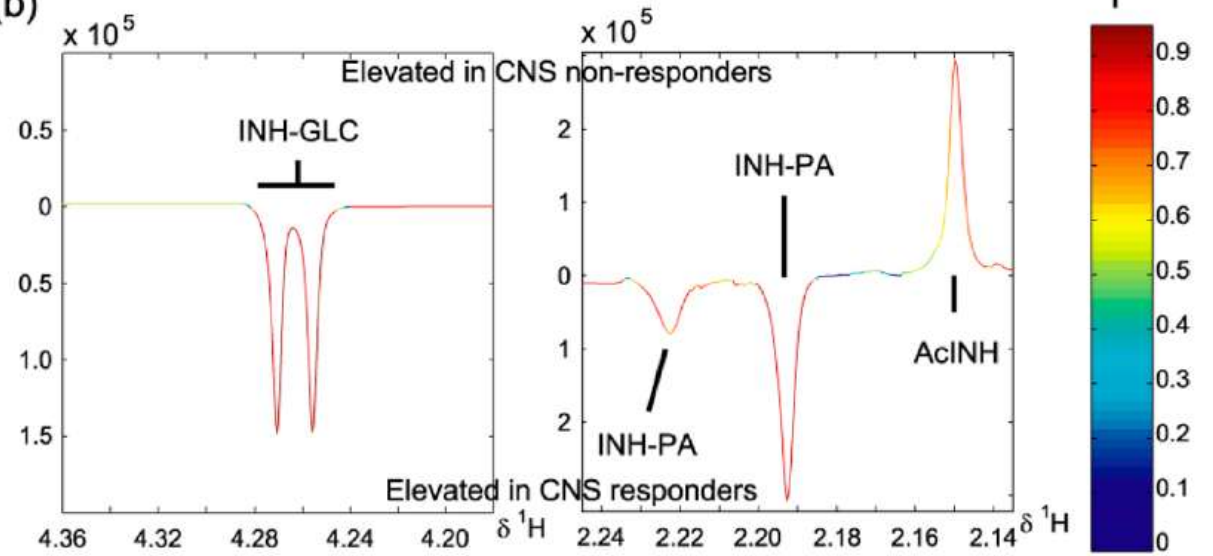
- xenobiotic metabolic profile of CNS-responders differs from non-responders
  - increased levels of INH-PA and INH-GLC and low levels of AcINH
  - lack of acetylation capacity leads to alternative toxic pathways

Principle Components Analysis (PCA) of urinary xenobiotic metabolite data at 0 to 7 hours post-dose for high dose INH

(a)



(b)



(c)

	AcINH: INH	INH-PA: AcINH	INH-GLC: AcINH
Low Dose (*n*=10)	0.97 ± 0.74	0.80 ± 0.35	0.36 ± 0.10
High Dose CNS Non-Responders (*n*=5)	0.70 ± 0.11	0.65 ± 0.15	0.31 ± 0.05
High Dose CNS Responders (*n*=3)	0.37 ± 0.13 *	3.26 ± 0.41 **	5.75 ± 1.53 *

* = p < 0.05 and ** = p < 0.01 in Student's two-tailed t test for HD CNS Responders vs Non-Responders

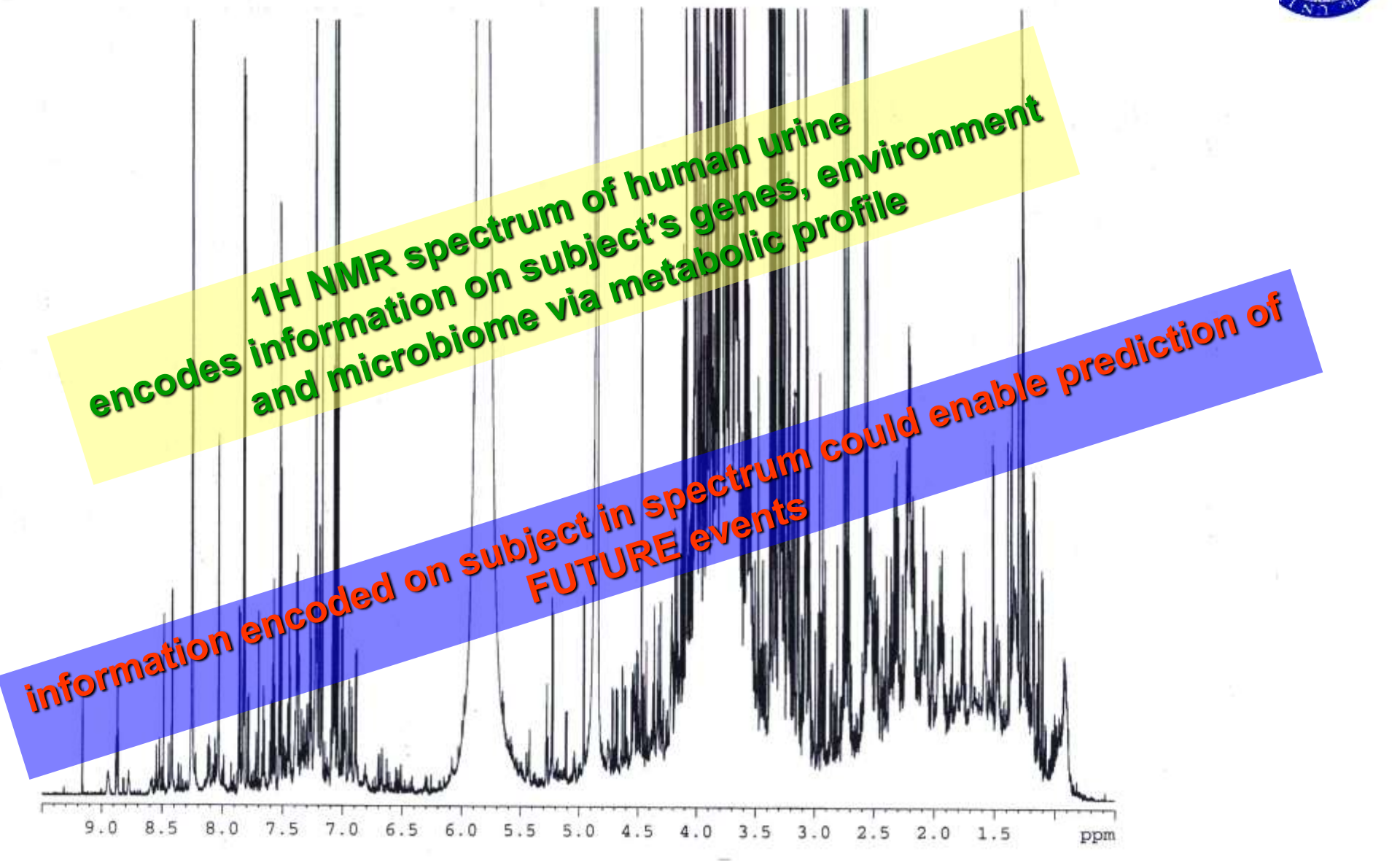
- CNS high dose responder
- CNS high dose non-responder
- CNS high dose non-responder B (high INH-PA and INH-GLC but no reduction in AcINH)

can we use metabonomics to predict the future?

- metabonomics typically studies effects of drugs after dosing
- can we use metabonomics to predict effects of drugs before drug dosing?
  - drug metabolism
  - efficacy
  - toxicology
- this would be pharmacometabonomics by analogy to pharmacogenomics

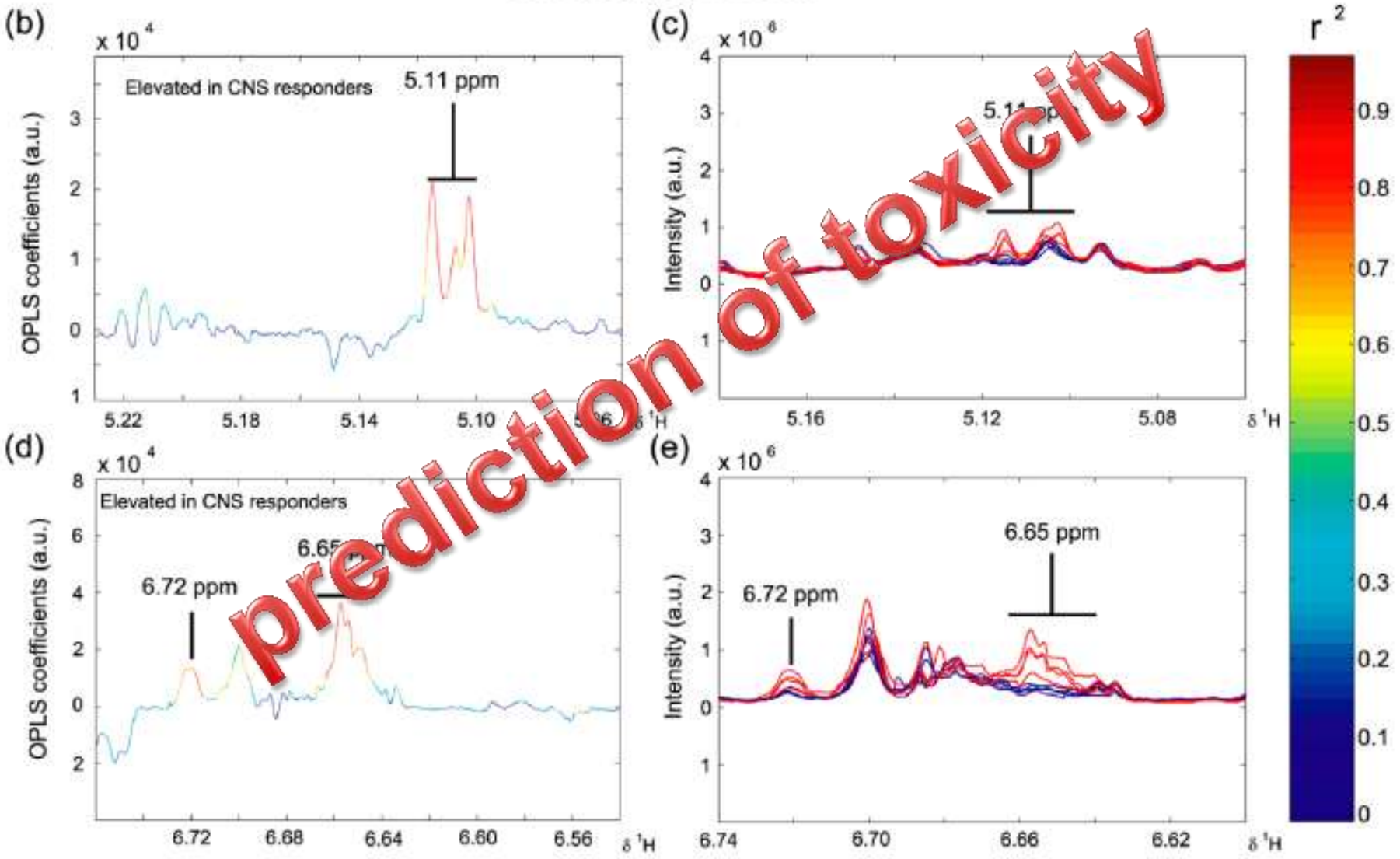
http://debralschubert.blogspot.co.uk

theoretically metabonomics could predict future

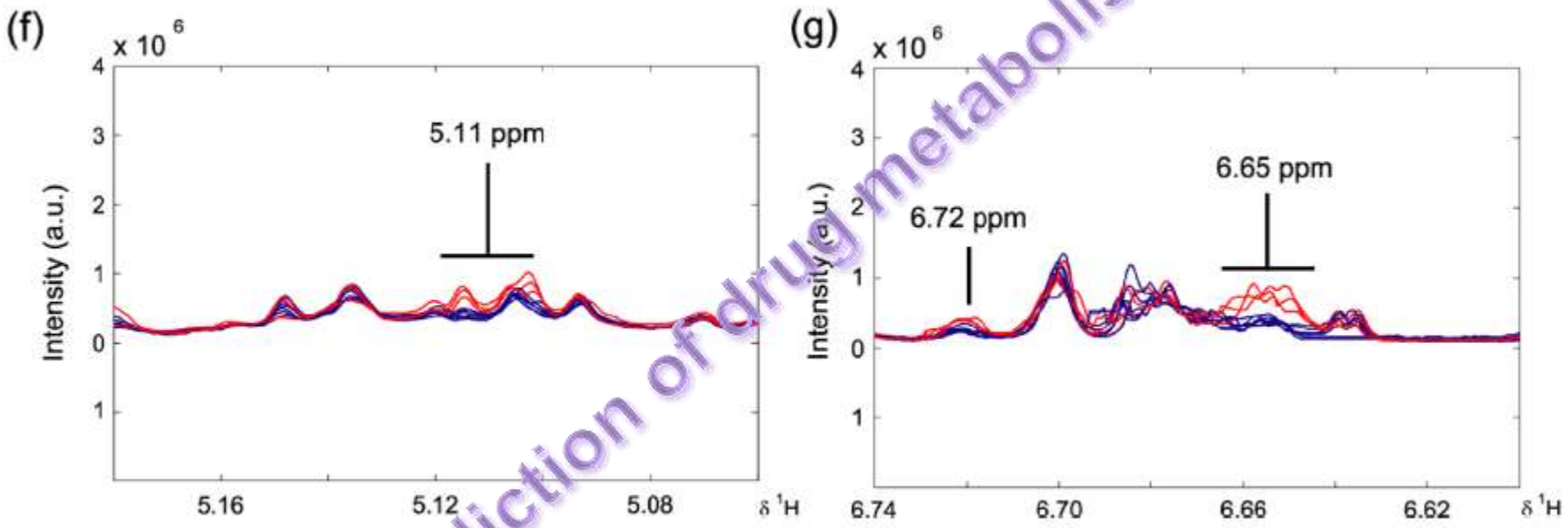


O-PLS-DA analysis of pre-dose urine spectra from high-dose isoniziad rats

spectra colour-coded on RHS by post-dose response: red = R: blue NR: ($Q^2Y = 0.34$, $R^2Y = 0.76$)



pharmacometabonomics and isoniazid: analysis of pre-dose spectra from LOW DOSE rats colour-coded by post-dose AcINH level: red < 3.0 x 10^8; blue > 3.5 x 10^8



Vol 440|20 April 2006|doi:10.1038/nature04648 nature

LETTERS

Pharmaco-metabonomic phenotyping and personalized drug treatment

T. Andrew Clayton[1], John C. Lindon[1], Olivier Cloarec[1], Henrik Antti[2], Claude Charuel[3], Gilles Hanton[3], Jean-Pierre Provost[3], Jean-Loïc Le Net[3], David Baker[4], Rosalind J. Walley[5], Jeremy R. Everett[5] & Jeremy K. Nicholson[1]

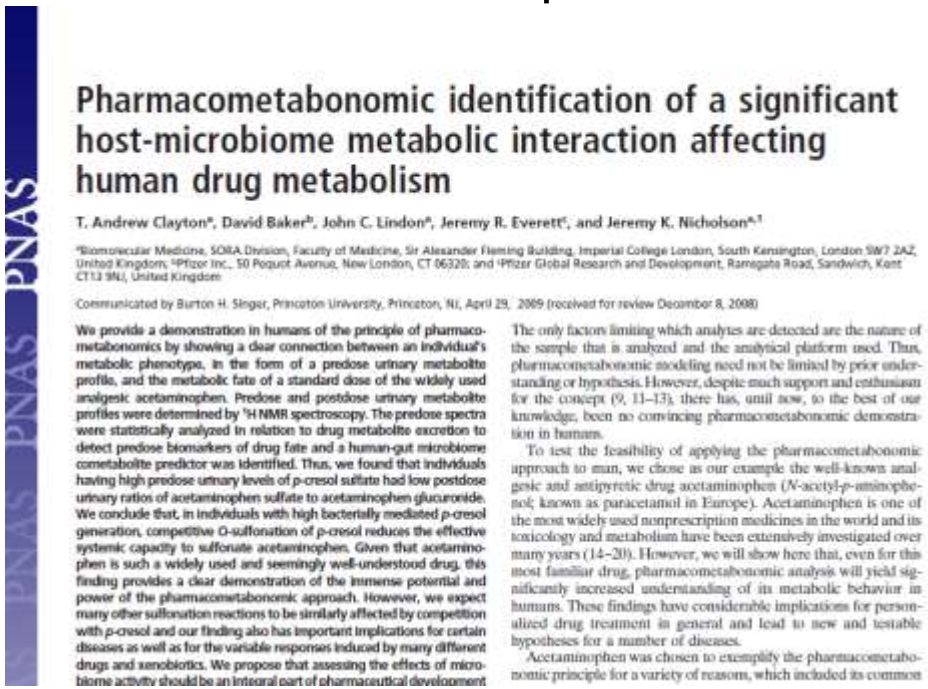
There is a clear case for drug treatments to be selected according to the characteristics of an individual patient, in order to improve efficacy and reduce the number and severity of adverse drug reactions[1,2]. However, such personalization of drug treatments requires the ability to predict how different individuals will respond to a particular drug/dose combination. After initial optimism, there is increasing recognition of the limitations of the pharmacogenomic approach, which does not take account of important environmental influences on drug absorption, distribution, metabolism and excretion[3–5]. For instance, a major factor underlying inter-individual variation in drug effects is variation in metabolic phenotype, which is influenced not only by genotype but also by environmental factors such as nutritional status, the

predictable, it suggested that information on individual responses to xenobiotics might be contained in the metabolite patterns of pre-dose biofluids. We thus conceived the possibility of 'pharmaco-metabonomics', which we define as 'the prediction of the outcome (for example, efficacy or toxicity) of a drug or xenobiotic intervention in an individual based on a mathematical model of pre-intervention metabolite signatures'.

We also conducted a larger study on the severity of liver damage induced in rats by allyl alcohol (50 mg kg^{-1}), and found a weak but statistically significant association between the extent of the induced damage and the pre-dose urinary data (Fig. 1b), although in this case the discriminating factor was the total pre-dose excretion of organic compounds as estimated by ^{1}H NMR spectroscopy, rather than the

human pharmaco-metabonomics

- ethically approved study of 100 fit, healthy, male volunteers
- oral dose of 2 x 500 mg paracetamol
- collection of pre-dose, 0-3 and 3-6 hour post-dose urines
- analysis of urine samples by NMR to establish if there was a relationship between pre-dose metabolite profiles and post-dose metabolic fate of paracetamol



Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism

T. Andrew Clayton[a], David Baker[b], John C. Lindon[a], Jeremy R. Everett[c], and Jeremy K. Nicholson[a,1]

[a]Biomolecular Medicine, SORA Division, Faculty of Medicine, Sir Alexander Fleming Building, Imperial College London, South Kensington, London SW7 2AZ, United Kingdom; [b]Pfizer Inc., 50 Poquot Avenue, New London, CT 06320; and [c]Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom

Communicated by Burton H. Singer, Princeton University, Princeton, NJ, April 29, 2009 (received for review December 8, 2008)

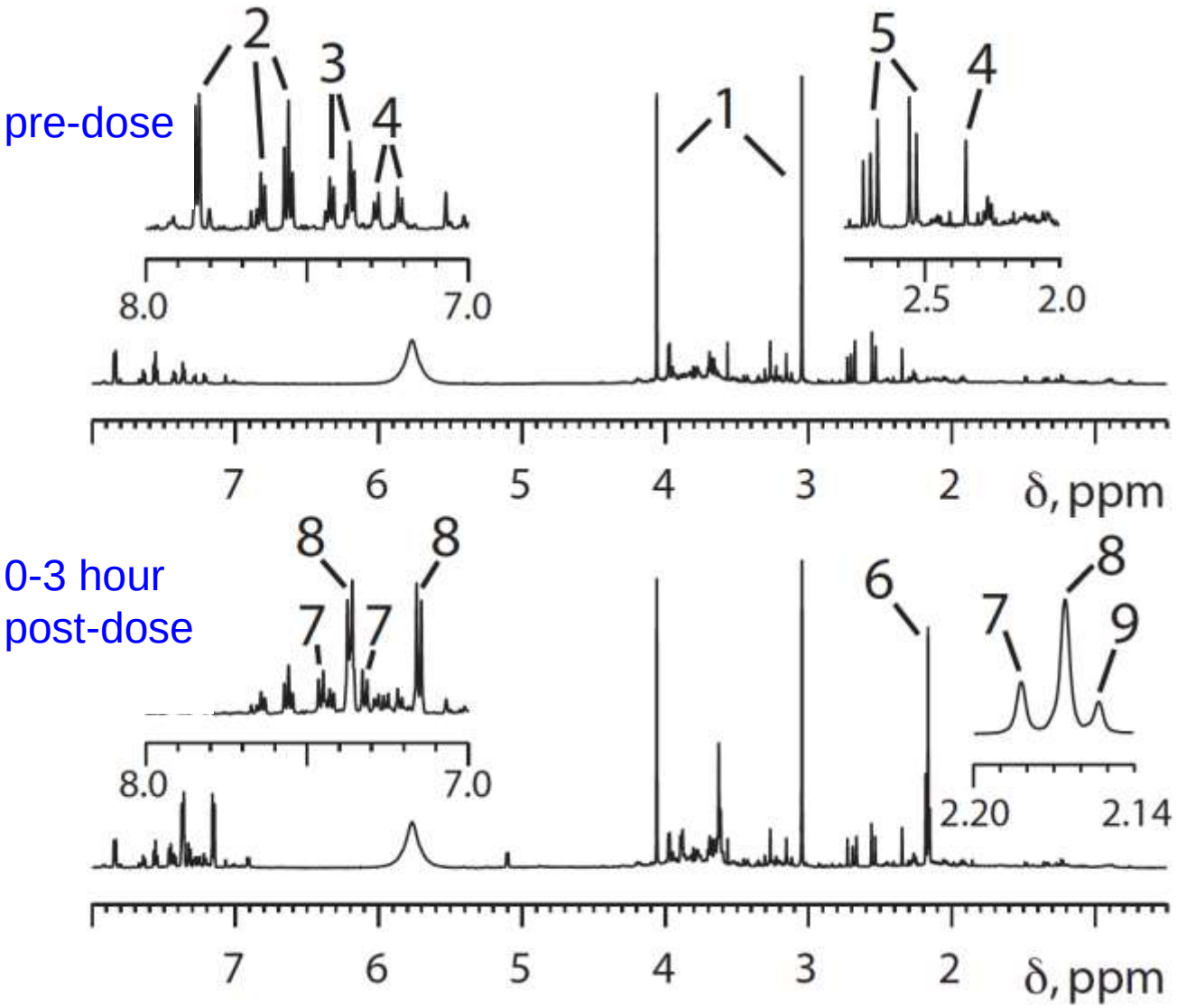
We provide a demonstration in humans of the principle of pharmacometabonomics by showing a clear connection between an individual's metabolic phenotype, in the form of a predose urinary metabolite profile, and the metabolic fate of a standard dose of the widely used analgesic acetaminophen. Predose and postdose urinary metabolite profiles were determined by ^{1}H NMR spectroscopy. The predose spectra were statistically analyzed in relation to drug metabolite excretion to detect predose biomarkers of drug fate and a human-gut microbiome cometabolite predictor was identified. Thus, we found that individuals having high predose urinary levels of *p*-cresol sulfate had low postdose urinary ratios of acetaminophen sulfate to acetaminophen glucuronide. We conclude that, in individuals with high bacterially mediated *p*-cresol generation, competitive O-sulfonation of *p*-cresol reduces the effective systemic capacity to sulfonate acetaminophen. Given that acetaminophen is such a widely used and seemingly well-understood drug, this finding provides a clear demonstration of the immense potential and power of the pharmacometabonomic approach. However, we expect many other sulfonation reactions to be similarly affected by competition with *p*-cresol and our finding also has important implications for certain diseases as well as for the variable responses induced by many different drugs and xenobiotics. We propose that assessing the effects of microbiome activity should be an integral part of pharmaceutical development

The only factors limiting which analytes are detected are the nature of the sample that is analyzed and the analytical platform used. Thus, pharmacometabonomic modeling need not be limited by prior understanding or hypothesis. However, despite much support and enthusiasm for the concept (9, 11–13), there has, until now, to the best of our knowledge, been no convincing pharmacometabonomic demonstration in humans.

To test the feasibility of applying the pharmacometabonomic approach to man, we chose as our example the well-known analgesic and antipyretic drug acetaminophen (*N*-acetyl-*p*-aminophenol; known as paracetamol in Europe). Acetaminophen is one of the most widely used nonprescription medicines in the world and its toxicology and metabolism have been extensively investigated over many years (14–20). However, we will show here that, even for this most familiar drug, pharmacometabonomic analysis will yield significantly increased understanding of its metabolic behavior in humans. These findings have considerable implications for personalized drug treatment in general and lead to new and testable hypotheses for a number of diseases.

Acetaminophen was chosen to exemplify the pharmacometabonomic principle for a variety of reasons, which included its common

^{1}H NMR spectra of pre- and post-dose urines from human volunteer taking paracetamol (1g, oral)



Key to numbered peaks:

1 creatinine

2 hippuric acid

3 phenacetylglutamine

4 ?

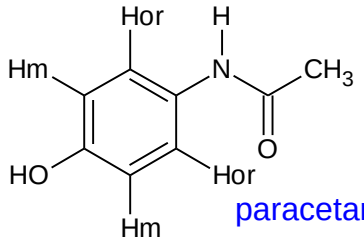
5 citrate

6 cluster N-acetyl groups from paracetamol-related compounds

7 paracetamol sulfate

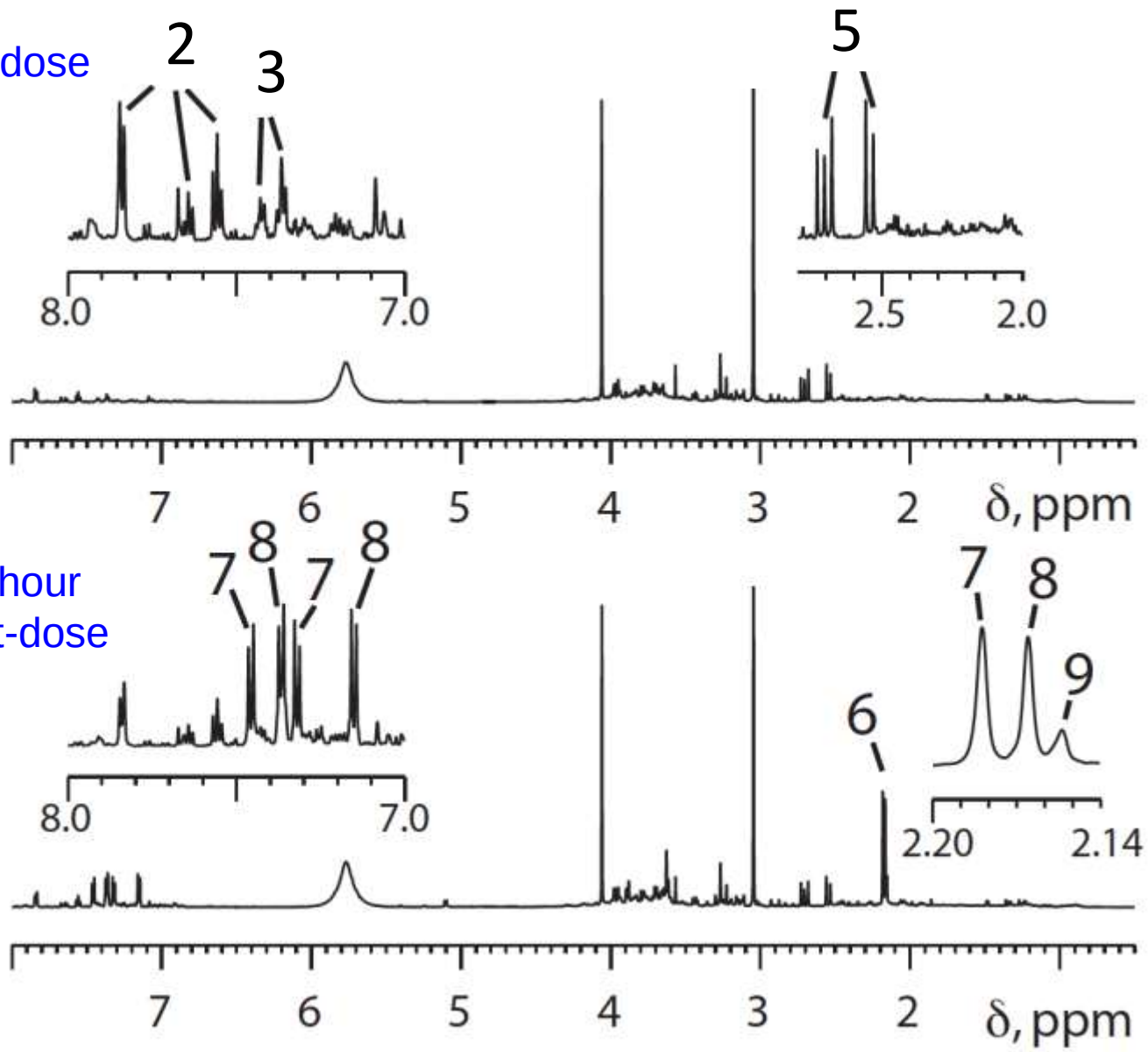
8 paracetamol glucuronide

9 other paracetamol –related compounds



paracetamol

^{1}H NMR spectra of pre- and post-dose urines from human volunteer taking paracetamol (1g, oral)



Key to numbered peaks:

1 creatinine

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3 phenacetylglutamine

4 ?

5 citrate

6 cluster N-acetyl groups from paracetamol - related compounds

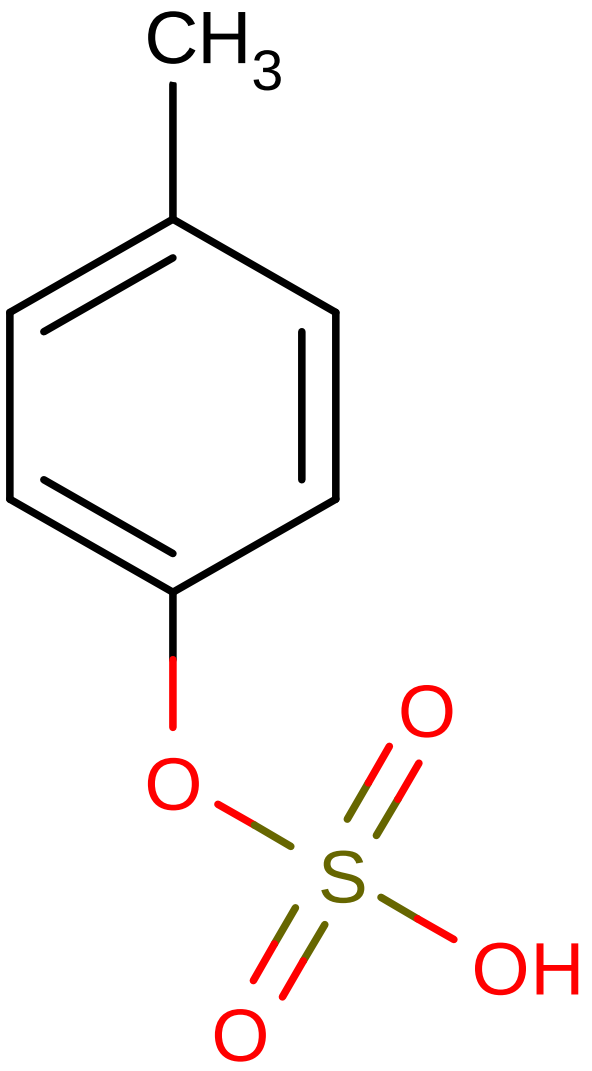
7 paracetamol sulfate

8 paracetamol glucuronide

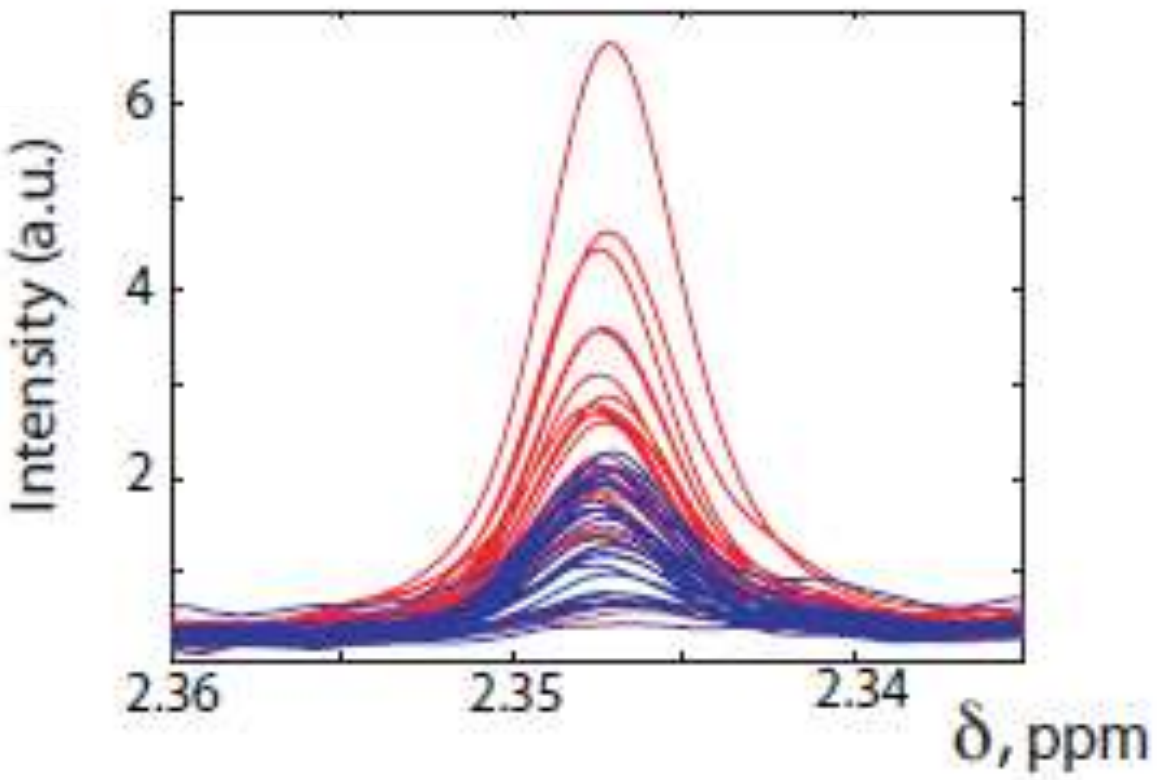
9 other paracetamol - related compounds

unknown 4

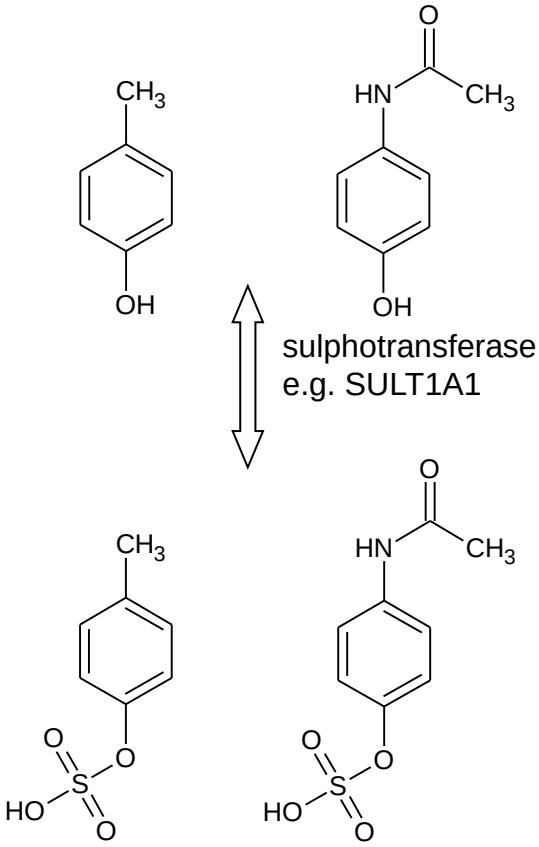
- methyl singlet at ca 2.35 ppm
  - probably CH3 – sp2C
- coupled aromatic doublets at ca 7.2 and 7.3 ppm
  - probably para disubstituted benzene ring
- isolated from urine and solved structure by NMR, MS and chemical synthesis
- para-cresol sulphate
  - not made by humans!



p-cresol sulphate H-1 NMR signals in pre-dose human urine



p-cresol paracetamol



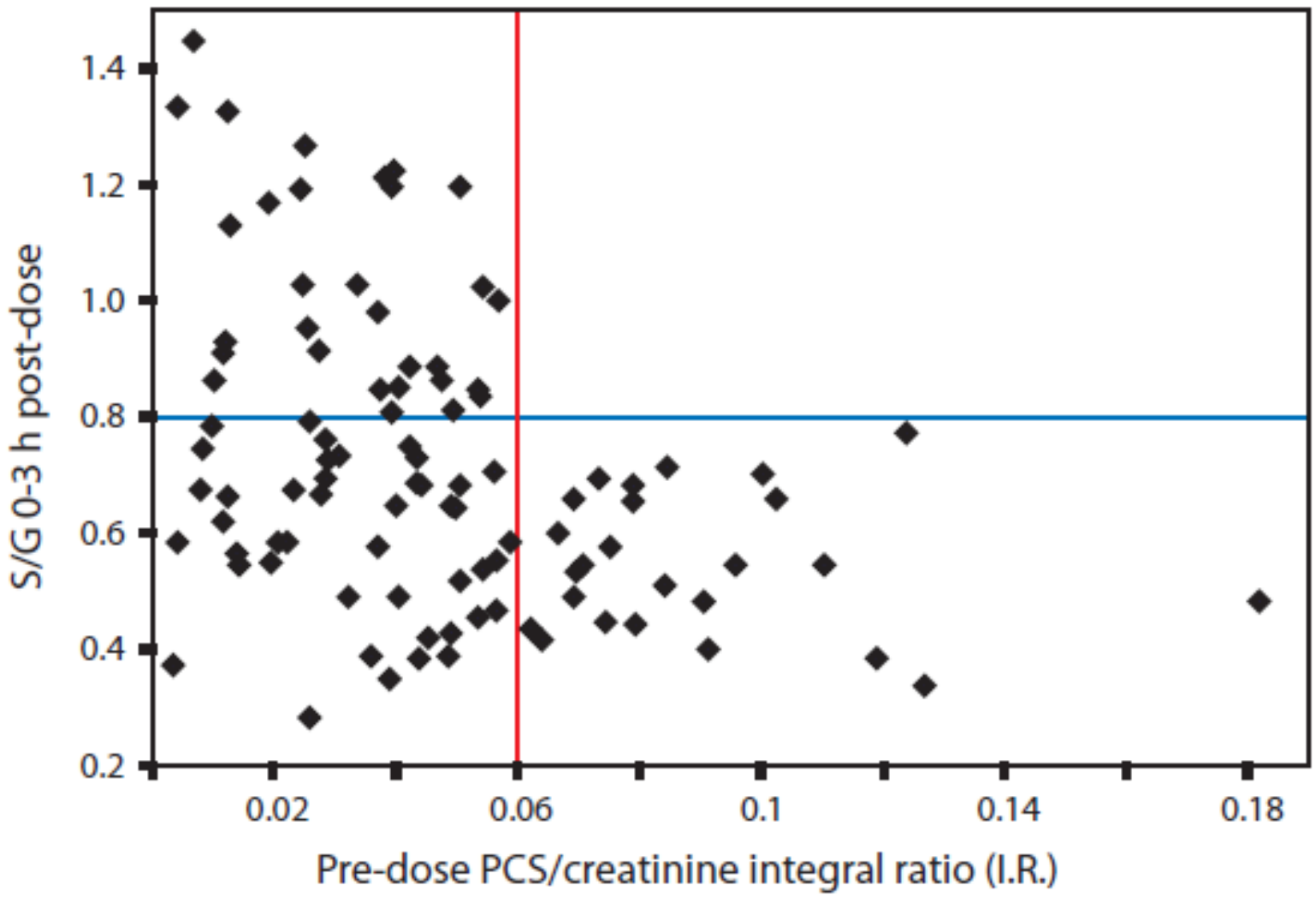
25 subjects with lowest S/G ratio in 0-3 hr post-dose urine

25 subjects with highest S/G ratio in 0-3 hr post-dose urine

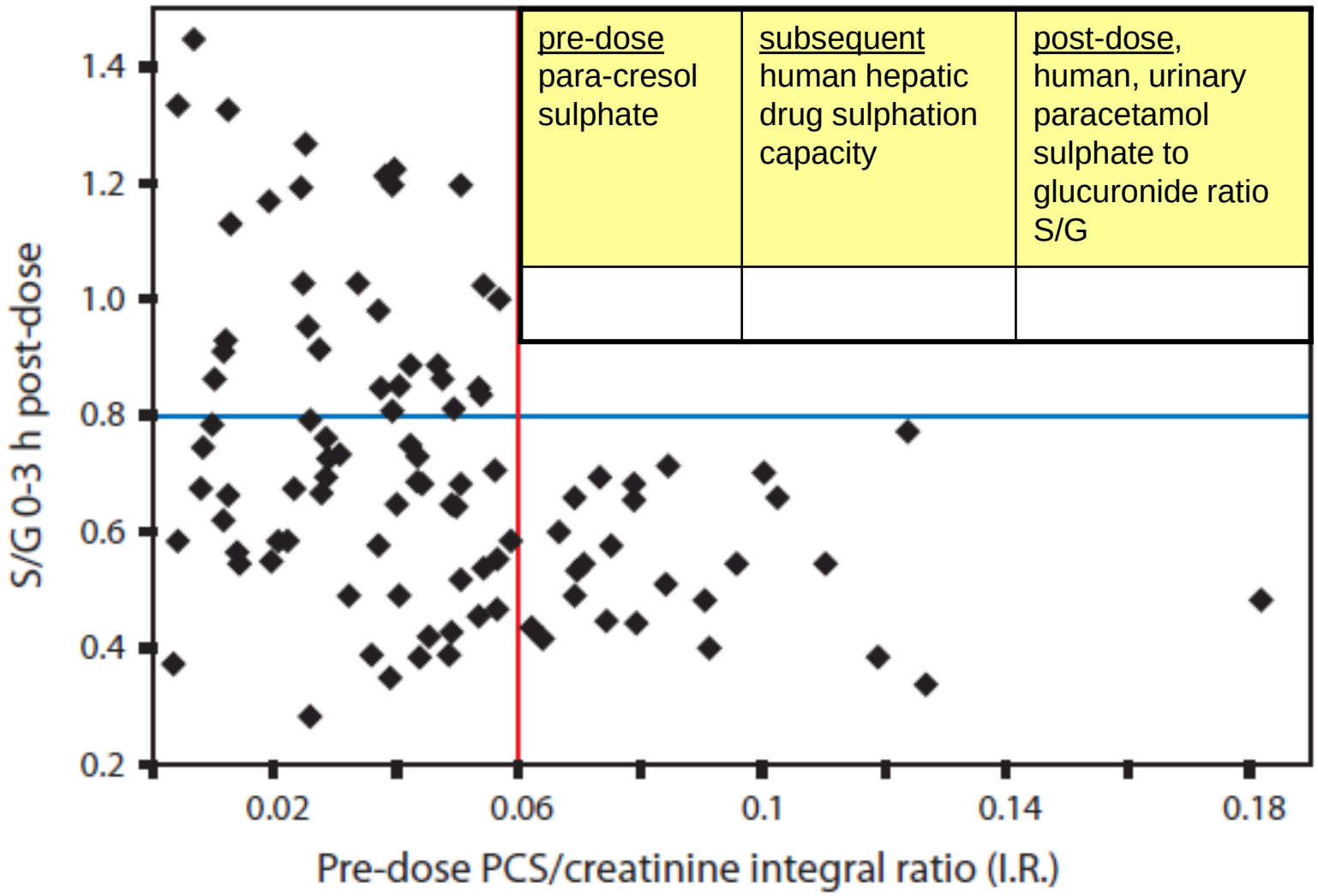
p-cresol sulphate

paracetamol sulphate (S)

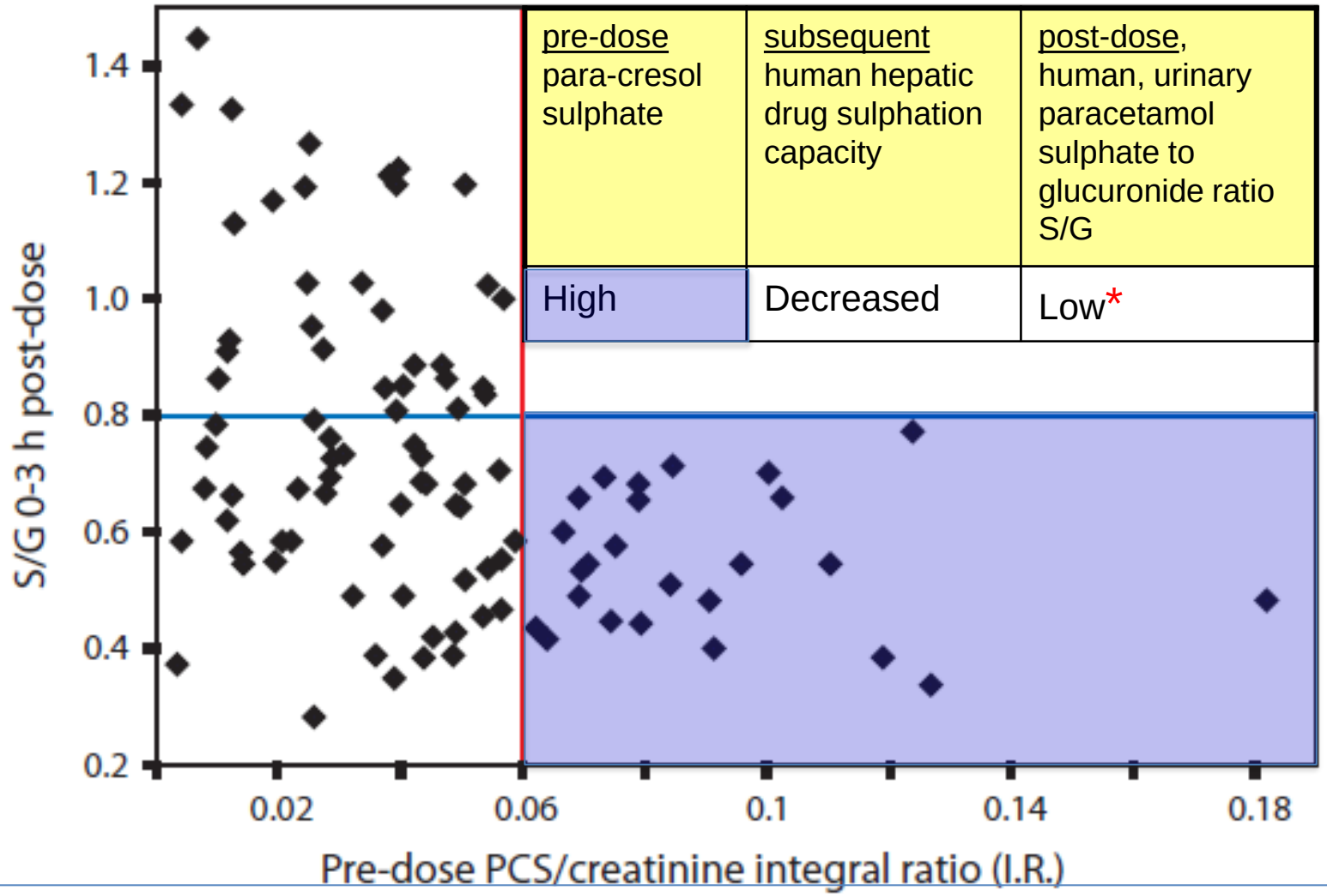
paracetamol S/G metabolite ratio in 0 – 3 hour post-dose urine related to pre-dose ratio of p-cresol sulphate to creatinine



paracetamol S/G metabolite ratio in 0 – 3 hour post-dose urine related to pre-dose level of p-cresol sulphate

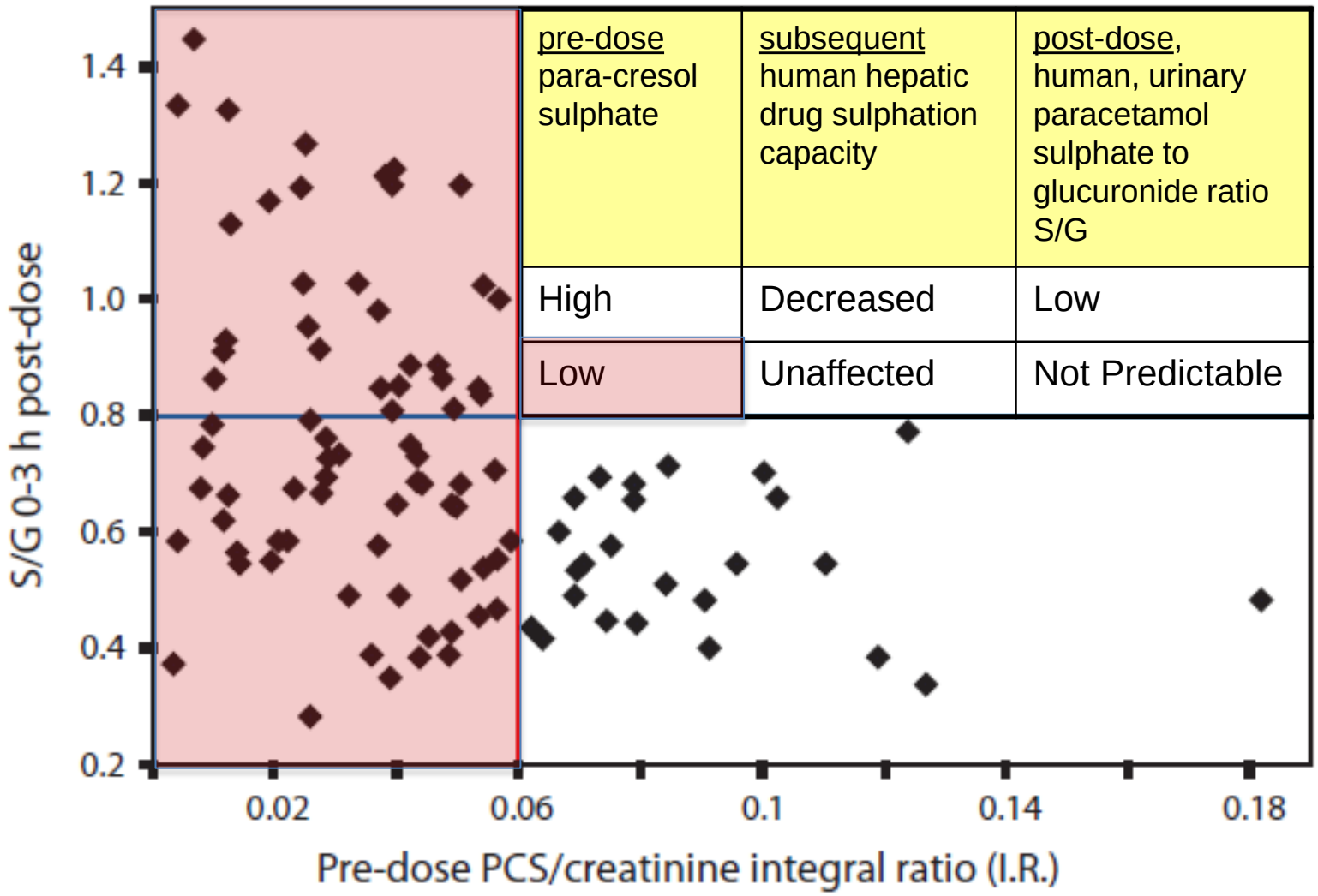


paracetamol S/G metabolite ratio in 0 – 3 hour post-dose urine related to pre-dose level of p-cresol sulphate



* P = 0.00010 in Mann–Whitney U test: in conjunction with Bonferroni correction (100), significant at 95% confidence

paracetamol S/G metabolite ratio in 0 – 3 hour post-dose urine related to pre-dose level of p-cresol sulphate



pharmaco-metabonomics: summary

- pharmaco-metabonomics demonstrated in animals and humans
  - first examples from our group further exemplified in 20 other publications from other groups
  - Everett et al, Ann. Clin. Biochem (2013)

- human metabolism of paracetamol – one of world's most prescribed and studied drugs is influenced by gut bacterial metabolite levels: radical new finding

- sulphation is a key metabolic process in the body in normal metabolism as well as drug metabolism: this finding could have wider implications

- study exemplifies concept of the super-organism and limitations of human genomics to fully explain human biology

- pharmaco-metabonomics will be complementary to pharmacogenomics in delivering the promise of personalised healthcare in future

potential uses of pharmaco-metabonomics: personalised medicine

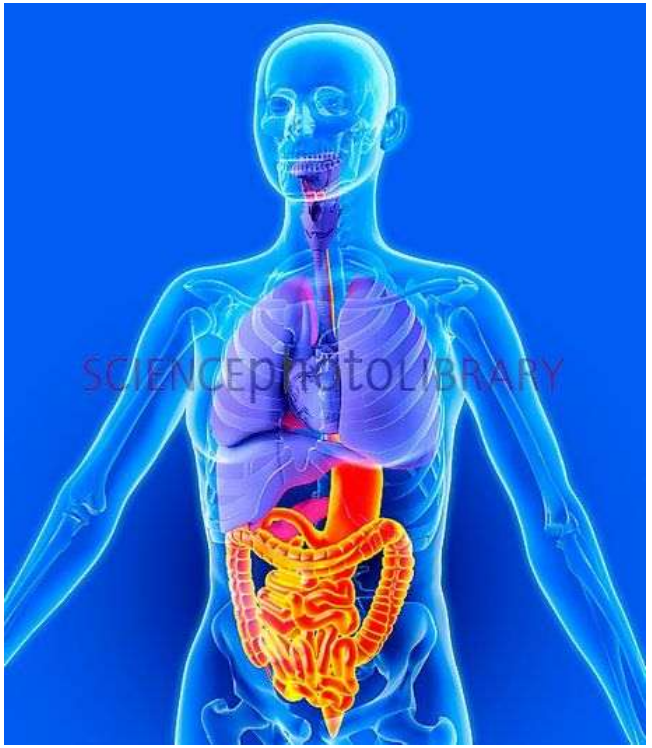
- patient selection to:
  - avoid toxicity in sensitive patients
  - improve drug efficacy (reduce/eliminate non-responders)
  - reduce variability and improve decision-making in Clinical Trials
- pharmacometabonomics directed pharmacogenomics (Rima Kaddurah-Daouk et al, Clin Pharmacol Ther.; 89: 97–104, 2011)

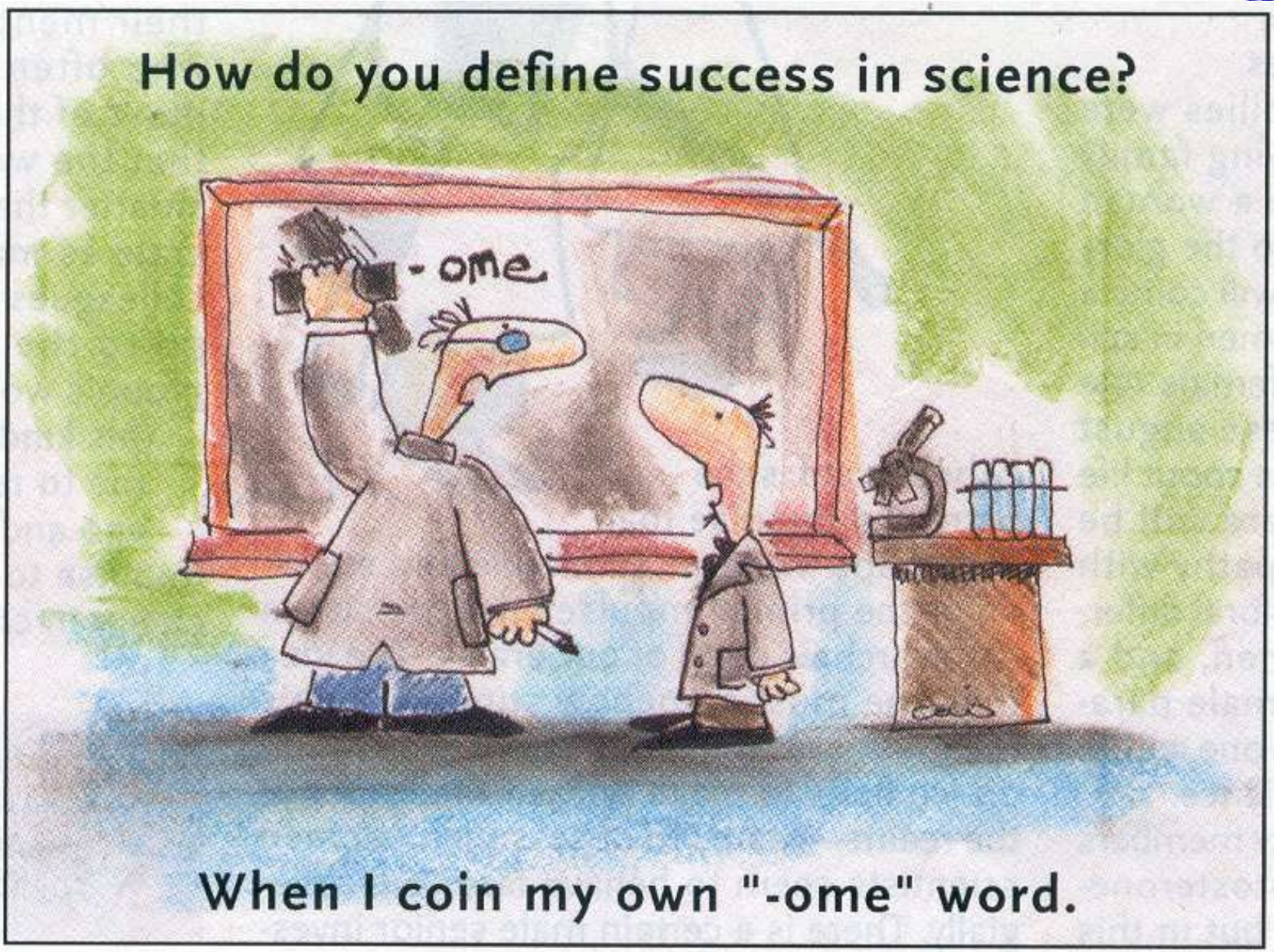
conclusions

- humans's are super-organisms whose health and response to medicines are influenced by
  - their **genome**
  - their environment, particularly their **microbiome**

- our genome is fixed but our microbiome changes with age, disease, nutrition, drugs, environment etc

- manipulation of the microbiome will play an important role in personalised medicine in the future (the right medicine to the right patient group)




How do you define success in science?
-ome
When I coin my own "-ome" word.

pharmacometabonomics

‘the prediction of the effect of a drug in an individual based on a mathematical model of pre-intervention metabolite signatures’.

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