Genómica nutricional
(interacciones genes-nutrientes)

Genes

Nutrigenética

Polimorfismo

Nutrigenómica

Expresión de genes

Nutrientes

Clinical Dietitian Routine
Case study

PSA, male, 7 years old

- Fever, abdominal cramps, steatorrhea, flatus, bloody stools, diarrhea, vomiting, lactose intolerance, abdominal cramps

Early exposition to cow milk (2 months of age); weight loss (8% in 2 weeks; anorexia)

- 21kg (p 50 = 23kg); 121 cm (p 50); Body mass index = 14.4kg/m^2 (p15 – p50)

Clinical Dietitian Routine
Case study

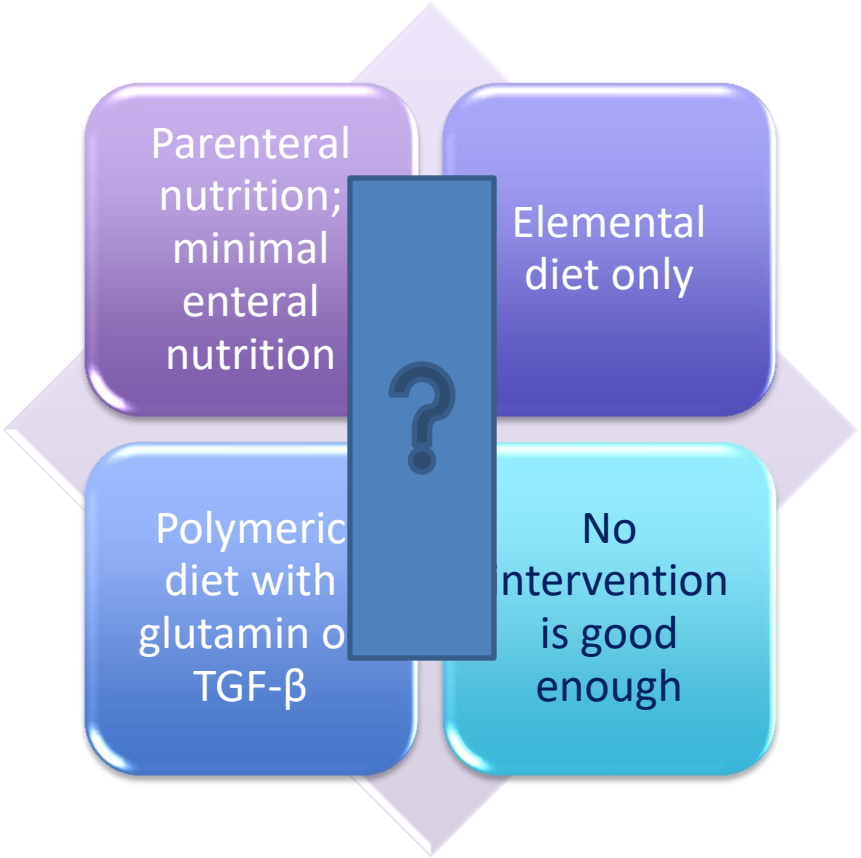
Leucocytes = 18000 cells/10^9; C-reactive protein = 33mg/dl; albumin = 2.7g/dl

- Acute Phase response
- Nutritional diagnosis: acute protein malnutrition

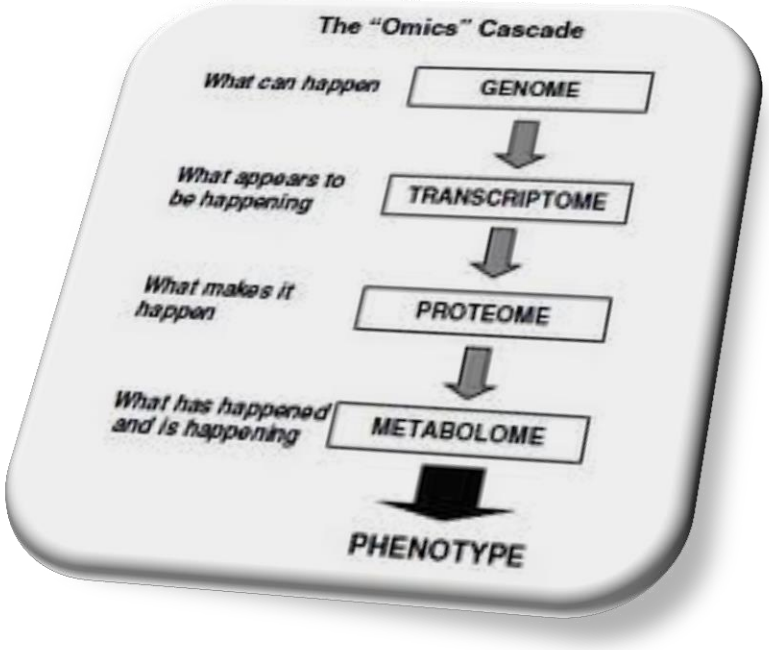
Malabsorption

- Lipid, electrolytes
- Medical Diagnosis: Crohn´s disease (disease activity index > 20)

Clinical Dietitian Routine
Clinical significance of intervention?



Systems Biology

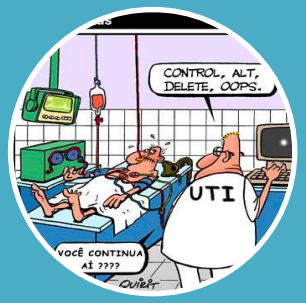


Variações das respostas às doenças

TIPO DE RESPOSTA METABÓLICA À INTERVENÇÃO NUTRICIONAL

Alguns respondem positivamente.

Alguns não respondem.



Alguns respondem negativamente.

Respondem devido às diferenças genéticas interagindo com o meio ambiente

Obesidade como exemplo...

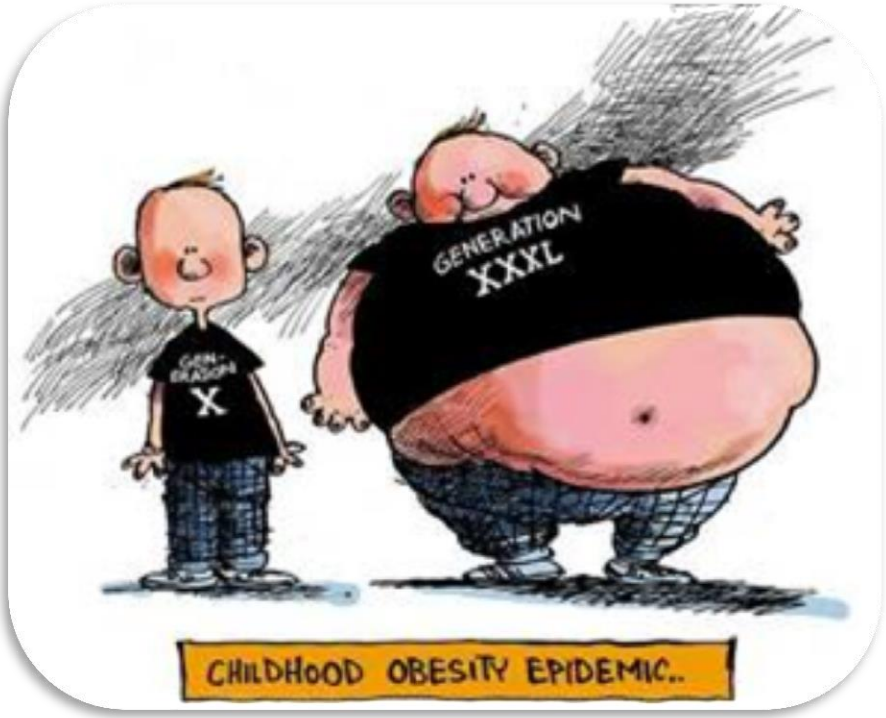
Uma dieta rica em lipídios não define que o indivíduo será obeso!...
Alguns estudos indicam que o consumo médio de alimentos está ↓ mas a obesidade está ↑...
Será que a obesidade não está necessariamente associada a alta ingestão alimentar? (Blundell JE & Cooling J, 1999)
A escolha por alimentos ricos em gordura é um fenômeno biológico ou do meio ambiente? E como as variações genéticas interferem neste processo?

Tabela 9 - Análise descritiva da ingestão energética e porcentagem de macronutrientes da dieta de adolescentes atendidos no CMSCVL e divididos por grupo, de acordo com o estado nutricional

Variáveis*	**Grupo 1**	**Grupo 2**	**Grupo 3**
Energia (Kcal)	2226 (1083-5092)	2199 (1294-3747)	2179 (1174-5489)
Proteínas(% VE**)	17,2 (10,7-24,1)	17,5 (13,3-30,9)	18 (11,1-26,6)
Carboidratos (% VE)	45,4 (25,5-59,9)	43,6 (27,6-52,2)	43,6 (34-51,7)
Lipídios (% VE)	36,6 (20,1-53)	38,3 (31-48,3)	38,2 (26,7-47,8)

* p > 0,05; **VE = Valor Energético

Medicina (Ribeirão Preto) 2011;44(3): 257-65



Respostas variadas ao tratamento

Alguns estudos não encontram associação entre ingestão alimentar e obesidade

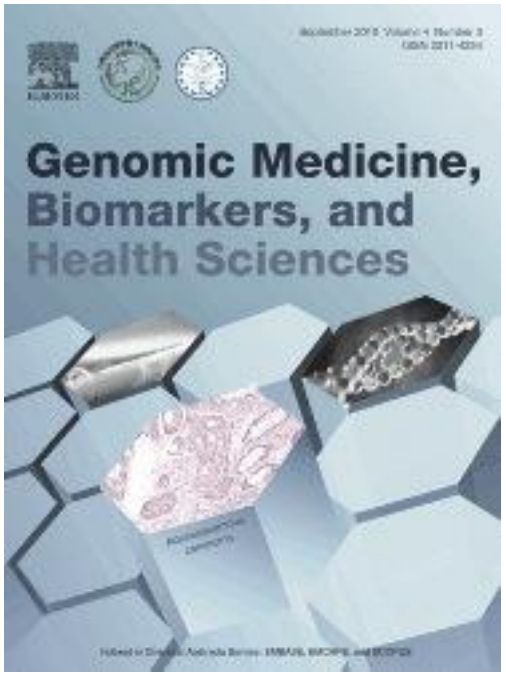
Diferentes necessidades nutricionais?

Qual a contribuição das variações genéticas?

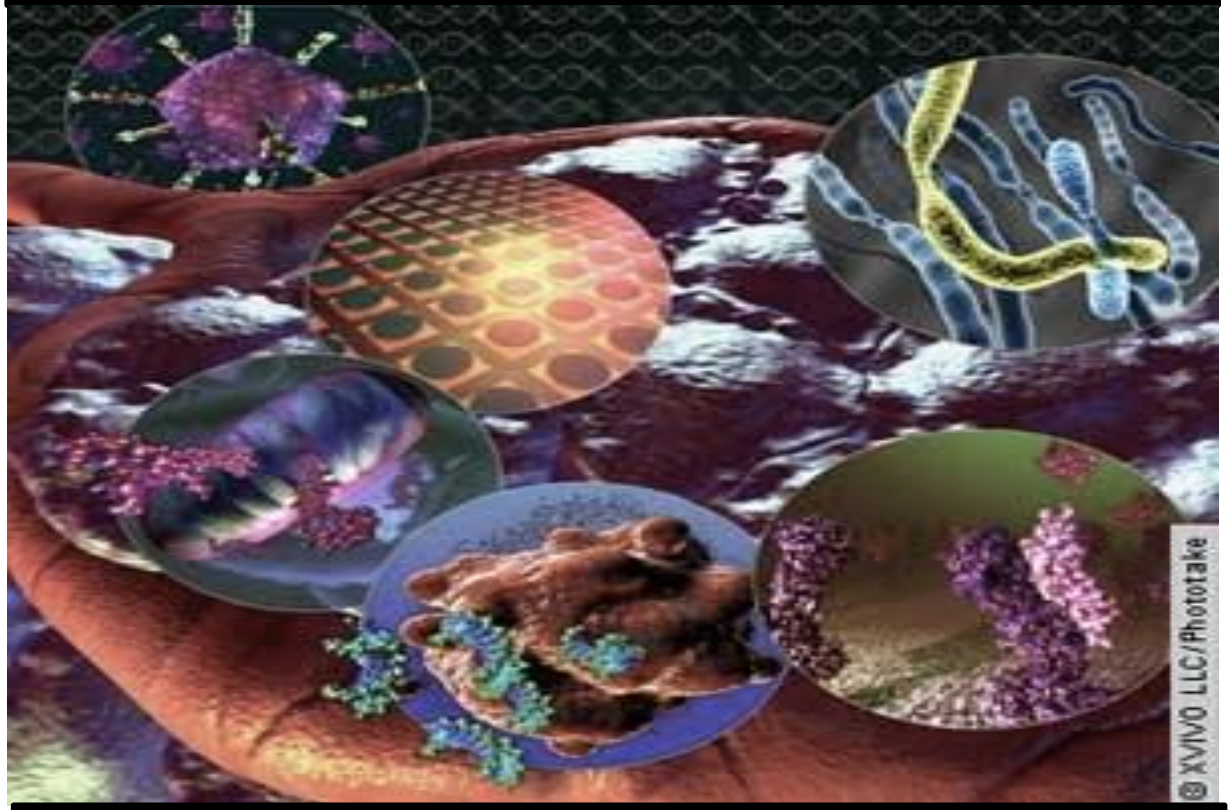
Interação gene-nutriente?

Bezerra IN & Sichieri R. 2011
Int J Behavioral Nut and Physical Activity

WIKIPEDIA
The Free Encyclopedia



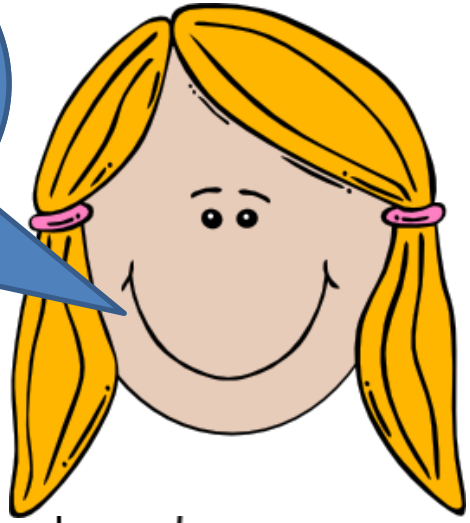
A **biomarker**, or **biological marker**, generally refers to a measured characteristic which may be used as an indicator of some biological state or condition.

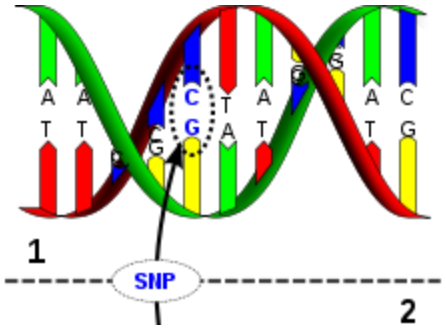


A **biomarker** can detect early stage of the development of a disease or indicate risk of a future disease.

Tendências da pesquisa sobre nutrição personalizada

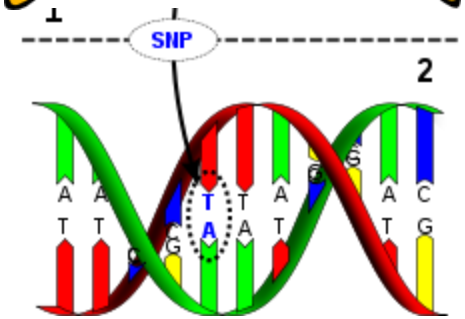
Se o nosso genoma é o mesmo porquê os cientistas estão dizendo que temos diferenças genéticas? Se isso é verdade então podemos ter diferentes necessidades nutricionais!!!!





Conceito alimento bom

Conceito alimento ruim



Rideout TC. 2011; Lovegrove JA & Gitau R. 2008

GENÔMICA DA NUTRIÇÃO NÃO É SIMPLES E ESTAMOS APENAS ENGATINHANDO...

Se você tem um polimorfismo na APOA1 do tipo GG você só terá ↑ HDL se PUFA < 4% VCT; agora se você tem polimorfismo do tipo GA, seu HDL vai ↑ quando PUFA > 8%

Doutora, porquê sigo tudo direitinho e meu LDL continua alto e meu HDL continua baixo?

Talvez não seja tão simples assim...

ESTUDAR UMA VARIAÇÃO GENÉTICA AJUDA?

Dieta pobre em lipídios e colesterol é boa para todos?

- Depende do polimorfismo da APOE

AJUDA MAS NÃO É SUFICIENTE PARA TRADUZIR A ADEQUADA INFORMAÇÃO PARA O PACIENTE…

APOE3/E3

APOE4/E4

APOE4/E3

Uusitupa MIJ et al.1992

Common genetic determinants of vitamin D insufficiency: a genome-wide association study

Thomas J. Wang e colaboradores

Lancet. 2010 July 17

Table 3

Genetic variants and risk of vitamin D insufficiency

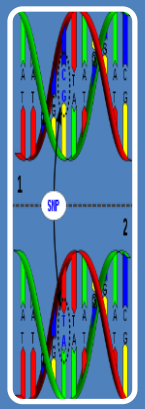
	< 75 nmol/L		< 50 nmol/L	
	Odds ratio*	*P*-value	Odds ratio*	*P*-value
Individual variants				
GC (rs2282679)	1.63 (1.53-1.73)	3.5×10^{-50}	1.49 (1.40-1.59)	7.5×10^{-33}
DHCR7 (rs7944926)	1.21 (1.14-1.29)	4.1×10^{-10}	1.21 (1.14-1.29)	4.7×10^{-09}
CYP2R1 (rs10741657)	1.21 (1.45-1.29)	9.4×10^{-11}	1.06 (1.00-1.13)	0.06
Genotype score				
Quartile 1	1.0 (Referent)		1.0 (Referent)	
Quartile 2	1.29 (1.15-1.46)		1.10 (0.97-1.25)	
Quartile 3	1.56 (1.39-1.75)		1.38 (1.22-1.57)	
Quartile 4	2.47 (2.20-2.78)		1.92 (1.70-2.16)	
P-for-trend	2.3×10^{-48}		1.0×10^{-26}	

AJUDA MAS NÃO É SUFICIENTE PARA TRADUZIR A ADEQUADA INFORMAÇÃO PARA O PACIENTE...

Gene da Glutationa Peroxidase-1 (GPx1)

37 obesas mórbidas

1 castanha durante 8 semanas (290µg de selênio por dia)



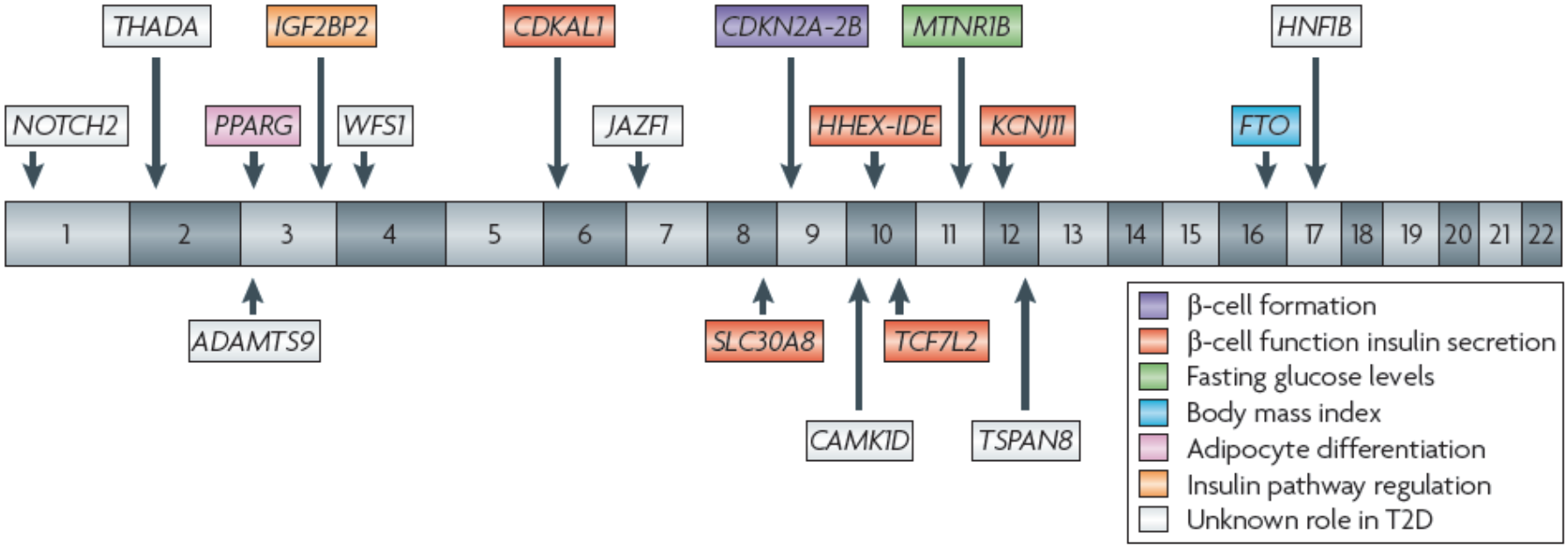
Melhora do estado nutricional relativo ao selênio

- Diferentes respostas de acordo com o polimorfismo Pro198Leu (Pro/Pro; Pro/Leu; Leu/Leu)

AJUDA MAS NÃO É SUFICIENTE PARA TRADUZIR A ADEQUADA INFORMAÇÃO PARA O PACIENTE...

Cominetti C et al. 2011 Nutrition

T2DM Genes (GWAS)

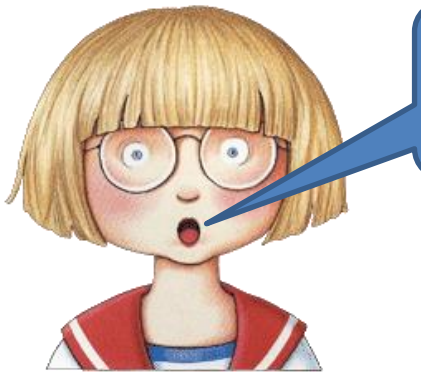


Tag SNP for a LD bin *statistically associated* with trait

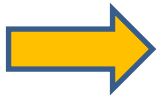
Collectively "explains" ~0.5% - 2.4% of individual's risk

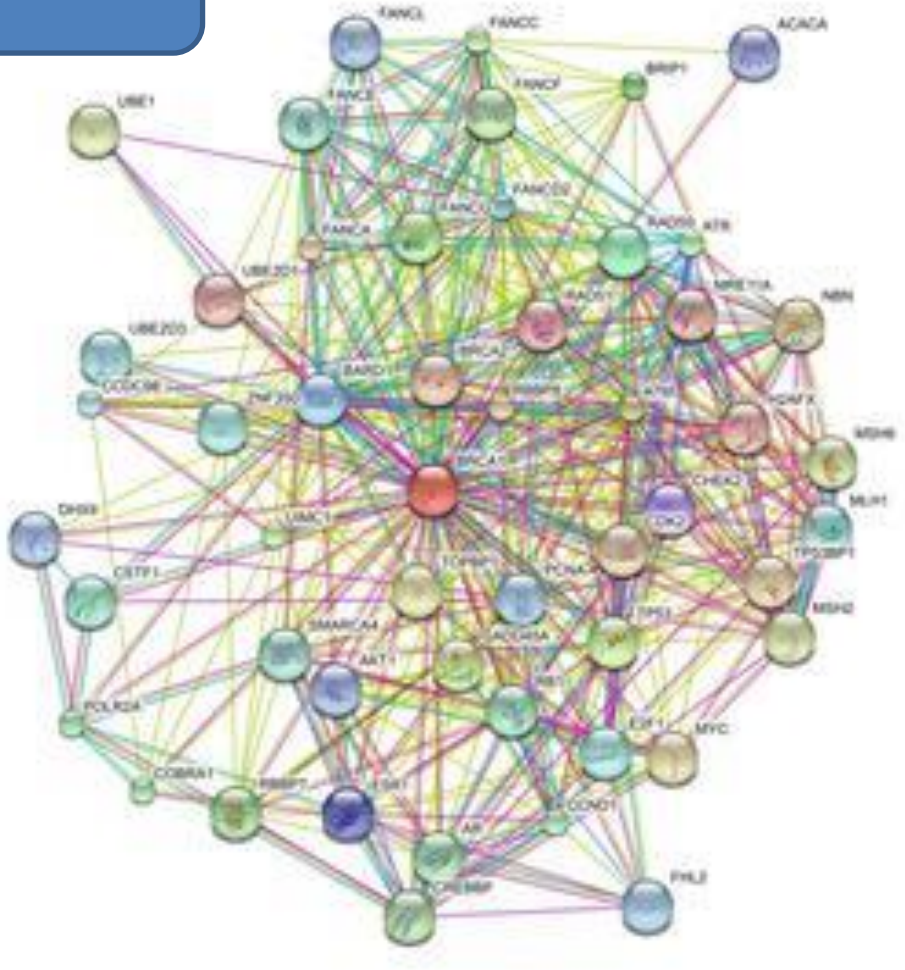
~96% of participants were Europeans

1 interação gene-nutriente no contexto de Sistemas Biológicos?



Complexa e delicada rede de interações que desafia a simplicidade e torna a orientação nutricional um desafio





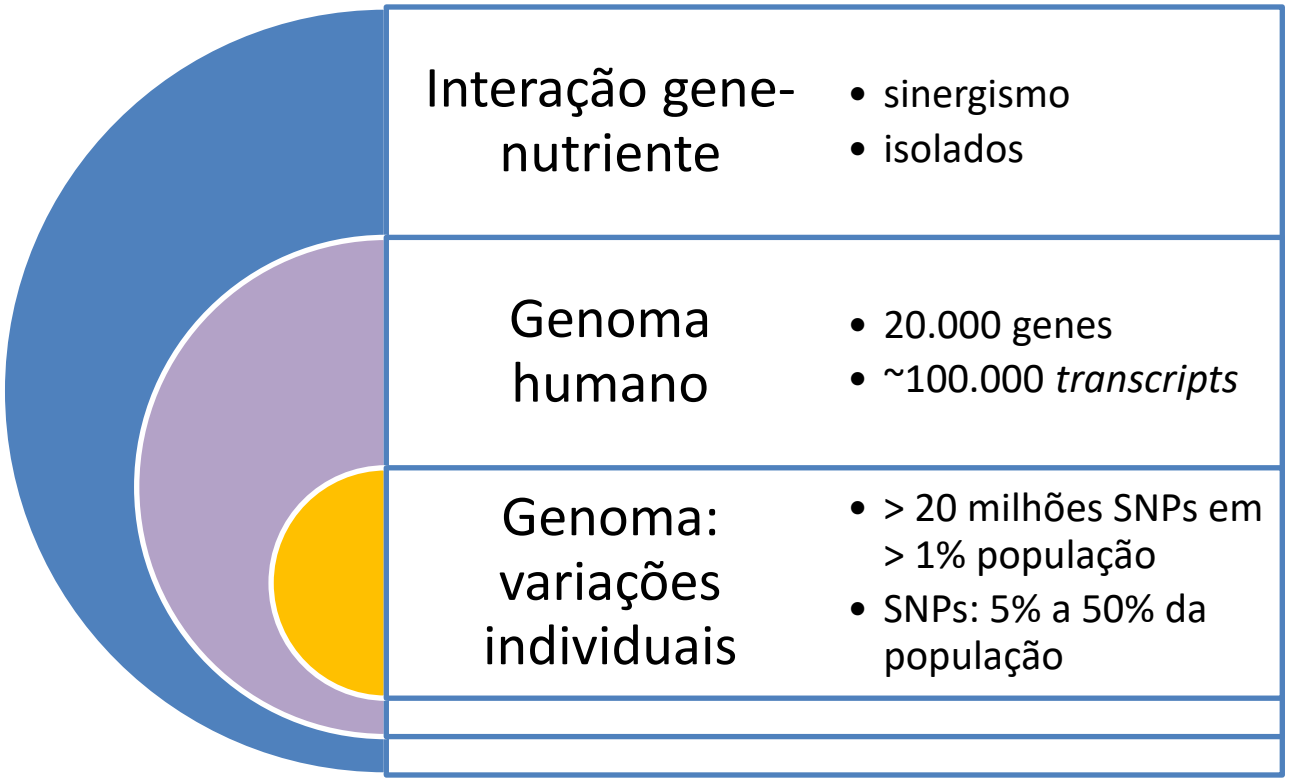
Many nutrient-nutrient interactions exist and are fundamental part of the growth and development process

Nutritional Systems

Fe

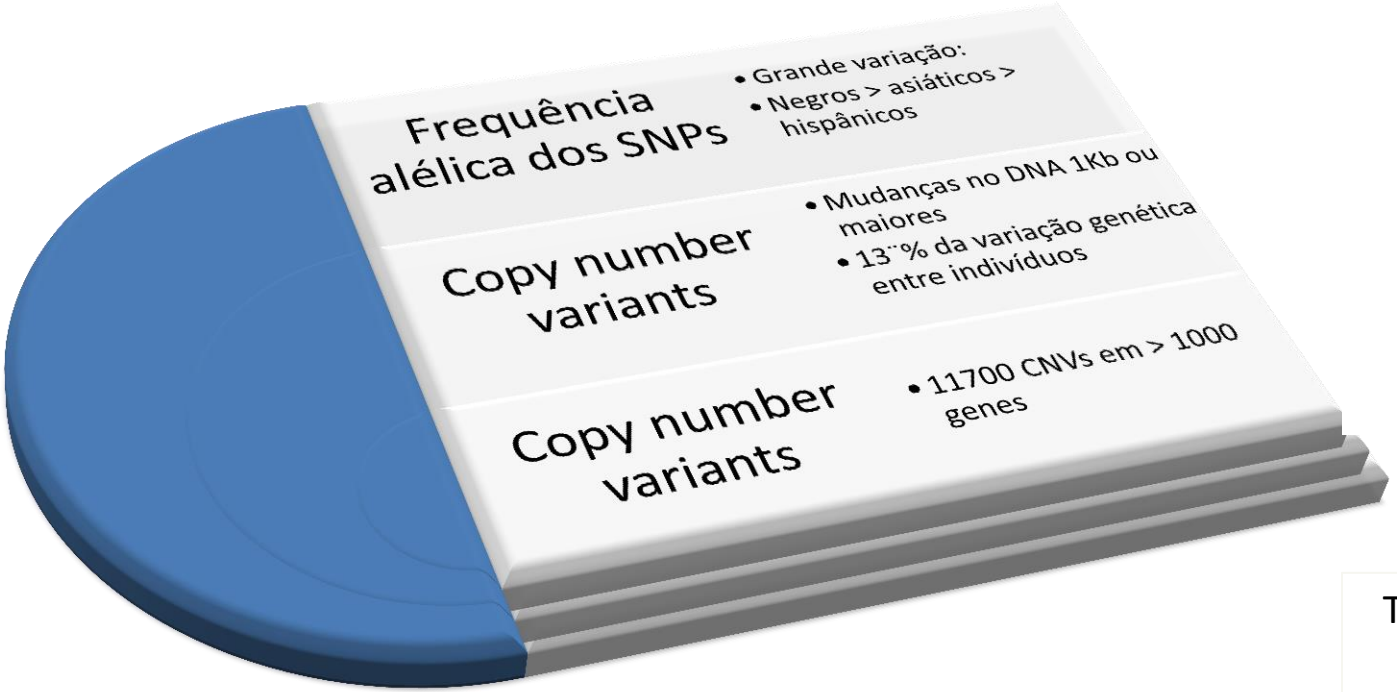
Focusing on a single nutritional cause obscured the broader nutritional phenotype that exists in a person

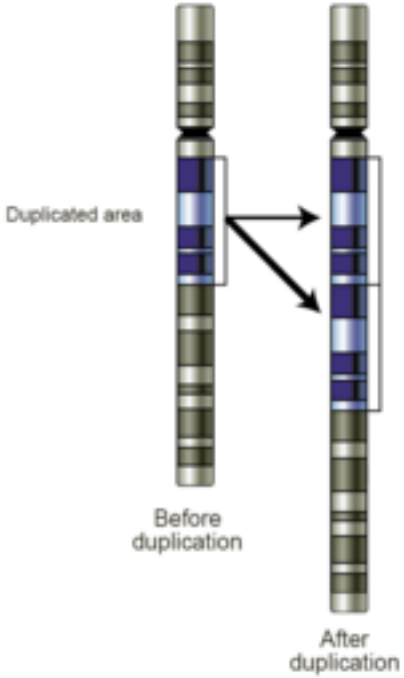
Darby 1966; Kaput et al. 2014



McVean G et al. 2005

Personalized Nutrition Research in the Era of Nutrigenomic

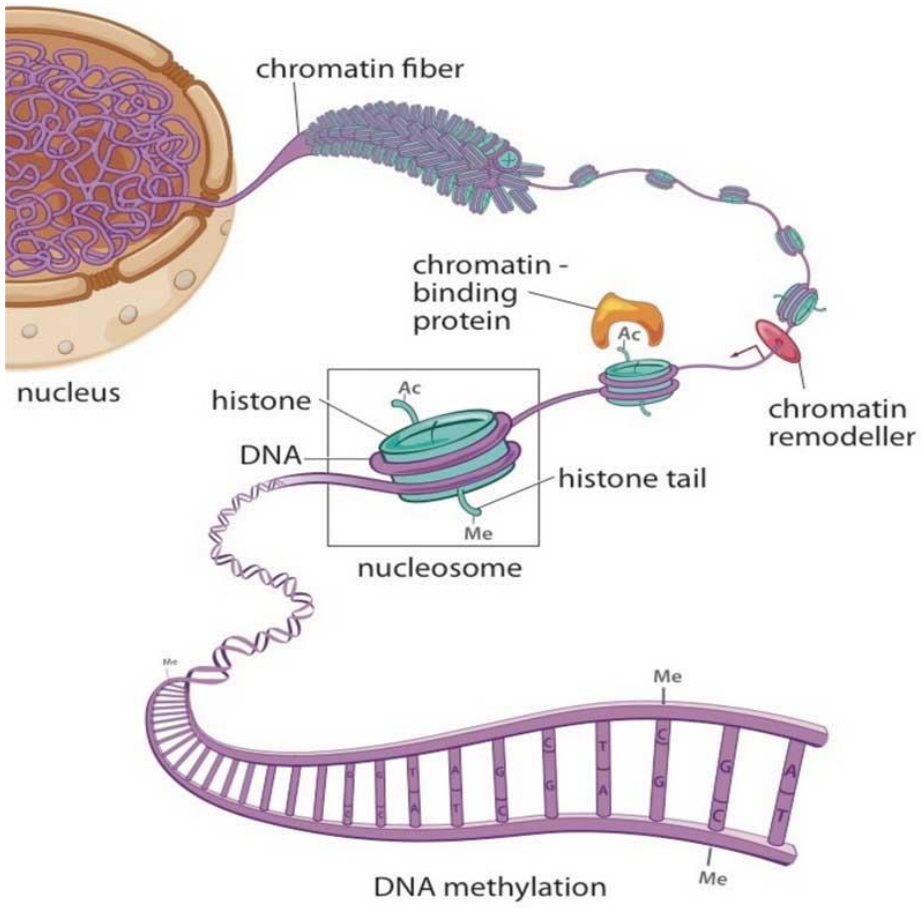




This gene duplication has created a copy-number variation. The chromosome now has two copies of this section of DNA, rather than one.

Varma V et al. 2010; Myles S et al. 2008
Simopoulus AP. 2010

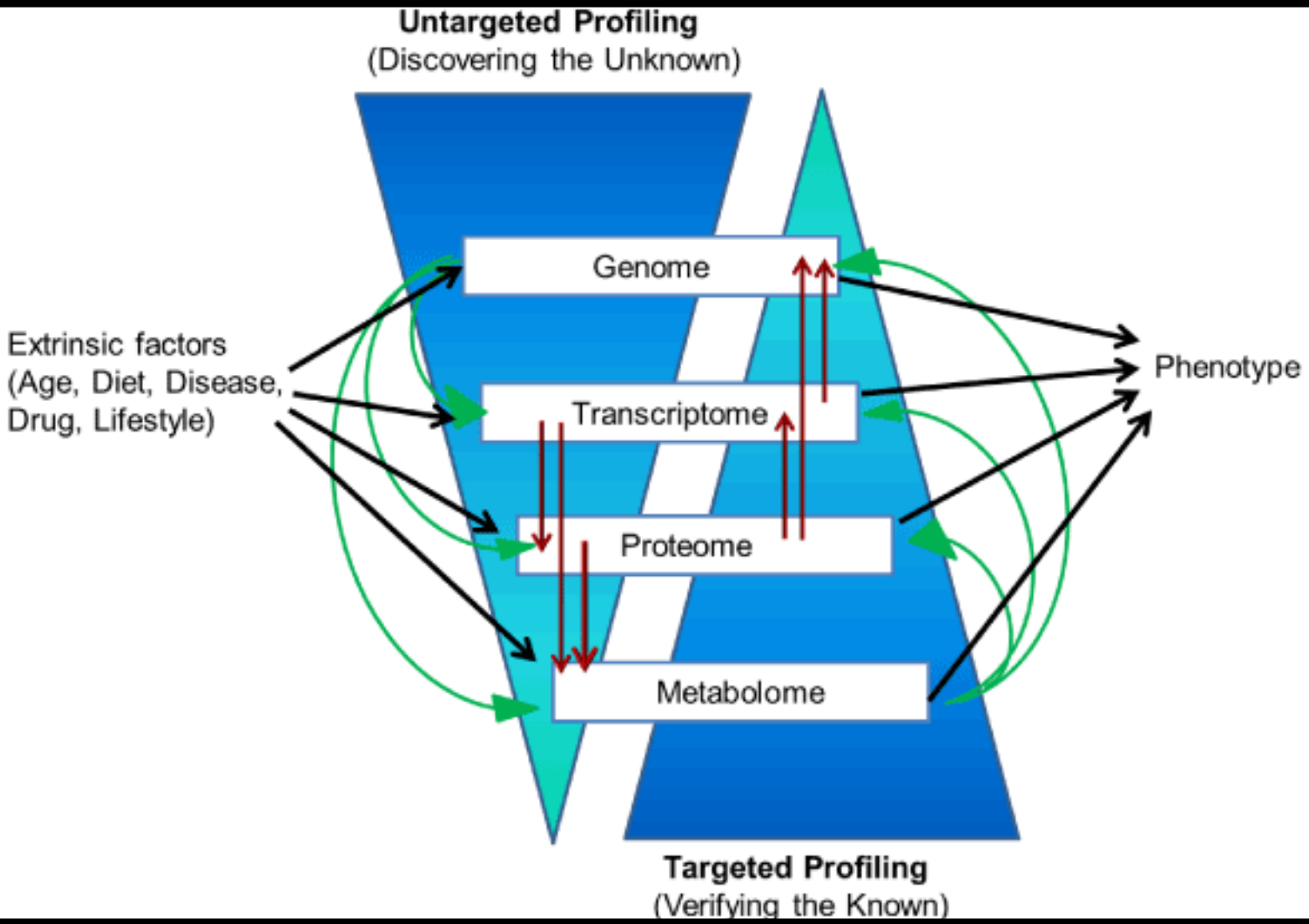
Metilação de DNA



Nutriente altera a estrutura do DNA mas não a sequencia de nucleotídeos

Zeisel SH. 2007

Ciências ômicas se referem a categorias de biomarcadores moleculares sendo estudados, mensurados ou descritos.



Fenótipo metabólico é o termo para um conjunto de metabólitos cuja abundância em fluídos biológicos reflete o estado de saúde/doença de um indivíduo. É preciso construir e validar ferramentas que forneçam mais informações sobre os processos que integram

Compostos bioativos são constituintes "extra-nutricionais" que ocorrem em pequenas quantidades nos alimentos.

Technologies All models – SYSTEMS BIOLOGY

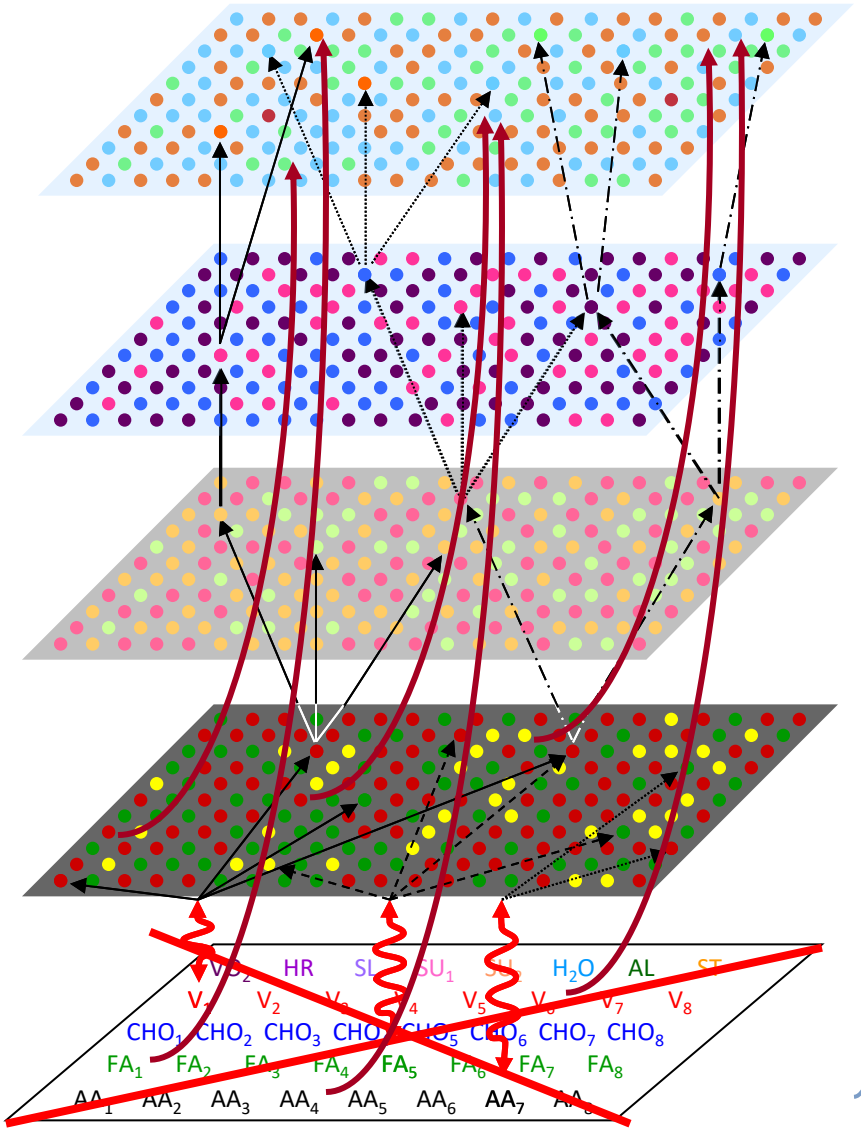
Clinical & Metabolomics

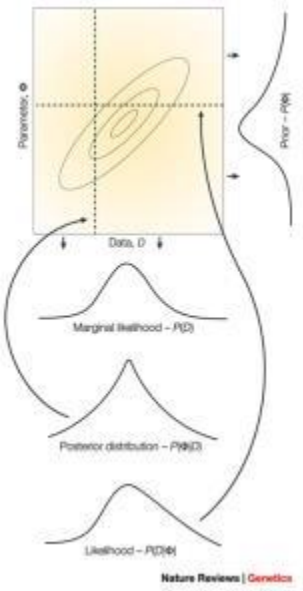
Proteomics

Transcriptomics

Genomics & Epigenomics

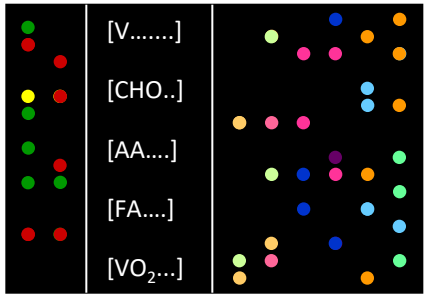
Diet & Lifestyle





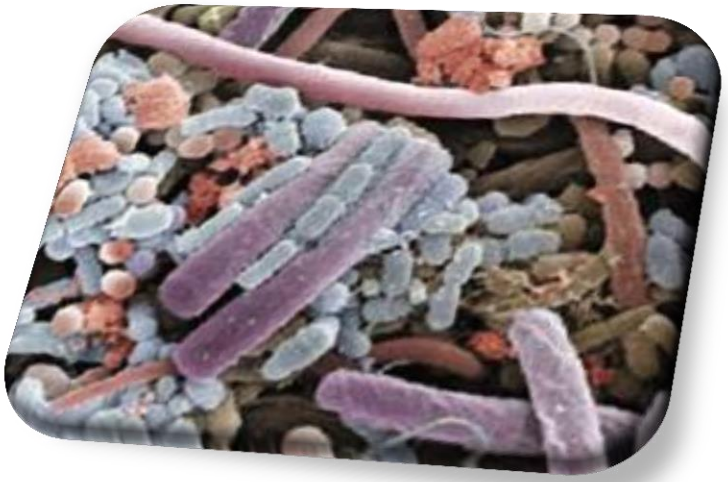
NRG 5:251 (2004)

$\int\Sigma$ (GxE) | Group



DESAFIOS AO ANALISAR AS INTERAÇÕES GENE-NUTRIENTE

Heterogeneidade genética e as interações gene-gene

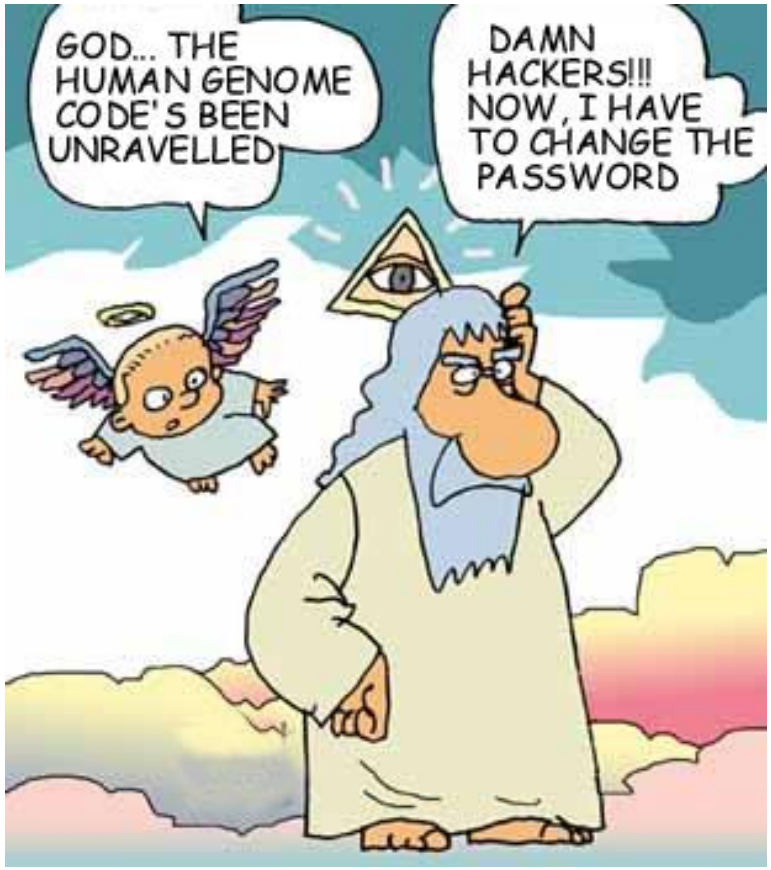


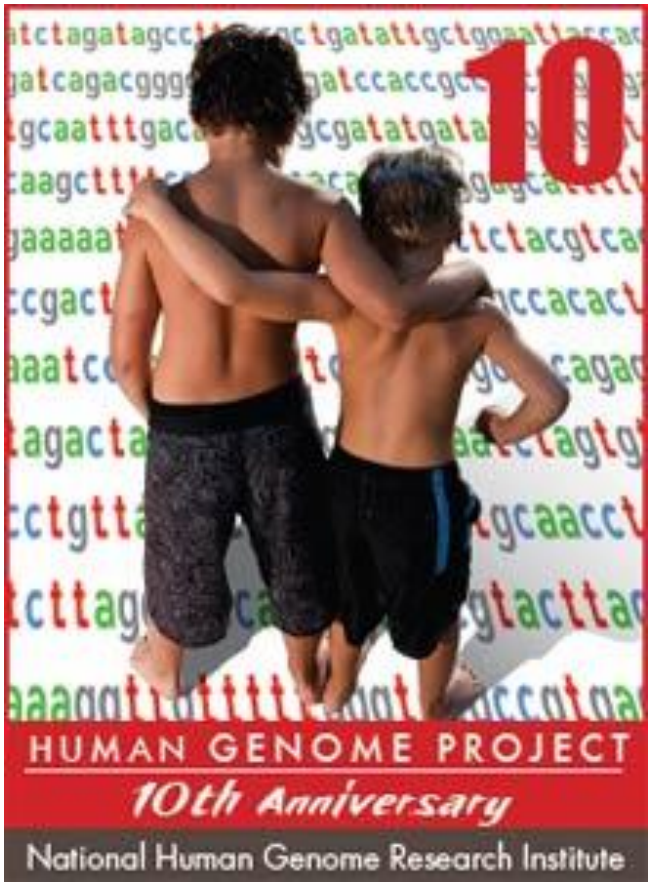
Microbiota

Diferenças nas

Meio ambiente: Diferenças nos Padrões Dietéticos e Interação

Approximately 228,000 human genomes have been sequenced by the start of 2015 and estimates are that 1.6 million genomes were sequenced in 2017



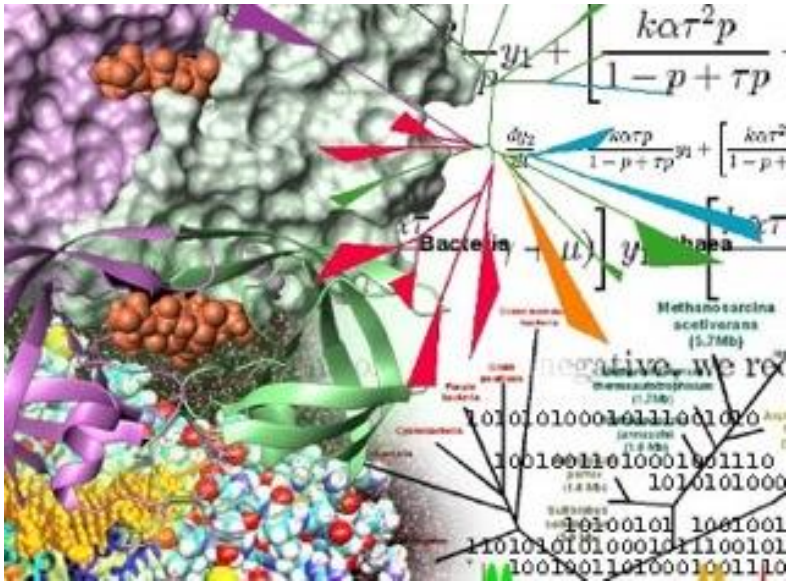


Each new genome sequence confirms that individuals are genetically unique and hence will have unique responses to environmental factors including diet, lifestyle, and medicines

Regalado 2014; Olson 2012

Algorítmo e Sistemas Biológicos

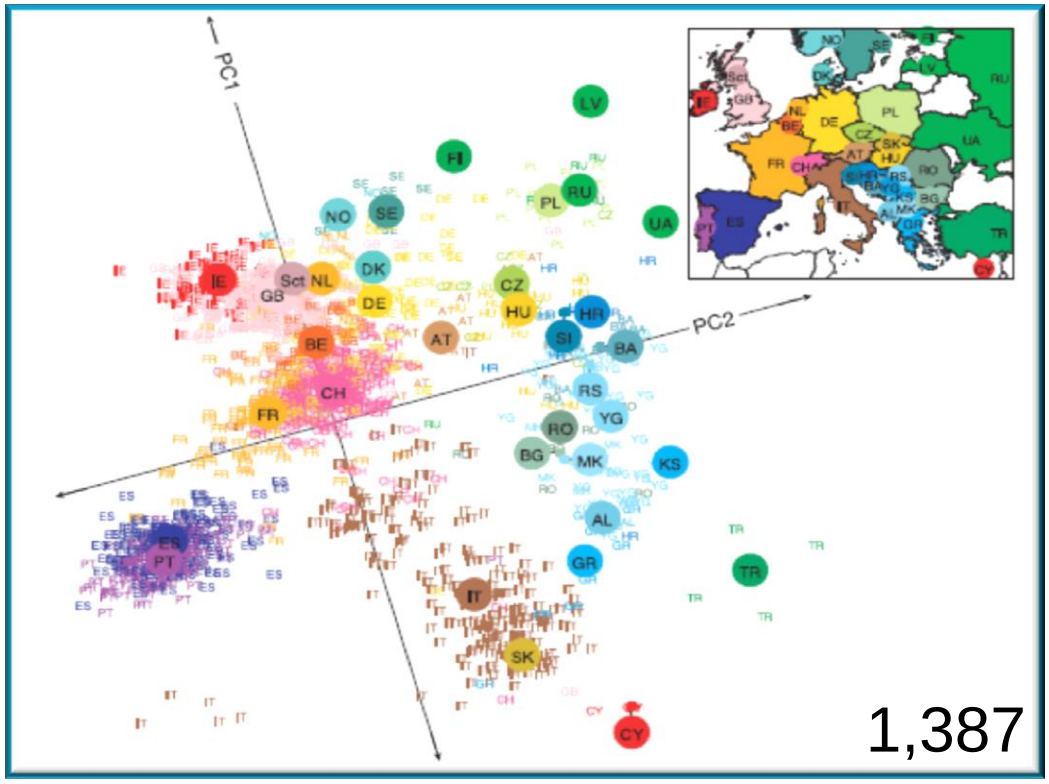
Permite identificar padrões, agrupar genes relacionados que regulam as vias metabólicas e agrupar vias metabólicas relacionadas

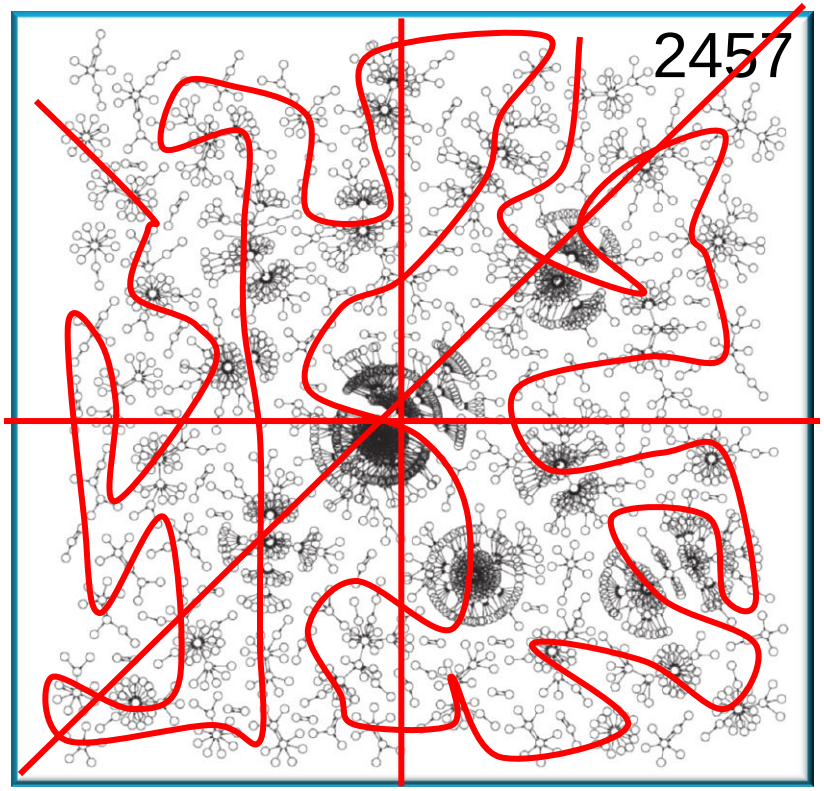


(1) Baseiam-se em uma codificação do conjunto das soluções possíveis;
(2) os resultados são apresentados como uma população de soluções e não como uma solução única;
(3) não necessitam de nenhum conhecimento derivado do problema, apenas de uma forma de avaliação do resultado;
(4) usam transições probabilísticas e não regras determinísticas

Em genômica da Nutrição é difícil definir um grupo controle

Facts & Challenges Genetic Diversity



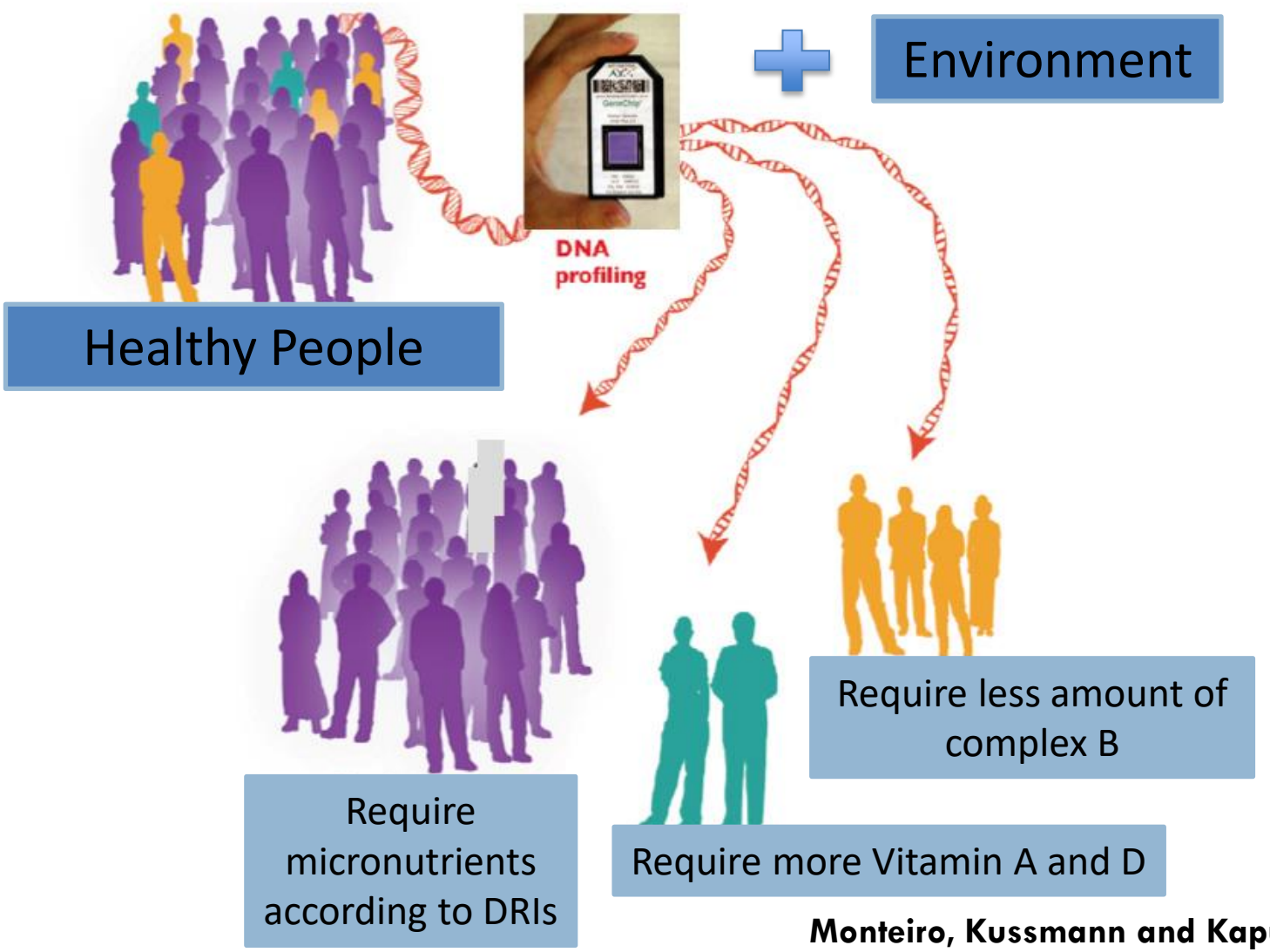


Novembre et al *Nature* 456, 98 (2008)

Lu et al *EJHG* 17, 967 (2009)

HOW TO SEPARATE CASES AND CONTROLS?

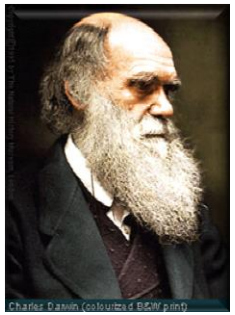
The conceptual basis of nutritional genomics is inter-individual variability in nutrient requirements



Monteiro, Kussmann and Kaput 2015

Basis of Nutrigenomics

A different effect of a *genotype* on disease in persons with different *environmental* exposures



Genotype X Environment Interactions

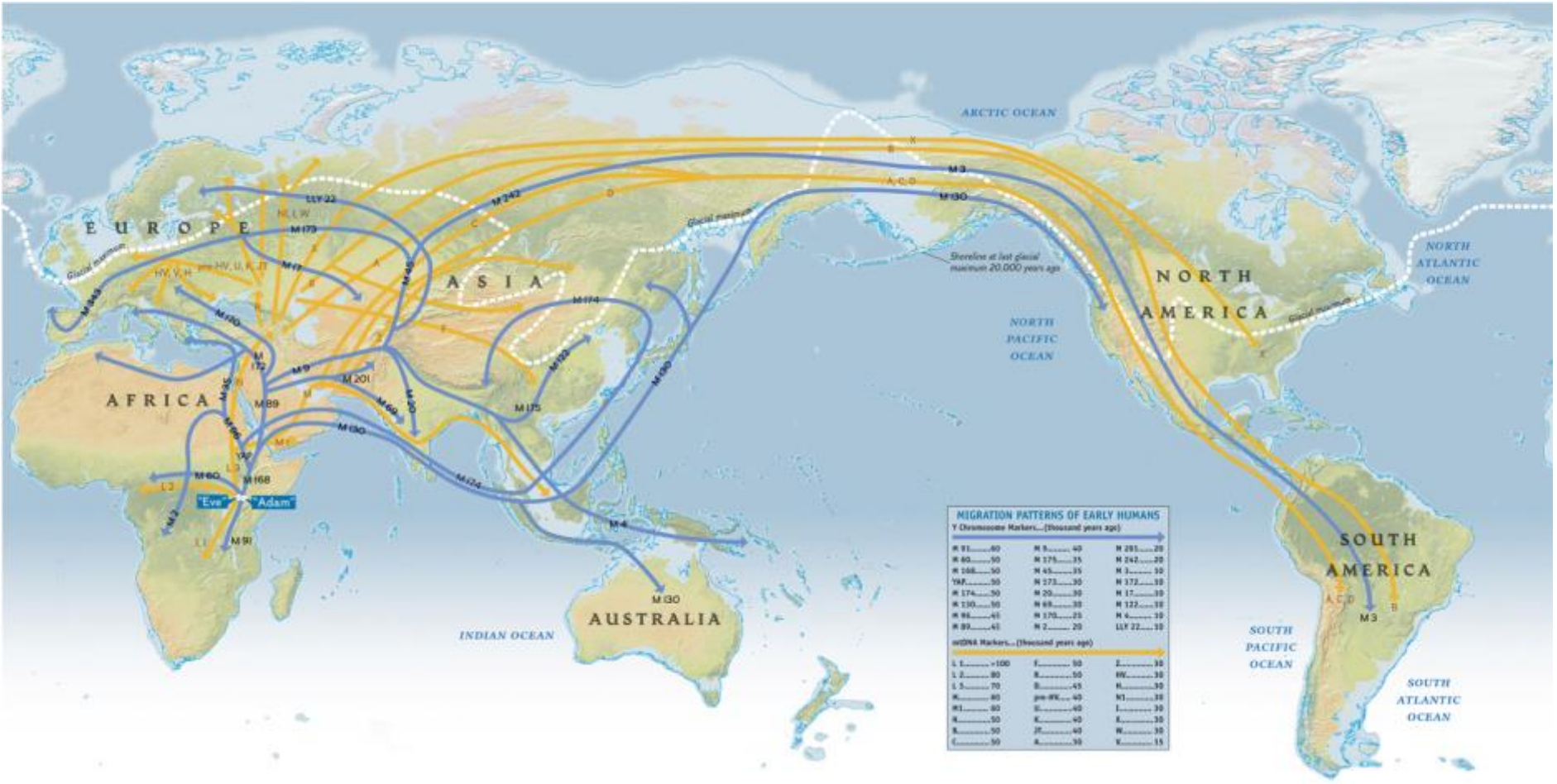
A different effect of an *environmental* exposure on disease risk in persons with different *genotypes*

Ottman, *Prev. Med* 25, 764 (1996)

Statistical Parlance

The *main effect(s)* may be *genotype x environment interaction(s)* for chronic diseases and modifying effects

Human Migrations - ancestry



Hypolactasia

N. European **Indian children**

Afr American kids **Indian adults**

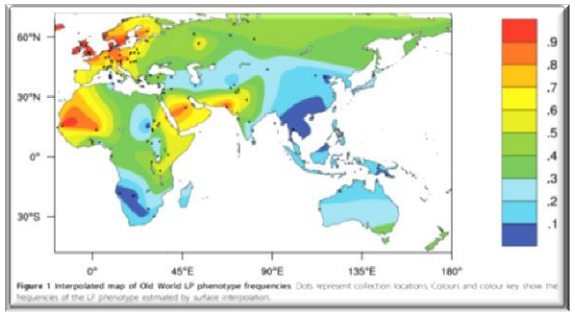
Mex American - adult **Cretans**

Cypriots **N. American Jews**

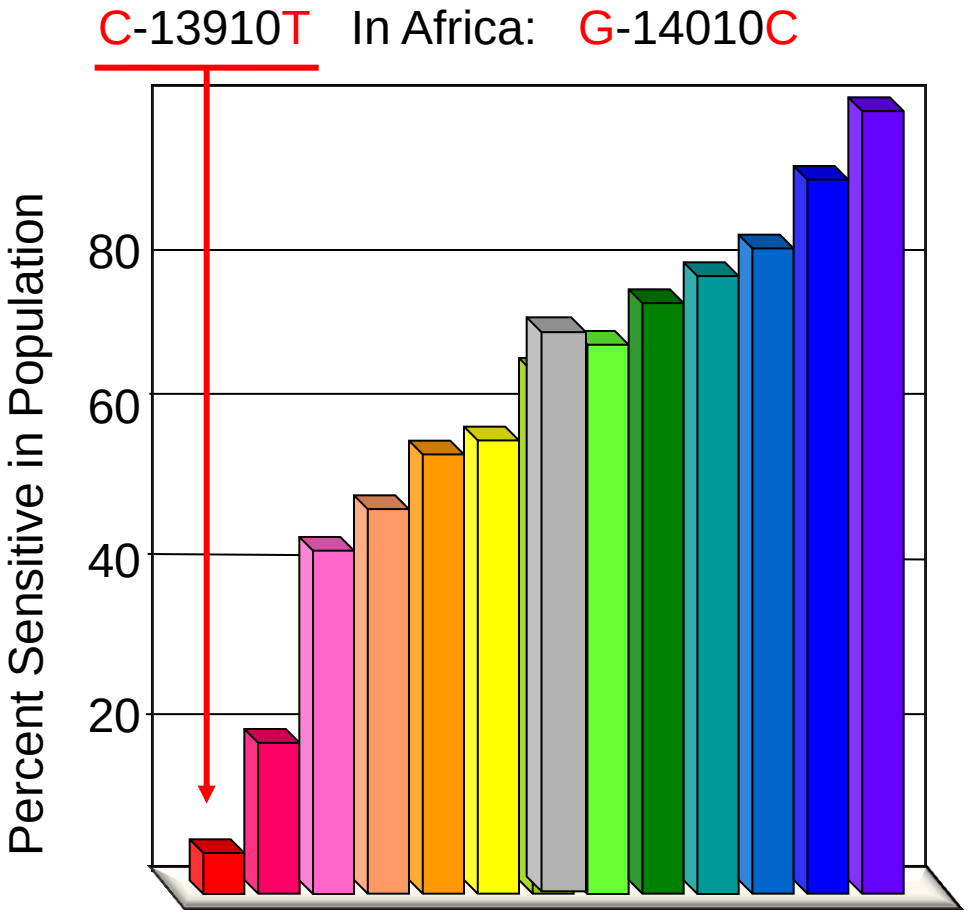
Mexicans - rural **SE Asians**

Eskimo **Afr American - adult**

Asian Americans



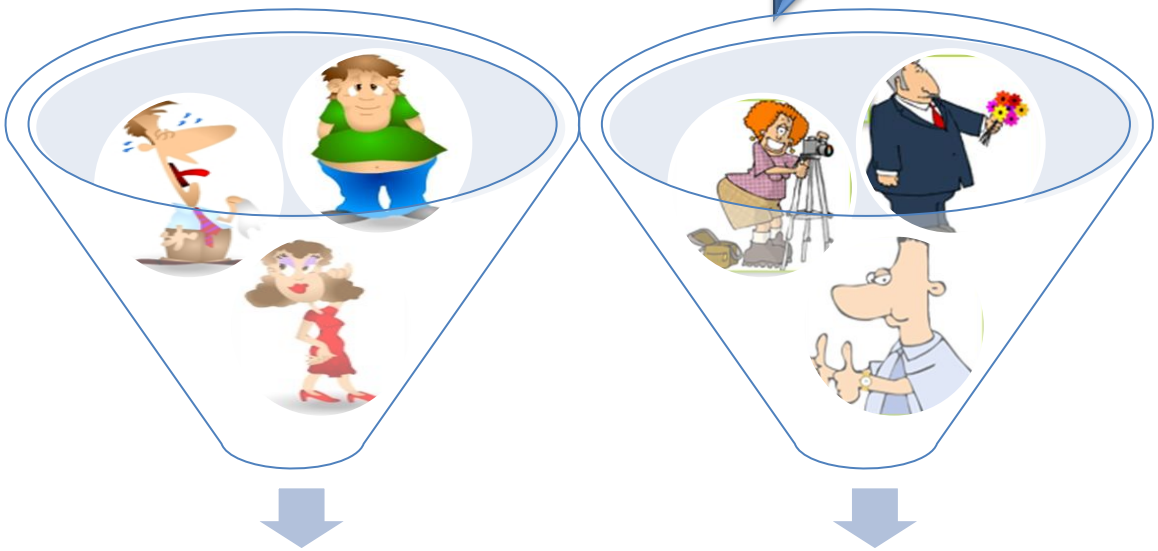
Itan *et al. BMC Evolutionary Biology* 2010, **10**:36
http://www.biomedcentral.com/1471-2148/10/36



Kaput and Rodriguez, *Physiological Genomics* 16, 166 (2004)

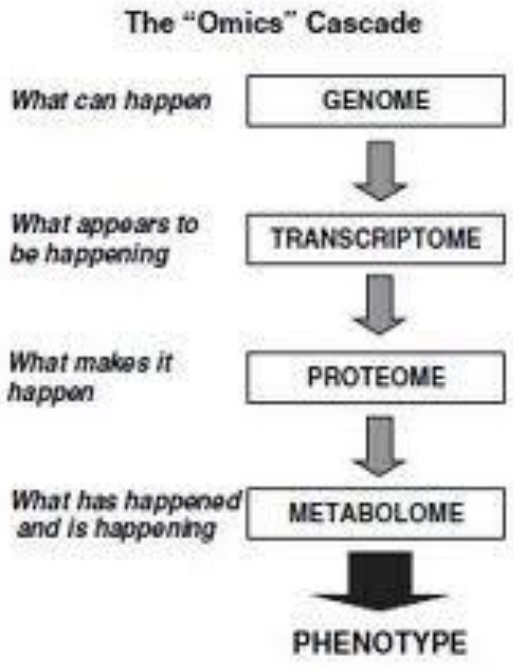
COMO PODEMOS MEDIR A MANEIRA COMO ELES RESPONDEM?

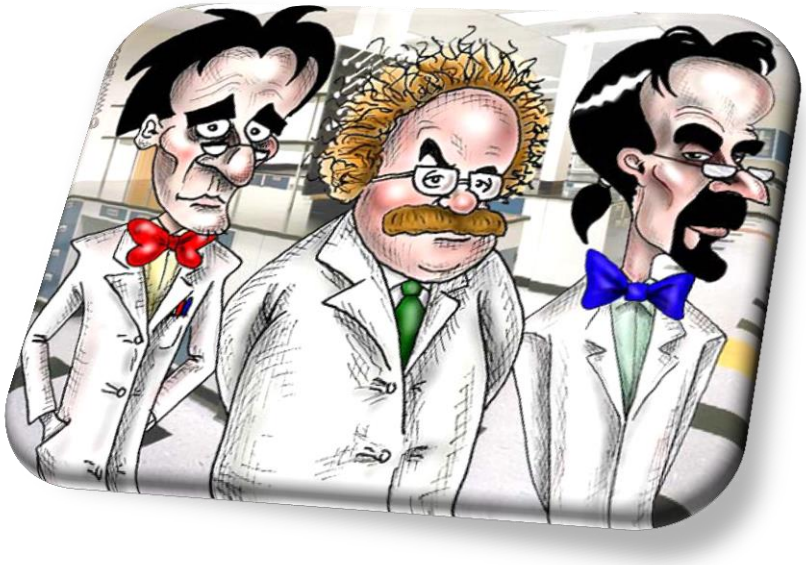
PROCURANDO POR DIFERENTES FENÓTIPOS METABÓLICOS.



FENÓTIPO METABÓLICO 1

FENÓTIPO METABÓLICO 2





Vamos padronizar nossos protocolos de pesquisa em diferentes populações mantendo a idéia original de procurar por diferentes FENÓTIPOS METABÓLICOS

N-of-1 Trials in the Medical Literature

A Systematic Review

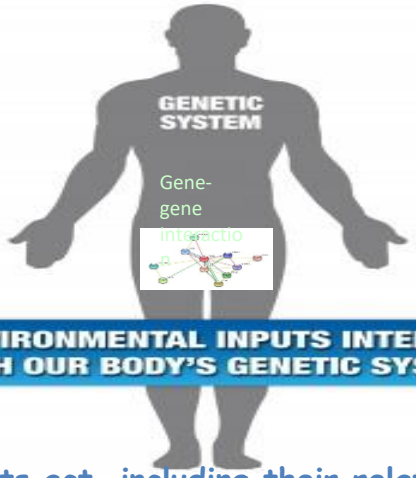
Nicole B. Gabler, PhD, MHA, Naihua Duan, PhD,† ‡*
Sunita Vohra, MD, FRCPC, MSc,§ and Richard L. Kravitz, MD, MSPH‖

Precisamos de mais estudos que considerem a resposta do INDIVÍDUO e, então, o classifique em grupos metabólicos semelhantes.

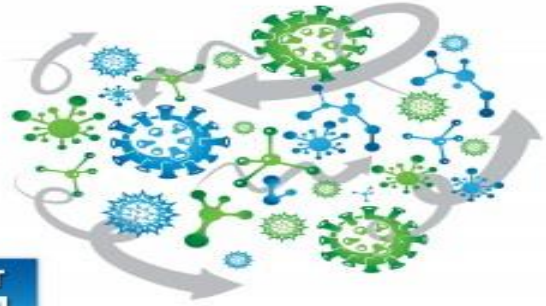
Os tradicionais ensaios clínicos randomizados resultam em uma política de "um tamanho serve para todos" em que o mesmo tratamento é aplicado a grupos heterogênios.

(*Med Care* 2011;49: 761–768)

ENVIRONMENTAL INPUTS
Exposures, Nutrition, Lifestyle



ENVIRONMENTAL INPUTS INTERACT WITH OUR BODY'S GENETIC SYSTEM



METABOLIC RESPONSE
Cancer Susceptibility Risk Factors and Other Health Outcomes

Systems thinking = a context in which components act, including their relationships and interactions in a non-static way

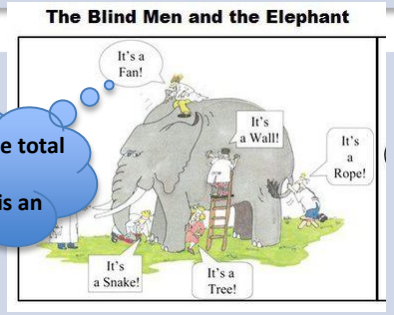
Prevent NCDs

Nutritional Systems Biology

What We Know About Diet, Genes, and Phenotype: Is There Potential for Translation?

Omics Technologies (genomics, transcriptomics, proteomics, metabolomics, bioinformatics)

To integrate all aspects of metabolism and gene response to nutrients



To generate knowledge about the normal range of metabolism

New biomarkers and metabolic profiles to characterize predisease phenotypes

Dietary recommendations customized at a sub-population level

LÉVESQUE L et al 2008

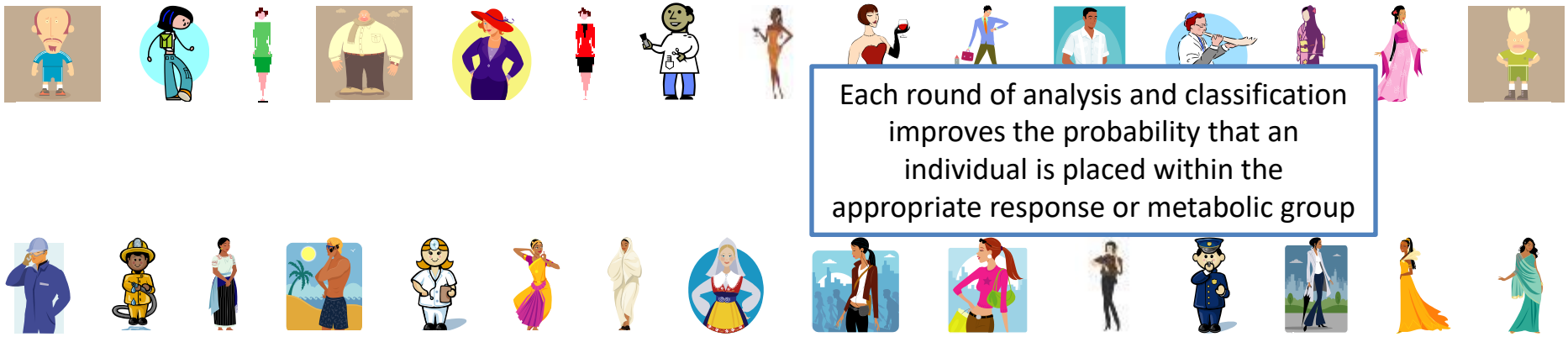
M. Joly, P.H.C. Rondó, 2015

Procurando por grupos metabólicos

Como fazemos esse tipo de pesquisa usando as ferramentas ômicas?

Experimental Design Concept n-of-1 to group level

N-of-1 trial = Detailed intervention study of one individual over time and in response to environment



Each round of analysis and classification improves the probability that an individual is placed within the appropriate response or metabolic group

Classification Algorithms

Nutritional status; Physical activity; Social, economic and behavior status; Genome, proteome, metabolome

Kaput & Morine, 2012; Monteiro et al. 2014; Morine & Monteiro 2014

Phillips County Arkansas: Marvell and Elaine

Obesity Prevention Summer Day Camp: USDA + FDA

Summer Day Camp at the Boys, Girls, and Adults Community Development Center

Blood (genotyping & metabolite analyses)

- @ beginning
- @ the end (5 weeks)
- @ one month following the end of summer camp

Breakfast, lunch, snack (Lowfat milk , fruits, vegetables)

- Three 24h recall and Health Eating Index
- Anthropometry

This study did not use a case-control design (105 individuals)

6 – 14 years old – 42% overweight

Individuals (represented in rows) with higher SAM/SAH tended to have higher plasma levels of fat-soluble vitamins A and D and medium or low plasma levels of vitamin E, thiamine, and pyridoxal. Individuals with low SAM/SAH tended to have the opposite patterns of these metabolites

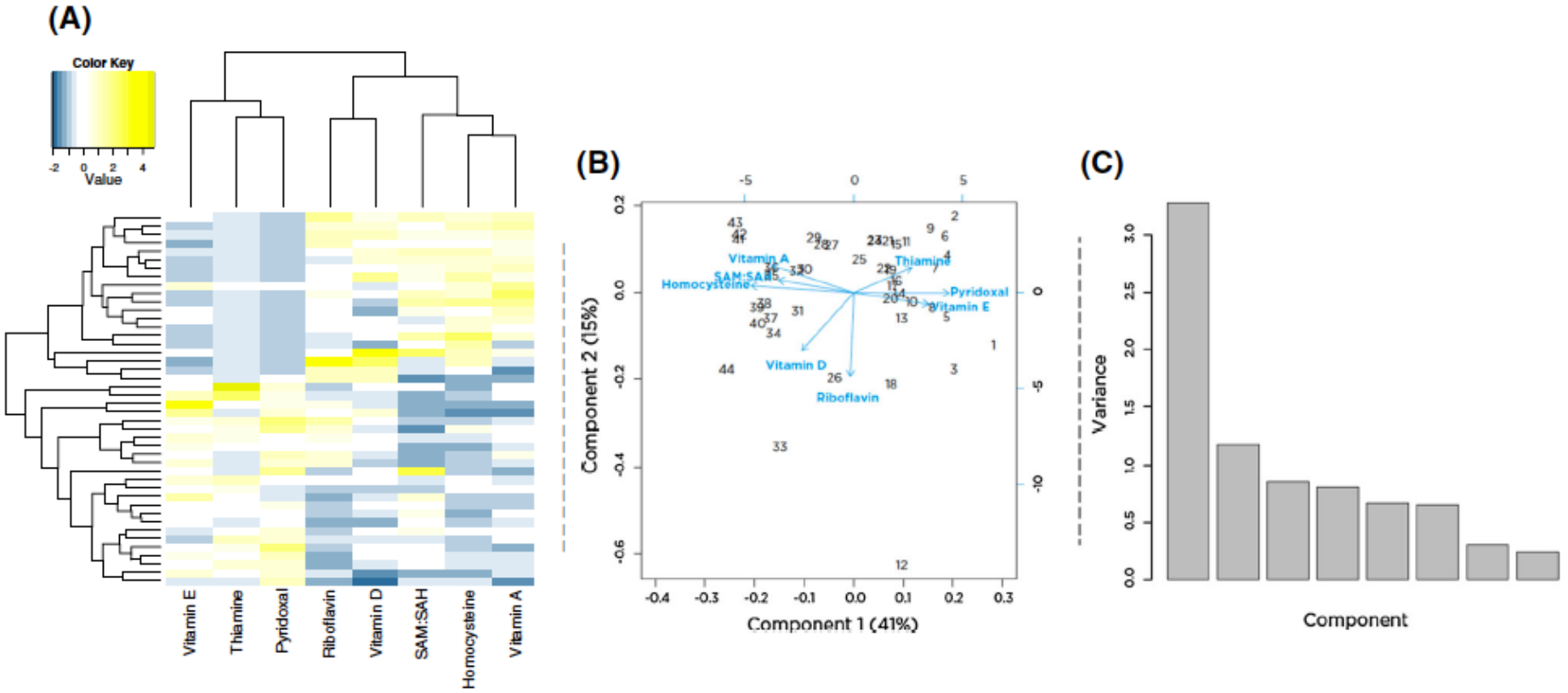
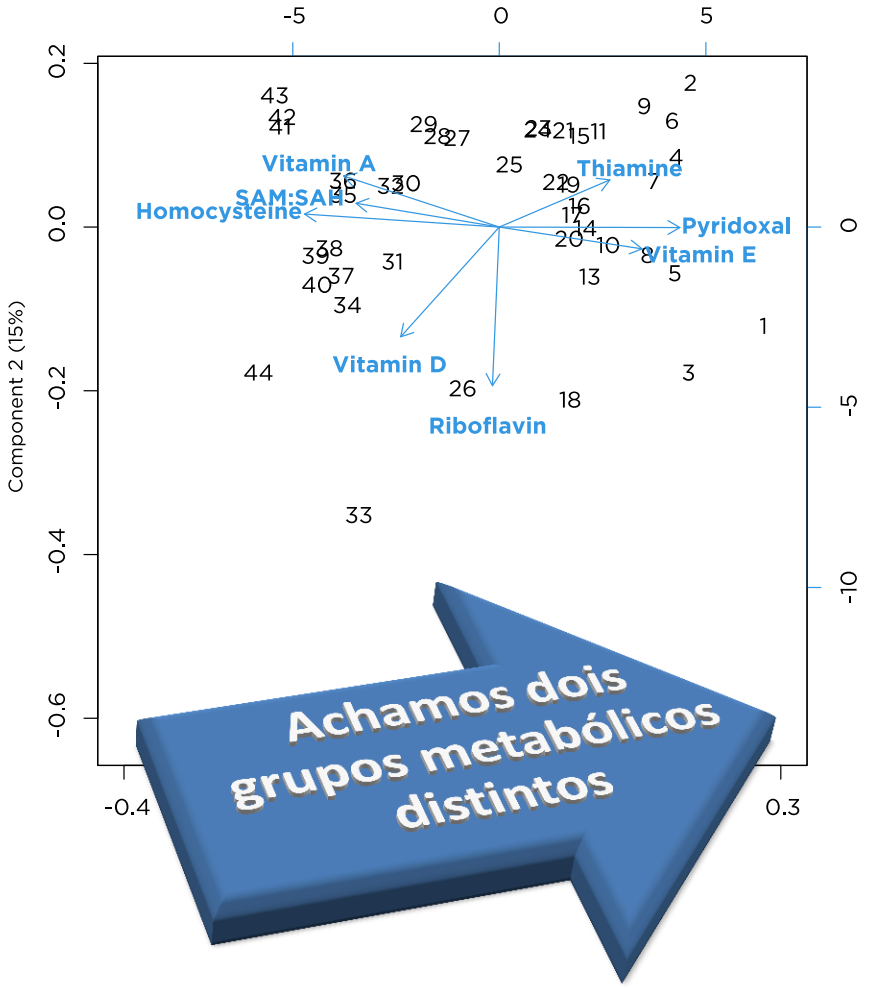
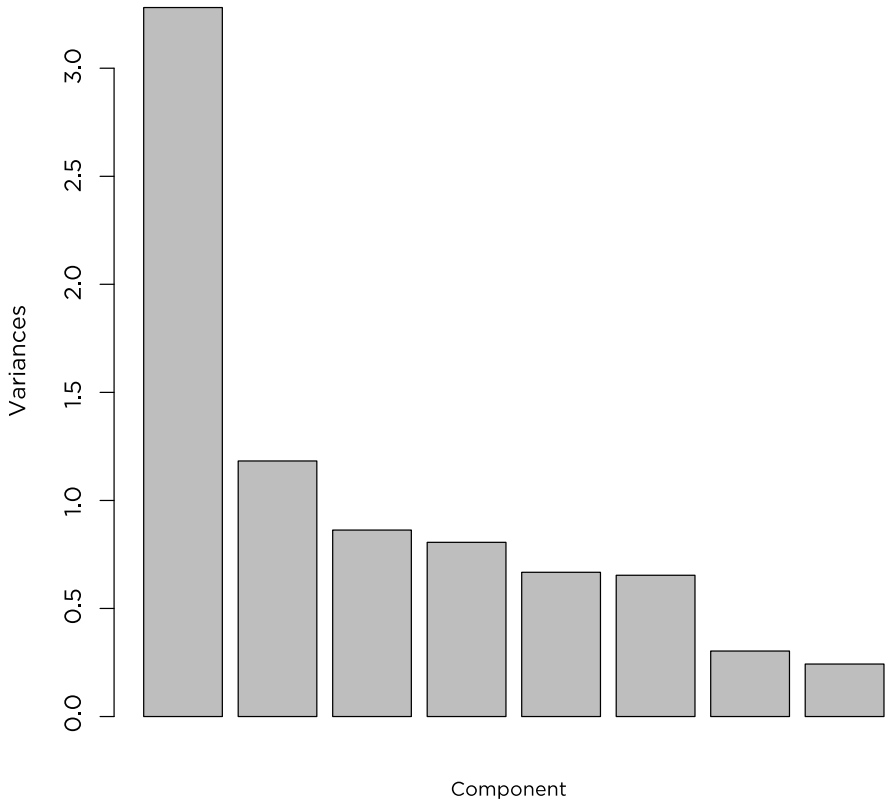


Fig. 1 Metabolite-level heat map and principal component analysis of vitamin levels. **a** Metabolite heat map where individuals are represented in the *rows*, and mean value of metabolite levels from three blood samplings is in the *columns*. **b** Principal component analysis of mean values of vitamin or metabolites. *Numbers* indicate values for individuals (**c**). Variances in each principal component (see "Materials and methods" section for details)

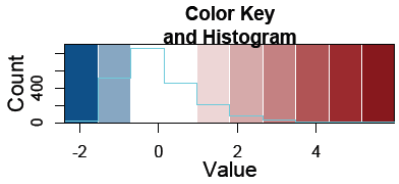
MICRONUTRIENT PROFILE - PCA

Plasma Metabolites





Proteomic data showed 51 proteins significantly associated with MET_PC1 variable



Functional analysis of branches

Met PC1

Vitamin A
SAM:SAH
Homocysteine

Pyridoxal
Thiamine
Vitamin E

COTL1 RPSA CDH12 IL1RAPL2 GNLY TNFSF15 IDS NAMPT CSF1 PGD SELE EPHA5 LY9 KLK11 BMP7 CST2 CD86 PGK1 IL22RA2 SLITRK5 SHH NBL1 LTA4H ENG CKB CKM PIGR C3 C3 C3 PTN IL12RB1 C1S CFH ESR1 PAK7 ANGPTL3 MMP10 AZU1 HSD17B1 DBNL HDGFRP2 TBP CMA1 PGAM1 MAPK1 EIF5 CSNK2A1 LRPAP1 FABP5 HDAC8 CDC2 CCNB1

Pro-Inflammatory

Anti-Inflammatory

+/- in inflammatory conditions

Plasma or membrane proteins	24/27 = 88.9%	5/12 = 41.6%
Cytosolic proteins	3/27 = 11.1%	7/12 = 58.4%

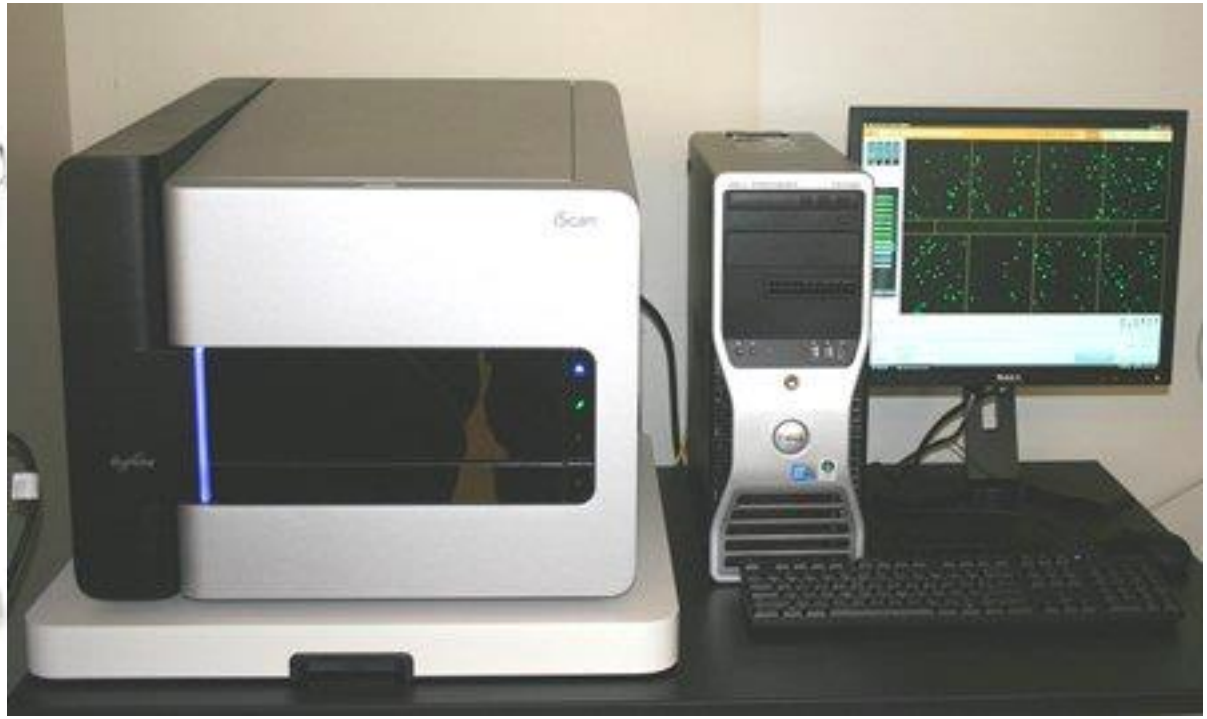
Monteiro JP et al 2014

S1_MetPC1_Prot_QTL

RAW SNP data

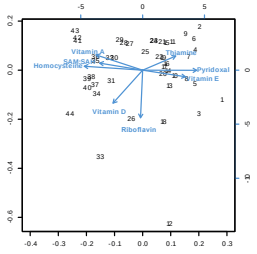
What are the SNPs and genes related to those 2 metabolic groups?

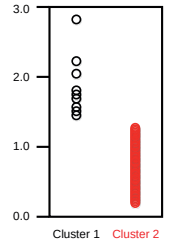
GENOTYPING



125959 SNPs

A pergunta que não quer calar…





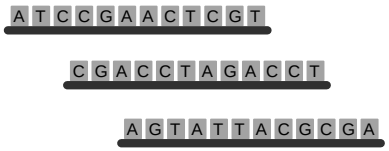
MICRONUTRIENT PROFILE DATA

Generalized Estimating Equation

GENOTYPE DATA

GEE

To connect 125959 SNPs with PCA1 (metabolic groups)



125959 SNPs

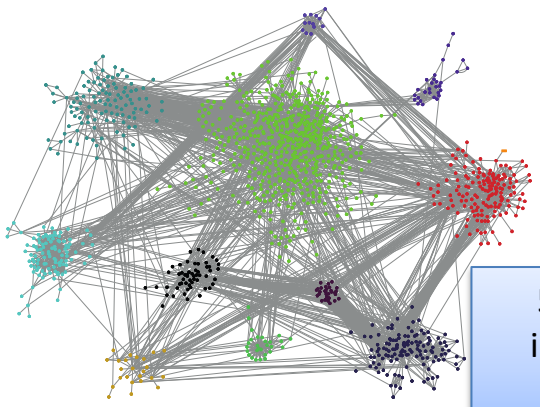
MICRONUTRIENT CORRELATED SNPS

3200 SNPs

VEGAS

MICRONUTRIENT CORRELATED GENES

1392 genes



TOPOLOGICAL MODULES

HYPERGEOMETRIC TEST

58 modules: 116210 interactions between 13705 genes

MICRONUTRIENT CORRELATED MODULES

4 modules (18, 52, 45, 2)

To reasonably represent the System Biology ,statisticians got a global networking using two manually curated protein-protein interaction database

Middle Out Enriched module – Genotype

Freq > 0.5%

Homozygote = 0

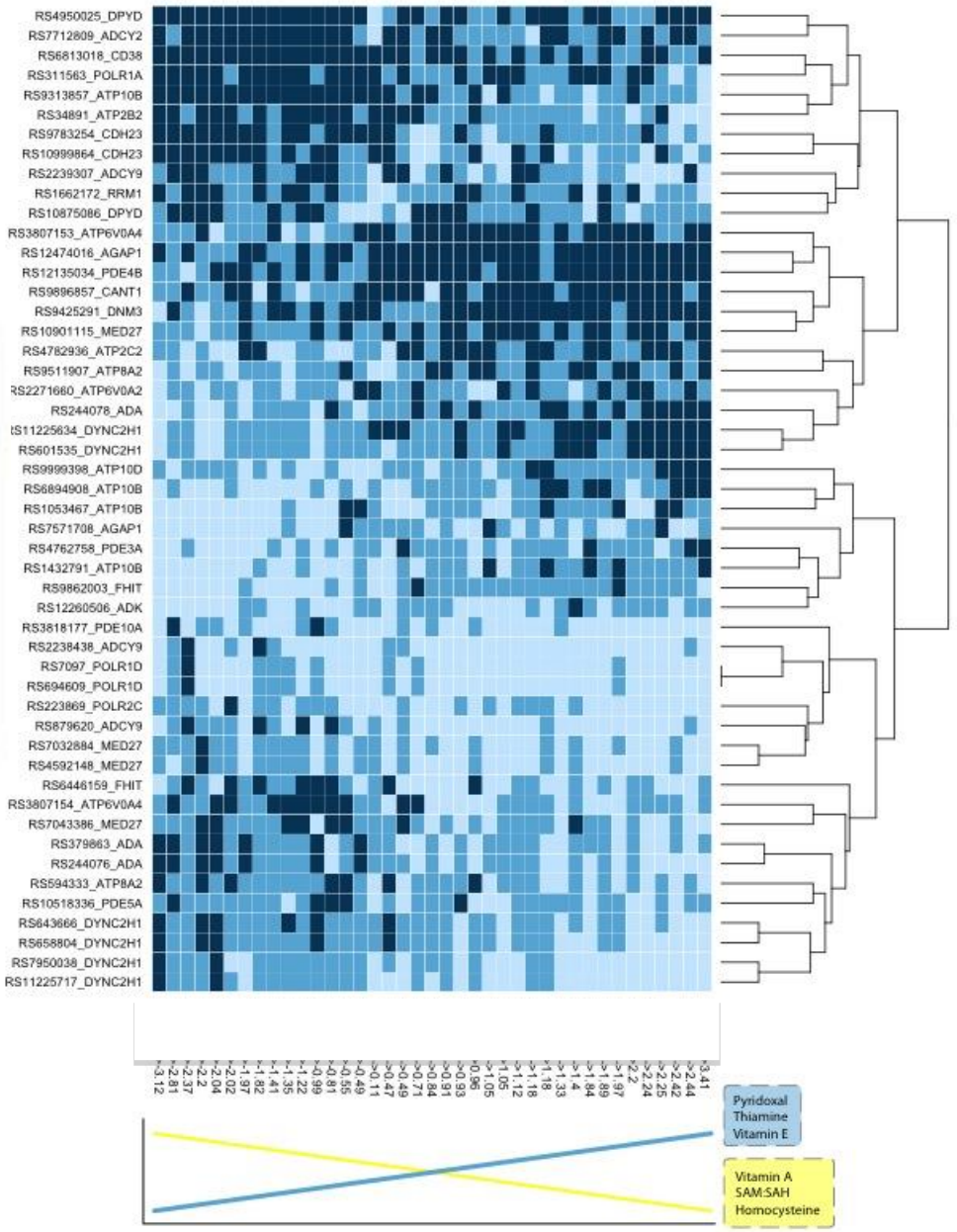
Heterozygote = 1

Homozygote = 2

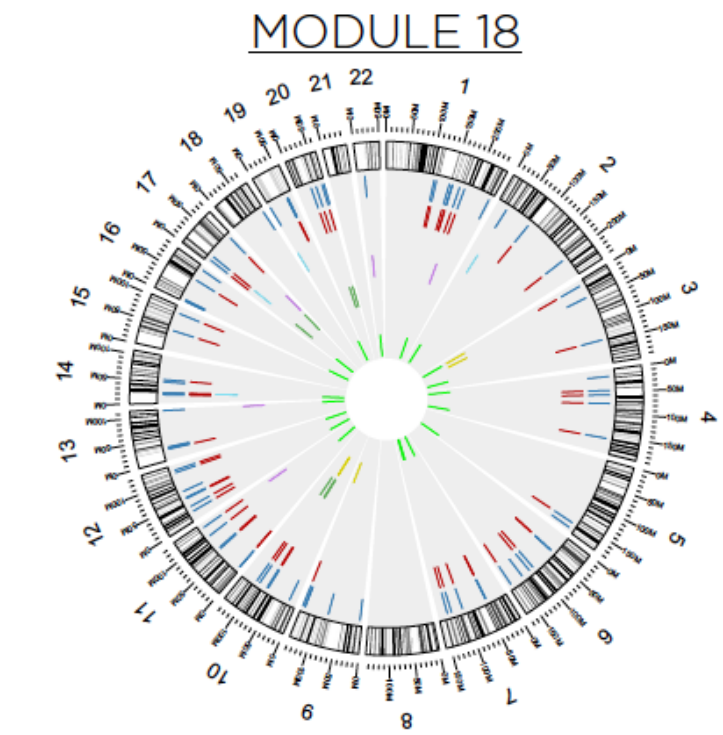
Module 18: 465 genes

Found 42 (2 metabolic groups)

Corrected modular p = 2.63E-19



Micronutrient module genes map to phenotype loci



42 DOS NOSSOS GENES ESTAVAM EM REGIÕES CROMOSSÔMICAS (QTL/LOCI) QUE CODIFICAM GENES ASSOCIADOS AO PESO CORPORAL, À LEPTINA, AO METABOLISMO DA GLICOSE

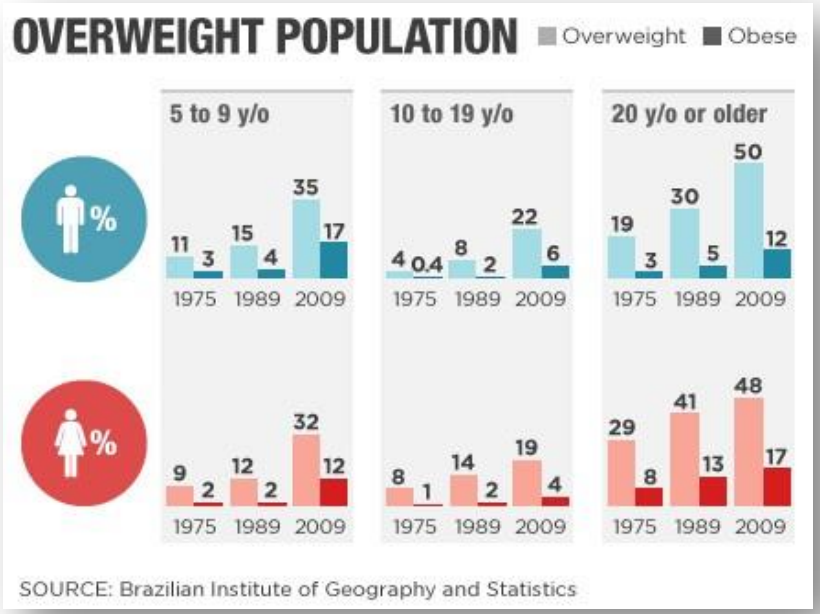
Location Ribeirão Preto, São Paulo, Brazil

Brazil Micronutrient Project

Nestlē Institute of Health Sciences

As amostras de sangue serão enviadas para análise no laboratório de pesquisa do Instituto de Pesquisa em Saúde da Nestlé (NIHS) na cidade de *Lausanne*, na Suíça.

Brazil Micronutrient Intervention Study



33.5% children are overweight in Brazil
(IBGE 2014)

Increased metabolic syndrome in children
eg. Dyslipidemia and glucose intolerance
(Harris et al. 2006)

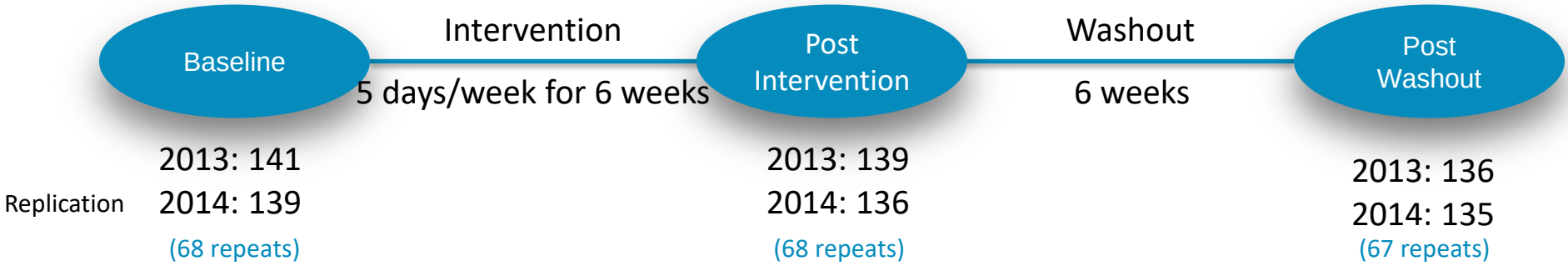
Obesity and metabolic syndrome are related to micronutrients deficiencies

Objectives

Primary Identify *responders & nonresponders* defined by glucose, LDL cholesterol, total cholesterol *to multiple micronutrients*

Secondary Develop methods to target appropriate combinations of micronutrients to individuals to improve metabolic health

Clinical Trial Design 2 tablets for 9, 10, 11 & 3 tablets for 12 & 13

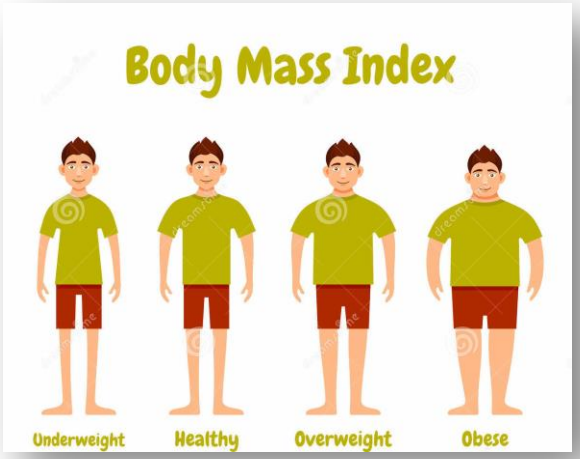


Milk Bar Composition Per 3 Tablets

	Amount	%RDI for 9, 10, 11, 12, 13
Vitamin A	801 µg	133.3%
Vitamin D	5.1 µg	50%
Vitamin E	9.9 mg	90%
Vitamin C	60 mg	133.3%
Vitamin B1	1.4 mg	155.5%
Vitamin B2	1.76 mg	178%
Niacin	18 mg	150%
Vitamin B6	2 mg	200%
Folate	200 µg	50%
Vitamin B12	1.1 µg	55.5%
Biotin	150 µg	750%
Pantothenate	6 mg	150%
Calcium	287 mg	22%
Phosphorous	217 mg	17.3%
Iron	6.5 mg	81.2%
Magnesium	125 mg	52%
Zinc	6 mg	75%

Measurements

- Anthropometric
- Clinical chemistry & CBC
- DNA damage
- 1,129 plasma proteins
- ~200 plasma metabolites
- 5M SNP plus whole exome
- Plus other



Selected Study Population Data

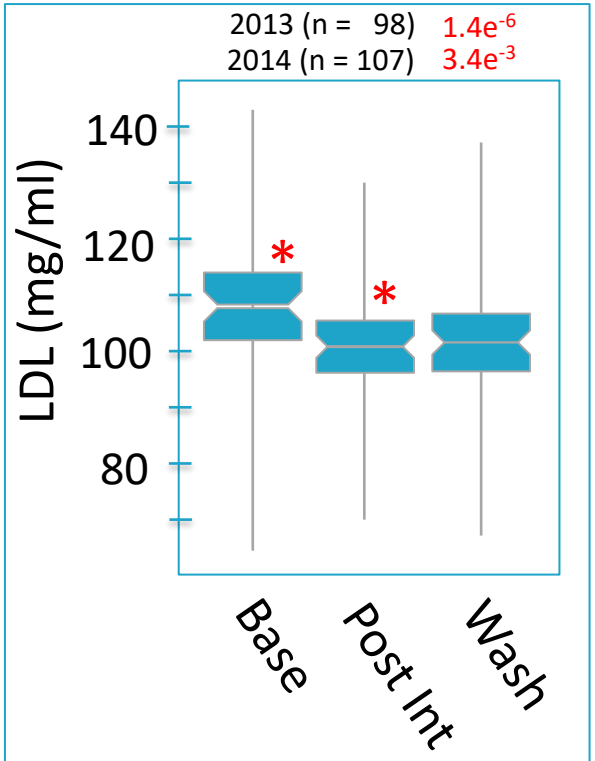
	2013	2014	2013 + 2014	2014 Validation	2014 Replication
Participants	141 ➔136	139 ➔135	280	69 ➔ 65	70 ➔70
Female	56.7%	54.0%	55.35%	44.9%	62.9%
Age (years)	11.4 ± 1.1	11.9 ± 1.0	11.6 ± 1.1	11.6 ± 1.1	12.2 ± 0.9
Mean weight - kg	48.8 ± 16.7	50.0 ± 14.6	49.4 ± 15.7	48.3 ± 11.8	51.7 ± 16.7
Mean BMI	20.6 ± 5.3	20.5 ± 4.9	20.5 ± 5.1	20.0 ± 4.1	21.0 ± 5.6
Overweight/Obese	48.9%	37.4%	43.2%	34.8%	40.0%

“Vitamin insufficiency”	Vitamin B12 insufficiency: 0%	Folate insufficiency: 5.2%	Riboflavin insufficiency: 19%	Beta-carotene: insufficiency: 44%	Retinol insufficiency: 48%

Validation = new recruits in 2014

Replication = returnees

Main Results Difference between Intervention & Baseline



	Glucose	Cholesterol
2013	$2.5e^{-4}$	$3.5e^{-5}$
2014	$4.0e^{-4}$	0.03

	HDL	TG	VLDL
2013	0.36	0.36	0.36
2014	0.94	0.08	0.08

*T test p values corrected for multiple testing (Benjamini-Hochberg)

	Responders		Non -Responders	
2013	#	Ave. Decrease	#	Ave. Increase
Glucose	77	4.7 mg/dl - 5%	31	3.9 mg/dl – 4%
LDL	78	13.4 mg/dl – 11%	24	8.5 mg/d – 5.2%
Cholesterol	62	17.8 mg/d - 13%	29	9.1 mg/dl – 8.8%

Statistical tests demonstrate that the fold change is due to the intervention

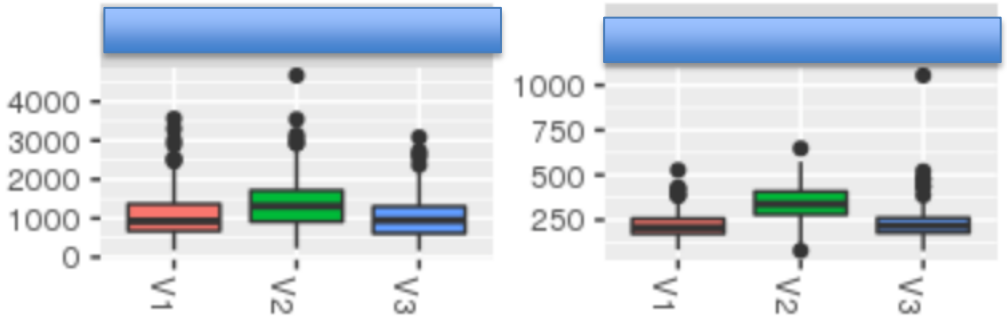
Conclusion

Multiple micronutrients alter lipid/glucose profile

Response is clinically relevant

Plasma Vitamin & Clinical Variables changed by Intervention

Examples



Subset of variables changed by intervention

	# changed	Total
Micronutrients	14	17
Clinical	3	6

Bodybugg at the same time...

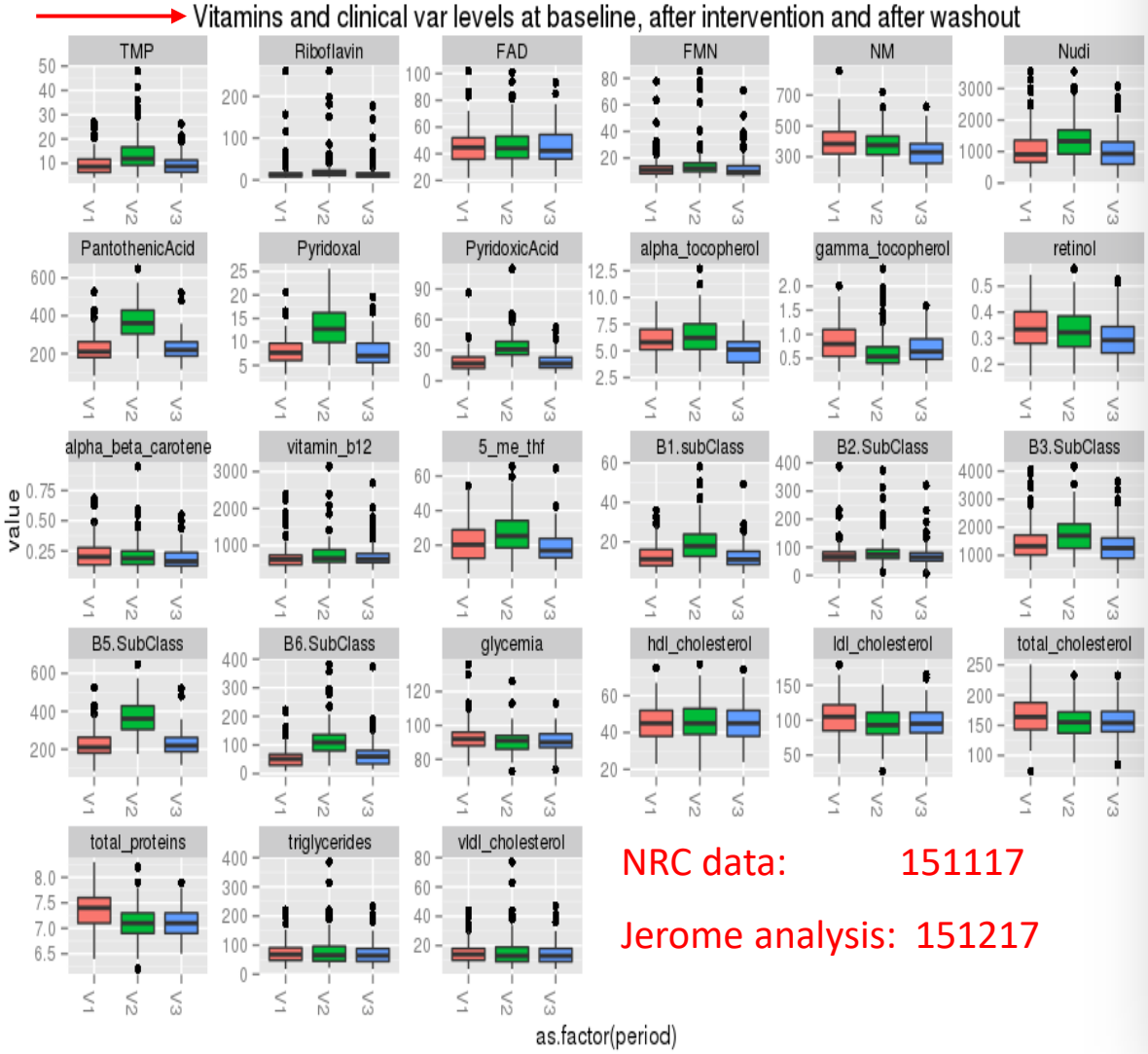
Three 24 hour recall (last week prior blood drawn and FFQ application)

Energy expenditure	Energy expenditure	Physical activity
• Skin temperature • Galvanic skin response	• Flux sensors • Resting energy expenditure	• Number of steps • Movment detector

jacque@fmrp.usp.br

Jacqueline Pontes Monteiro - University of São Paulo
Ribeirão Preto - Brazil

Plasma Vitamin & Clinical Variables



NRC data: 151117

Jerome analysis: 151217

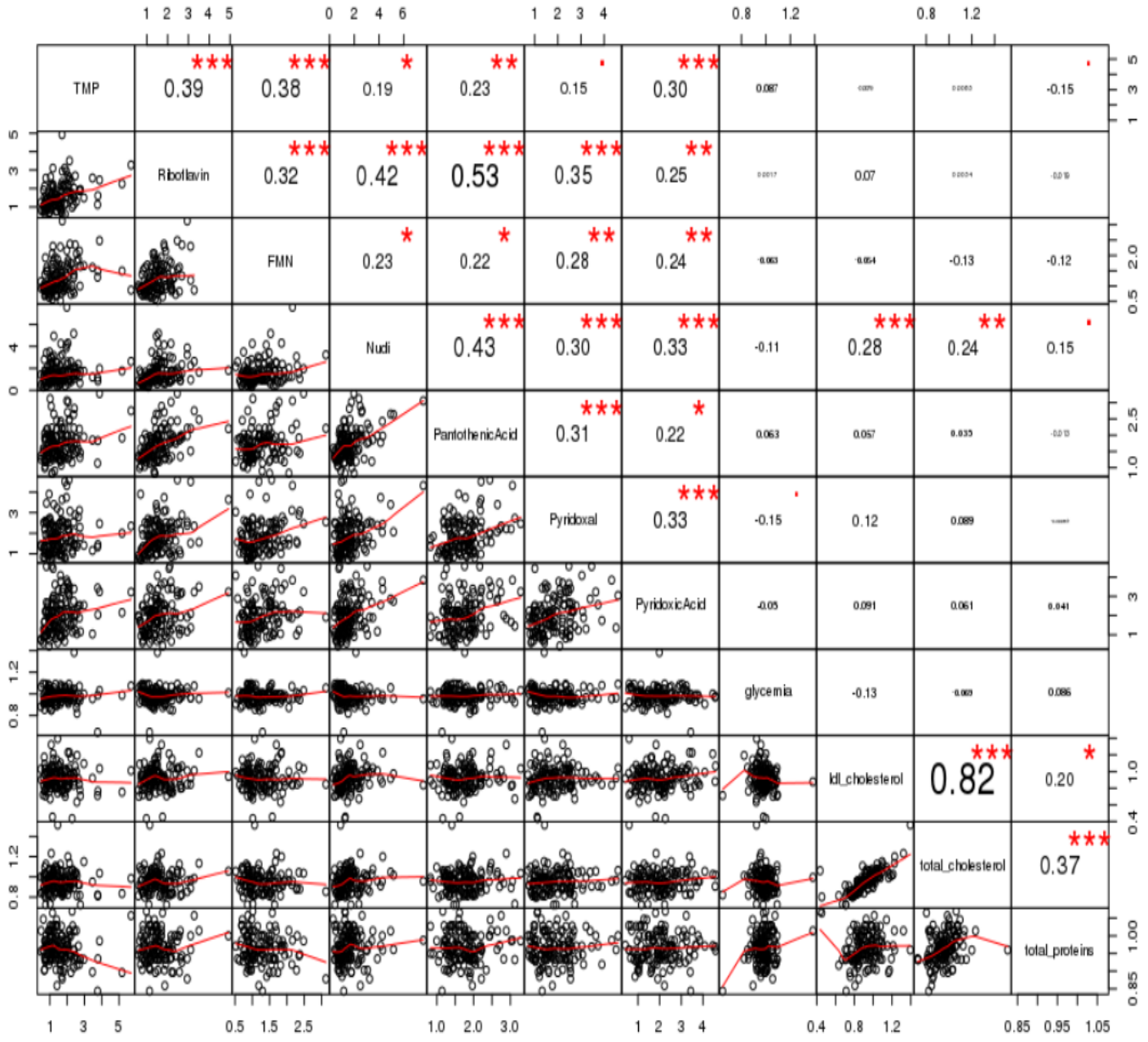
Effect of supplementation intervention on vitamins and clinical variables level

	Vitamins	Pvalue	Pcorrected
✓	TMP	1.2e-11	3.6e-11
✓	Riboflavin	8.9e-15	3.4e-14
	FAD	7.6e-01	0.76
✓	FMN	2.7e-05	4.6e-05
	NM	2.1e-01	0.26
✓	Nudi	9.2e-08	1.8e-07
✓	PantothenicAcid	2.2e-23	3e-22
✓	Pyridoxal	3.1e-20	2.1e-19
✓	PyridoxicAcid	1.1e-18	5.9e-18
✓	alpha_tocopherol	8.0e-03	0.011
✓	gamma_tocopherol	1.1e-09	2.7e-09
	retinol	8.2e-02	0.11
	alpha_beta_carotene	3.6e-01	0.41
✓	vitamin_b12	1.5e-03	0.0021
✓	5_me_thf	2.2e-08	4.9e-08
✓	B1.subClass	5.5e-15	2.5e-14
✓	B2.SubClass	3.8e-05	6e-05
✓	B3.SubClass	1.0e-06	1.8e-06
✓	B5.SubClass	2.2e-23	3e-22
✓	B6.SubClass	2.2e-20	2e-19
✓	glycemia	2.5e-04	0.00038
	hdl_cholesterol	2.7e-01	0.32
✓	ldl_cholesterol	1.1e-10	3e-10
✓	total_cholesterol	6.0e-08	1.2e-07
✓	total_proteins	1.7e-14	5.7e-14
	triglycerides	6.3e-01	0.68
	vldl_cholesterol	7.6e-01	0.76

✓ Statistically Significant for V1 to V2

Correlations

Fold Change of Vitamins & Clinical Data



Jerome: 151217

Statistical Tests of intervention

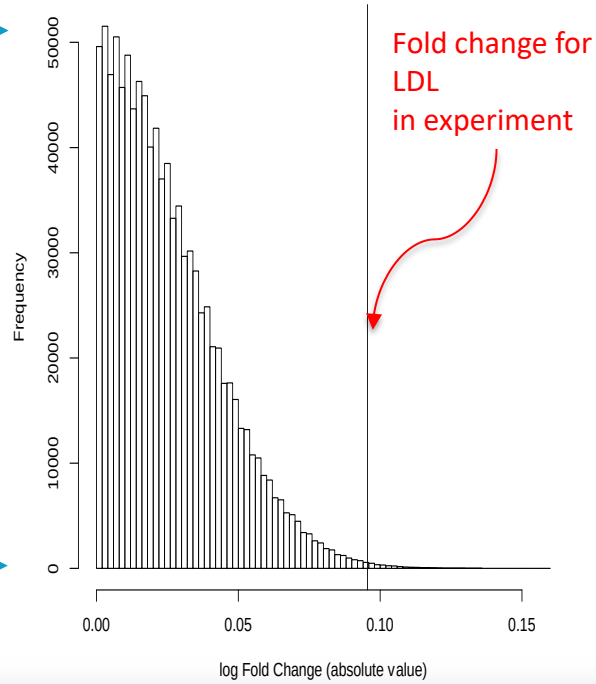
COSBI: 160117

Permutation

Procedure Pooled V2 & V1, randomly divided the data into two equal groups
Evaluated the significance of the response (V1 vs V2 with Student t-test)
Repeated 1,000,000 times

Results In 5% of permutations significant differences between both time points

Conclusions The LDL-C response to intervention was rarely (< 5%) observed by chance
The observed intervention effect on LDL-C was not driven by outliers



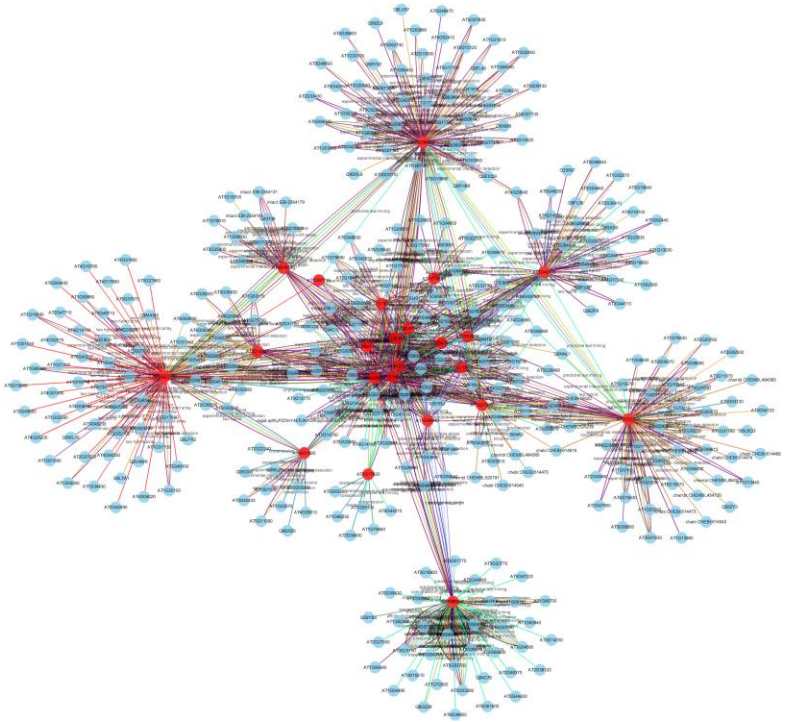
Regression to the mean

Variable	Effect	Down-Down treatment	Down-Down p-value	Down-Down washout	Down-Down p-value	Down-Up treatment	Down-Up p-value	Down-Up washout	Down-Up p-value	Up-Down treatment	Up-Down p-value	Up-Down washout	Up-Down p-value	Up-Up treatment	Up-Up p-value	Up-Up washout	Up-Up p-value
LDL	-12.0	1E-07	-8.2	1E-07	-14.7	5E-13	11.4	5E-15	8.9	2E-06	-12.8	3E-05	8.3	0.07	7.7	0.011	

Conclusion RTM & PM analysis indicate that the fold change is due to the intervention, not RTM or random chance

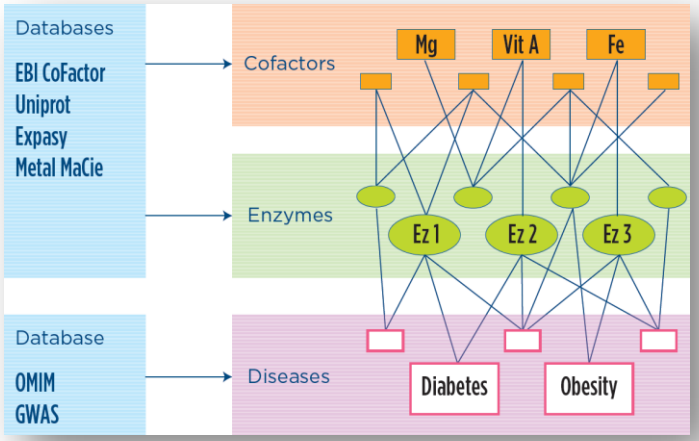
Systems modeling using protein-protein interaction

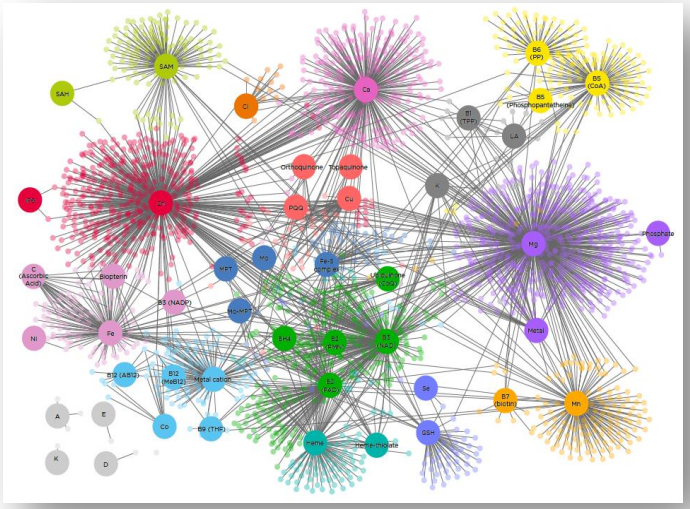
Modeling of metabolic networks is one powerful approach to allow a better understanding of nutrient behavior in cells both in normal and in pathogenic states

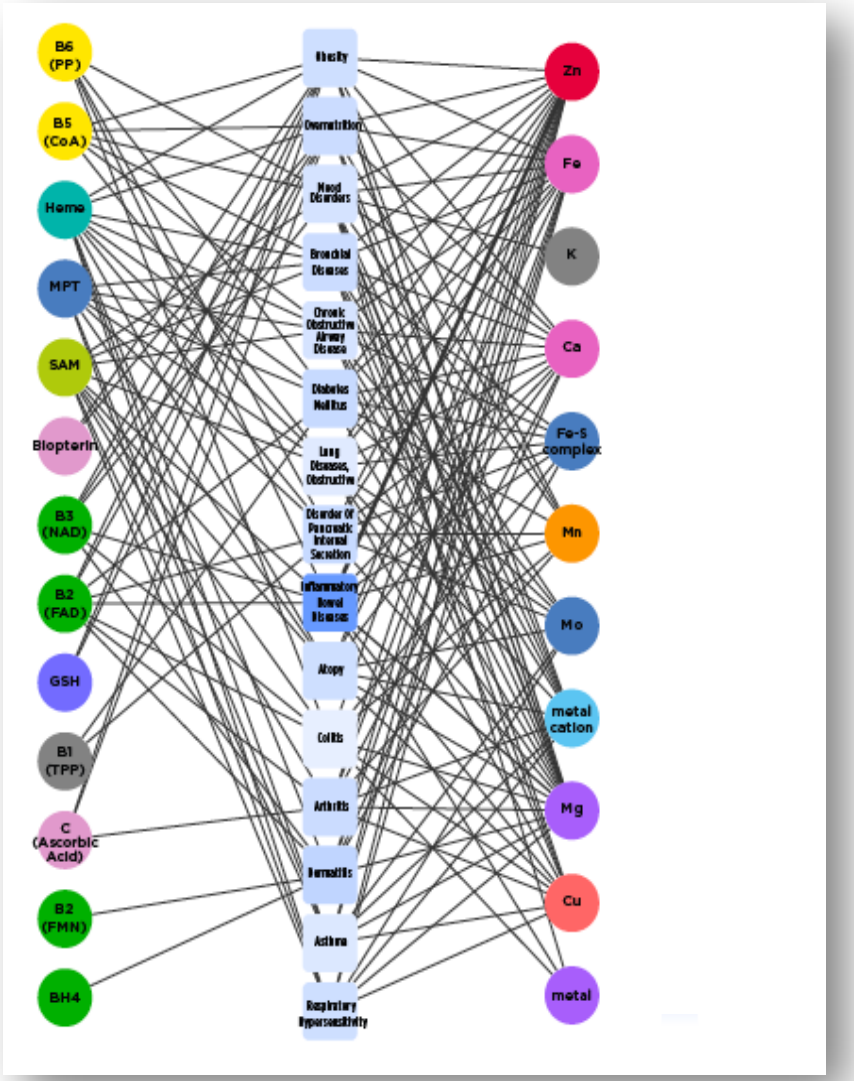


Nijhout et al. 2008; Reed et al. 2008; Scotti et al. 2013

Cofactor-Protein Database

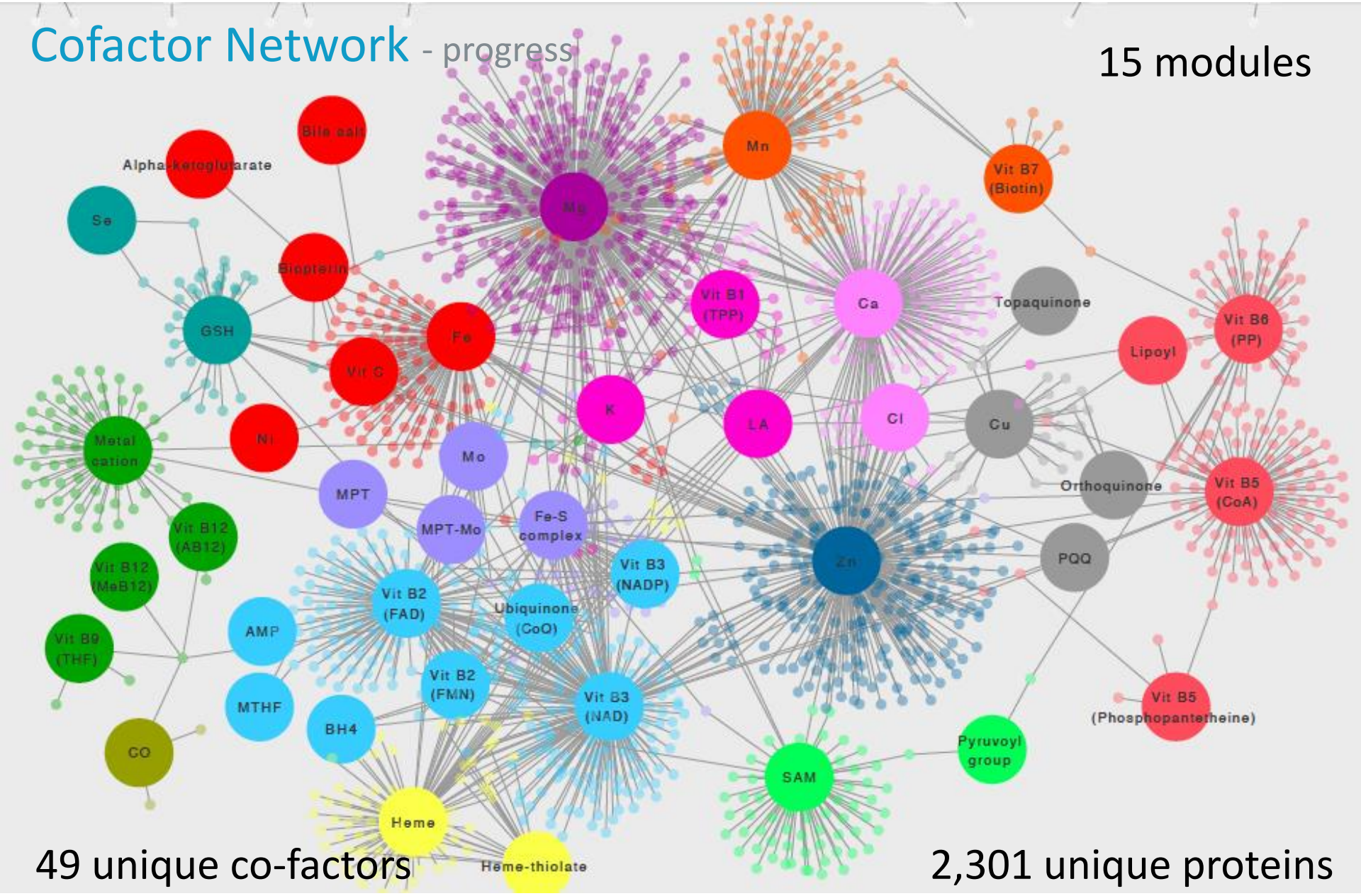






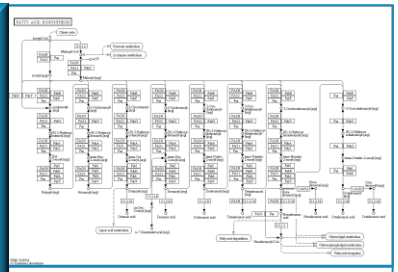
Sebastian Lecroix, Marie Pier Scott-Boyer, Mellissa Morine, Marco Scotti, Jim Kaput

Global effect of micronutrient: To associate genes identified by data mining methods with nutrients through cofactor network



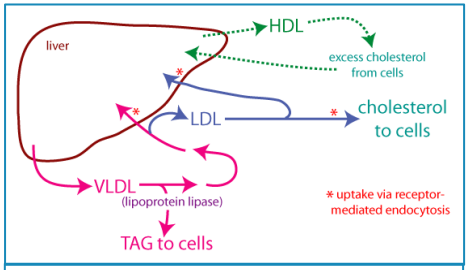
Cofactor Database

Provenance



Gene - pathway

Genetic Variation & Phenotype Associations



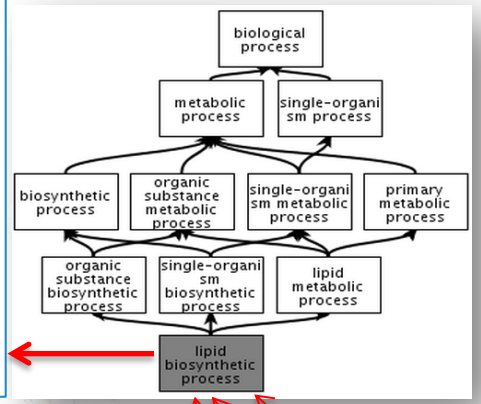
Gene - phenotype

Proteins that bind inorganic and organic (includes in vivo produced) cofactors identified in 4 databases (below)

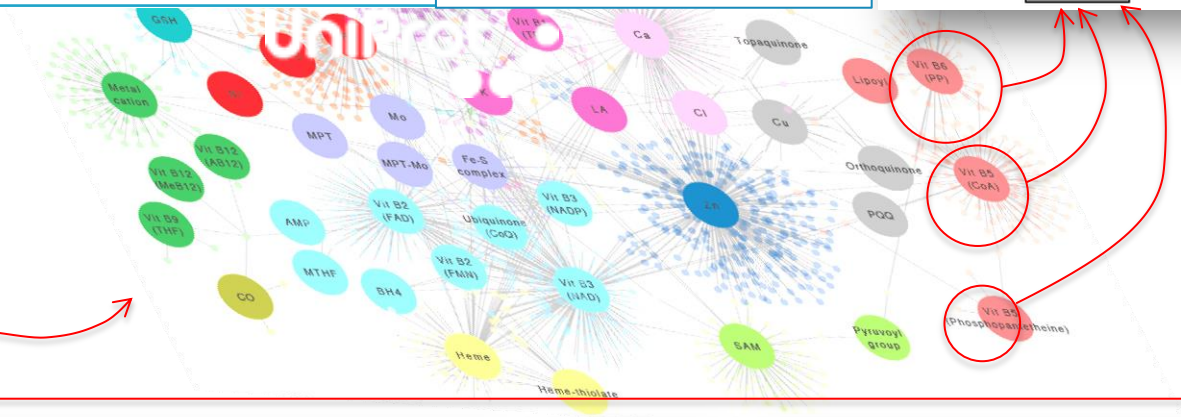
Genes mapped PPMI network, gene ontology & functional pathways to link processes to cofactors

Genes linked with disease and GWAS to associate cofactors to disease and phenotype

ABHD5	DGAT1	LPCAT4
ACAA2	DGAT2	MBOAT2
AGPAT1	FASN	MCAT
AGPAT2	GPAM	MOGAT1
AGPAT3	GPAT2	MOGAT2
AGPAT4	HMGCR	MOGAT3
AGPAT5	HMGCS1	OLAH
AGPAT6	HMGCS2	OXSM
AGPAT9	LCLAT1	SCP2
AWAT1	LPCAT1	SPTLC1
AWAT2	LPCAT2	SPTLC2
CPT1B	LPCAT3	SPTLC3



Marie Pier Scott-Boyer
Sebastien Lacroix



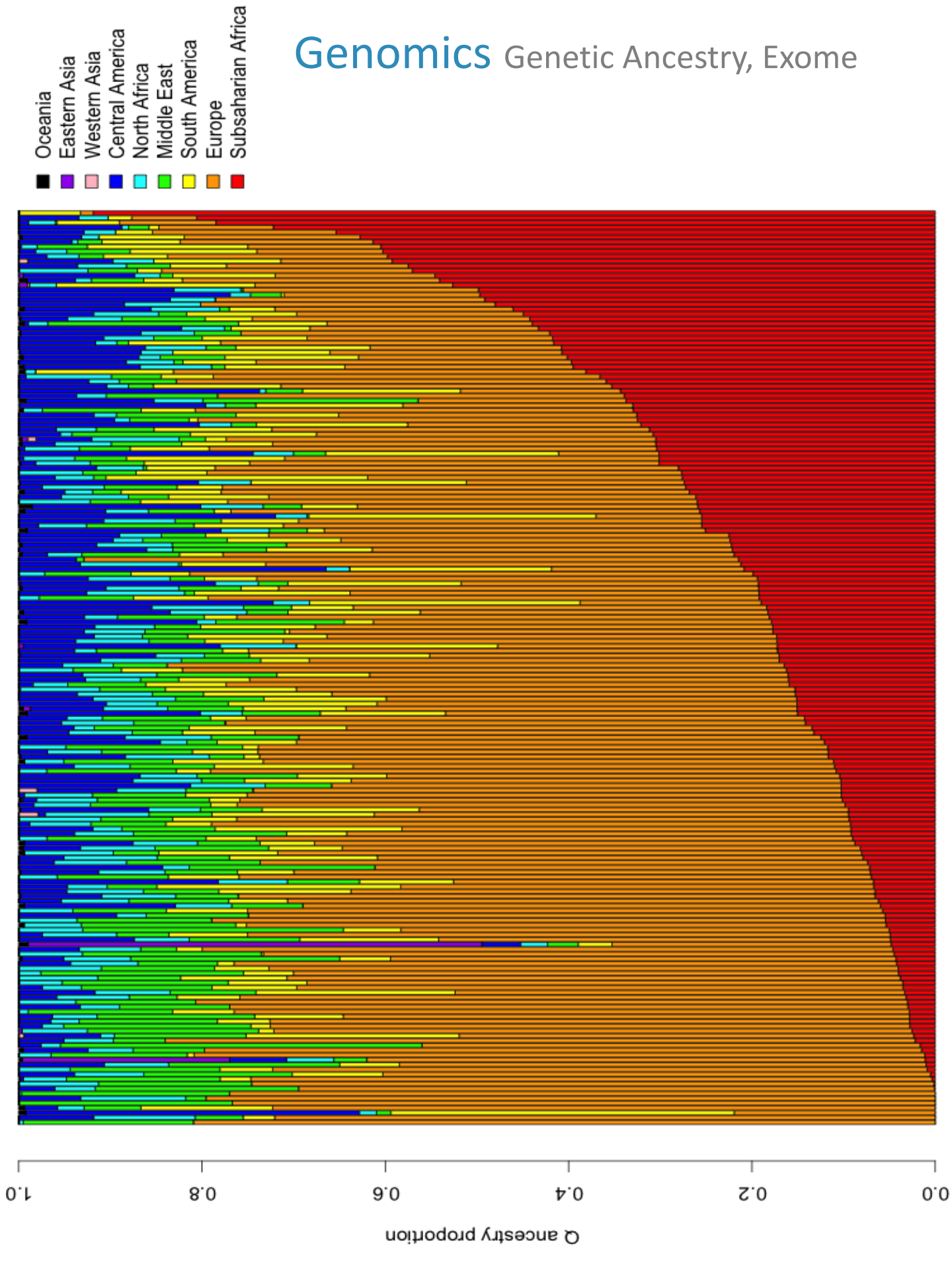
PLP (Vit B6)
CoA
Pantothenic (Vit B5)

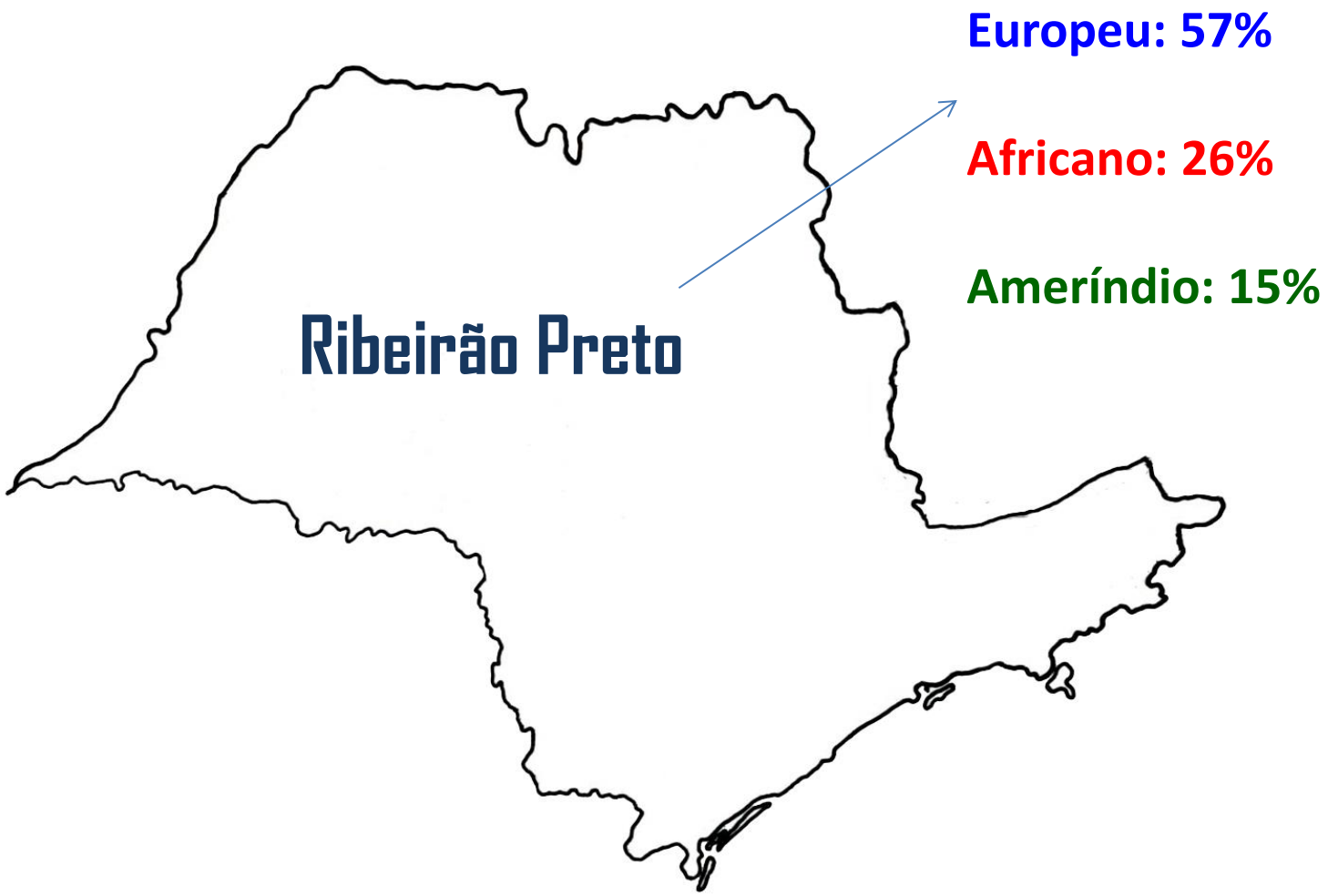
MetalMACiE: the database of catalytic metal ions

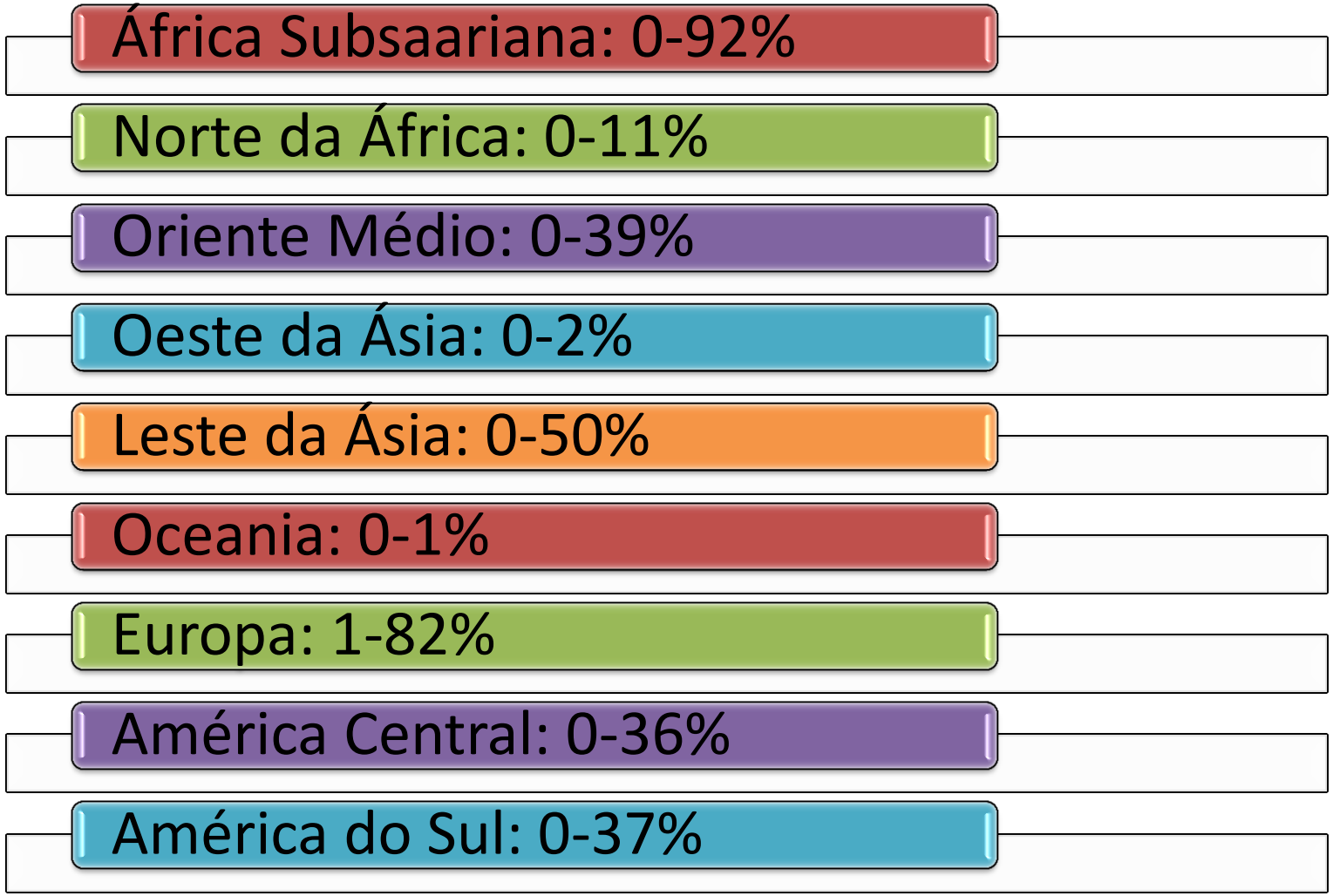
UniProt

SIB ExPASy Bioinformatics Resource Portal

Genomics Genetic Ancestry, Exome




Europeu: 57%
Africano: 26%
Ameríndio: 15%
Ribeirão Preto


África Subsaariana: 0-92%
Norte da África: 0-11%
Oriente Médio: 0-39%
Oeste da Ásia: 0-2%
Leste da Ásia: 0-50%
Oceania: 0-1%
Europa: 1-82%
América Central: 0-36%
América do Sul: 0-37%

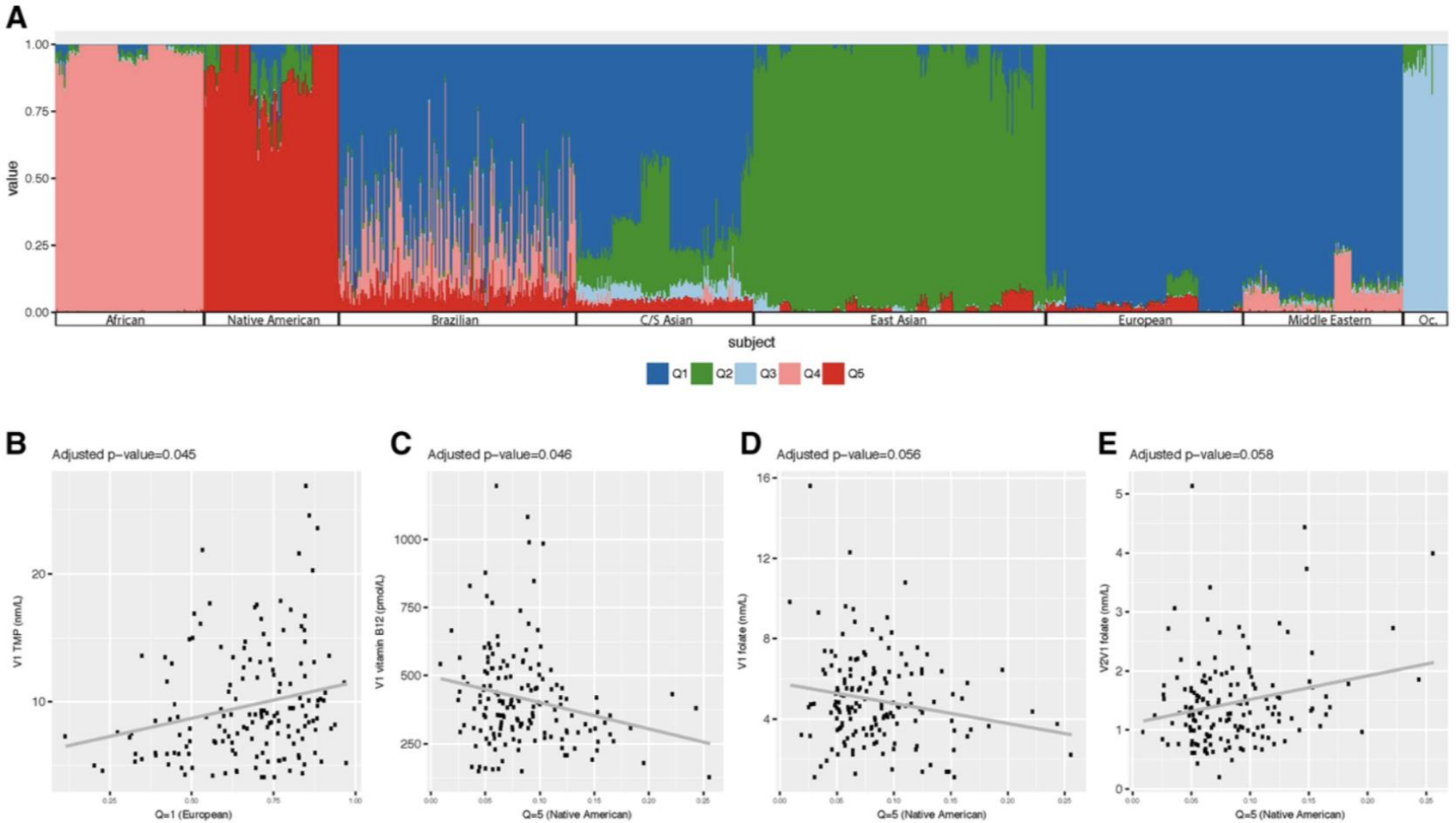


Figure 2. Admixture analysis. Influence of genetic ancestry on baseline vitamin levels. Ancestry markers from the Human Genome Diversity Project (HGDP) reference populations were used A) to identify admixture in data from unrelated participants from both years as per methods. To test whether linear regression between the ancestral components and baseline vitamin levels existed, a $k = 5$ model was used to the following covariates: trial year, sex, age, fat mass, and tanner score. Adjusted p-value of 0.05 was used as significance threshold. B) Baseline TMP and Q1 (Europe) with estimate of regression coefficient (ERC) 4.57, C) baseline vitamin B12 and Q5 (Native American) ECR = 186.53, D) baseline folate and Q5 (Native American) ECR = 2.13, and E) folate response as ratio of V2/V1 and k5 (Native American) with ECR = 0.77.

Brazil Micronutrient Project

Food history and micronutrient profile and their relation to DNA damage in children in Brazil

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Background

Dietary pattern of children and adolescents:

↑ High energy density food ↓ Fruits and vegetables

→ Insufficient intake of vitamins and minerals

Micronutrients → cellular protection as antioxidants

Repair DNA damage

Unrepaired DNA damage can lead to the development of carcinogenic or mutagenic changes in cells

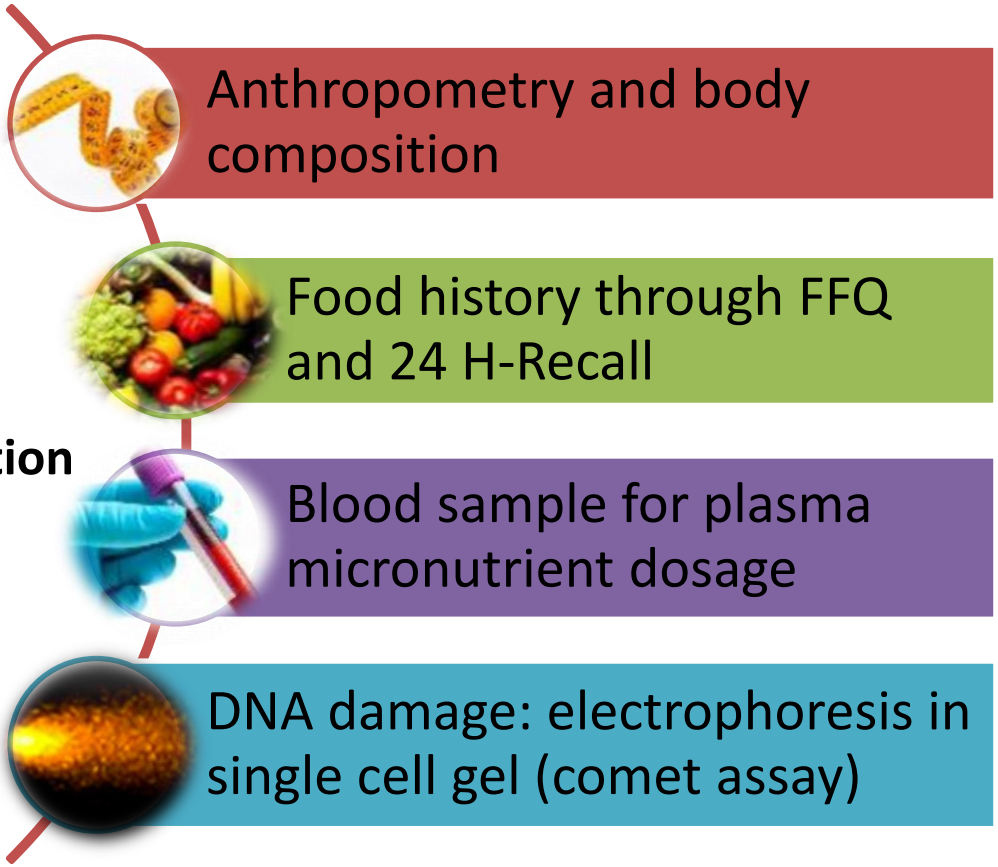
TORAL N et al, 2007. BENEDET J. et al, 2013. KRAUSE, 2002. BENEDET J et al, 2013. COMINETTI C et al, 2011.

Objective

To investigate the association between DNA damage and nutritional status in 9 to 13 years old children and adolescents

Methods

Data collection

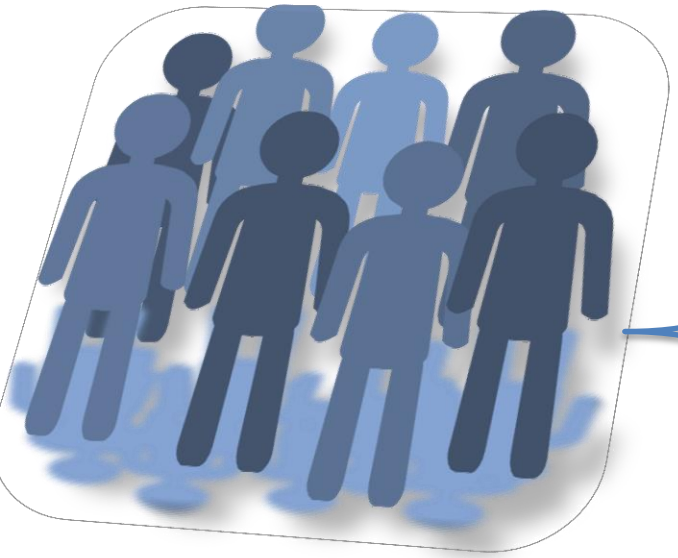


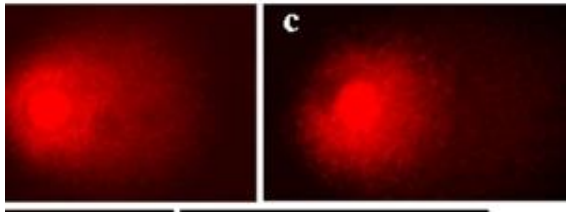
9 to 13 years old healthy children
Total of 141 subjects

After exclusion of under and over diet reports: 120 subjects

Software SPSS 20.0 for Statistical Analysis

Results – Population data



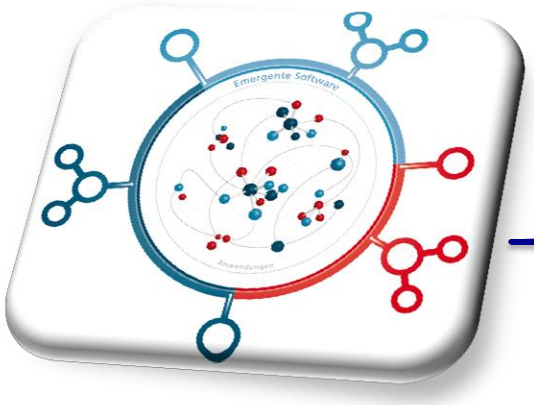


Tail intensity: 10.32% ± 4.35
Tail moment: 4.78 ± 2.46
(no differences by sex and pubertal status)

Body Mass Index/Age*		Frequency (n / %)
< percentil 3	Very thin	4 (3.3%)
percentil 3 - percentil 15	Slimness	9 (7.5%)
percentil 15 - percentil 85	Eutrophic	51 (42.5%)
percentil 85 - percentil 97	Overweight	27 (22.5%)
> percentil 97	Obesity	29 (24.2%)

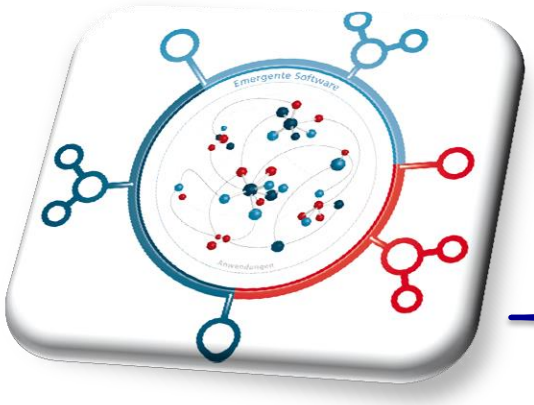
Body Mass Index according to OMS 2007 5 to 19 years

Results – Cluster Analysis (Tail intensity/tail moment)



Variables	Cluster 1 n = 73	Cluster 2 n = 47	p value
Age (years)	11.49 ± 1.04	11.28 ± 1.16	0.29
Sex (% M/F)	43.8/56.2	46.8/53.2	0.75
Fat mass (% body weight)	24.77 ± 6.72	24.37 ± 7.69	0.76
Tail intensity (%)	7.08 ± 2.24	15.35 ± 2.40	< 0.001
Tail moment	3.38 ± 1.23	6.97 ± 2.30	< 0.001

Results – Cluster Analysis (Tail intensity/tail moment)

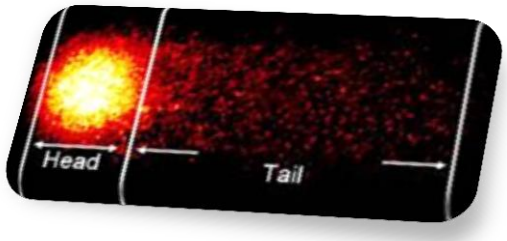


Variables	Cluster 1 n = 73	Cluster 2 n = 47	p value
Total vegetables	3.13 ± 2.04	2.36 ± 1.93	0.041
Green, yellow, orange and red vegetables	3.47 ± 2.10	2.67 ± 2.19	0.047
Milk and dairy	4.92 ± 3.62	6.63 ± 3.02	0.008
Meat	10.0 (10.0 – 10.0)	10,0 (8.8 – 10.0)	0.022
Healthy Eating Index	56.7 ± 10.0	52.7 ± 9.2	0.03
Plasma riboflavin (µmol/L)	4.17 ± 3.11	2.68 ± 1.83	0.03

Moser et al 2011
Collins, 1998.
Konopacka, 2000

Separation into 2 groups according DNA damage

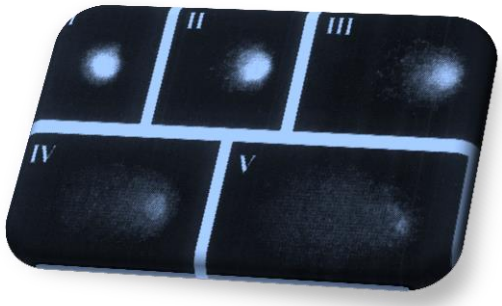
(Wollowski et al. 1999; Giovannelli et al 2002)



- **Comet assay:** fragments of damaged DNA are dragged in electrophoresis, forming a tail

- Tail intensity values:

  → Measures % of DNA in tail (damaged DNA)



- Classification proposed by Wollowski et al (1999)
- **Group 1:** 0 to 17% of damage (n=108)
- **Group 2:** ≥17,1% of damage (n=12)

class I: 0–6%; class II: 6.1–17%; class III: 17.1–35%; class IV: 35.1–60%; class V: 60.1–100%

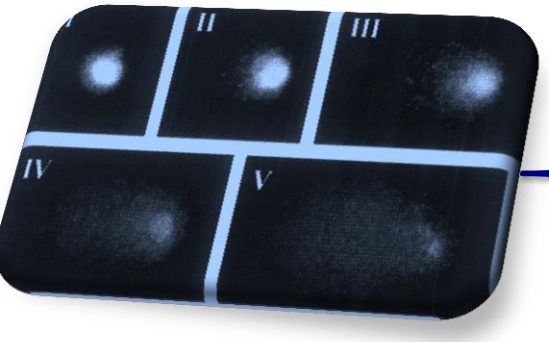
Results – Cluster Analysis (Wollowski et al. 1999; Giovannelli et al 2002)

COLLINS et al, 1998

MANTHEY et al, 2006

MINNET et al, 2011

KONOPACKA et al, 2001
MORIN et al, 2007

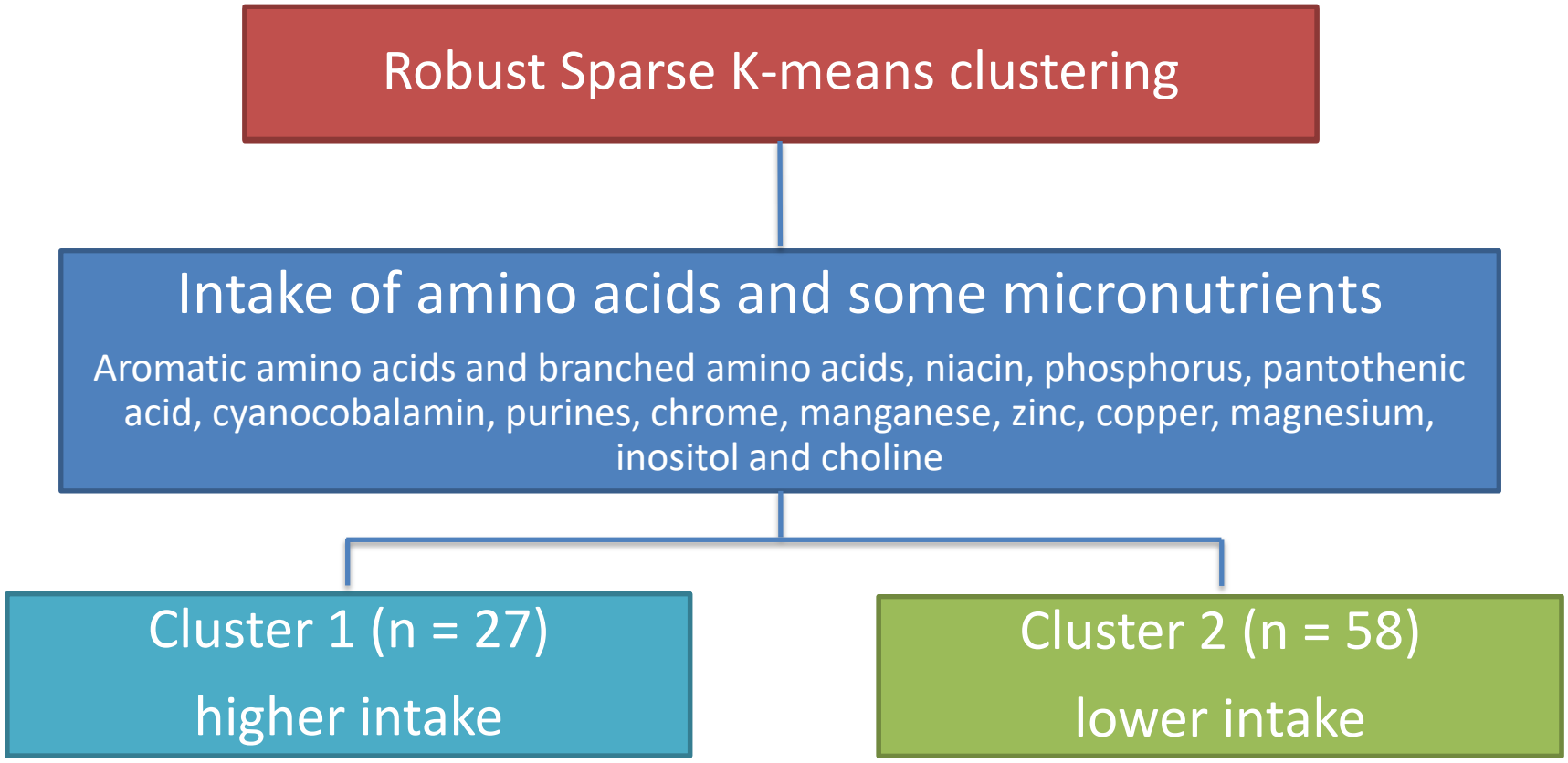


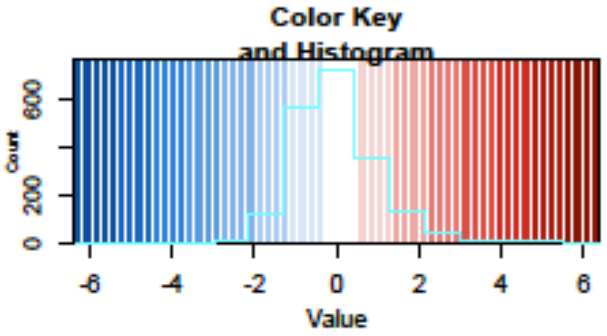
Tail intensity

class I: 0–6%; class II: 6.1–17%; class III: 17.1–35%; class IV: 35.1–60%; class V: 60.1–100%

Variables	Cluster 1 n = 108	Cluster 2 n = 12	p value
Age (years)	11.43 ± 1.09	11.25 ± 1.14	0.60
Sex (%, M/F)	46.3/53.7	33.3/66.6	0.40
Tail intensity (%)	9.39 ± 3.91	18.68 ± 0.93	< 0.001
Fat mass (%)	25.02 ± 7.05	20.97 ± 6.56	0.06
Overweight (%)	50.6	8.3	0.047
Plasma retinol (µmol/L)	0.35 ± 0.08	0.27 ± 0.09	0.01
Plasma – βcarotene (µmol/L)	0.22 ± 0.13	0.15 ± 0.10	0.02
Plasma riboflavin (µmol/L)	3.10 (1.57 – 5.07)	1.57 (1.13 – 3.00)	0.04

Nutrient intake patterns





Heatmap: separation of the accurate FFQ reporters into 2 dietary patterns

n = 27

n = 58

Tail intensity (%)
9.41 ± 4.17

Energy intake (kcal)
1854.51 ± 322

Tail intensity (%)
11.54 ± 4.90

Energy intake (kcal)
2234 ± 536

p = 0.06

p = 0.001

ffq_valine
ffq_phenylalanine
ffq_leucine
ffq_tryptophan
ffq_isoleucine
ffq_aromatic_aas
ffq_aas_branched
ffq_phosphorus
ffq_pantothenic
ffq_cyanocobalamin
ffq_proteins
ffq_niacin
ffq_purines
ffq_chrome
ffq_uric_acid
ffq_sulfur
ffq_zinc
ffq_manganese
ffq_inositol
ffq_magnesium
ffq_copper
ffq_acid
ffq_choline

This study confirms the protective effect of micronutrients against DNA damage

Children are increasing energy density food intake lacking in micronutrients

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