Nutritional Epidemiology in the Genomic Age

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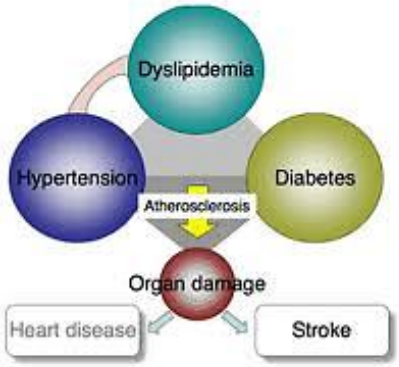
Learning objectives

- Types of genetic association approaches and their relevance to nutritional research

- Models used to analyze associations of genetic variants with disease phenotypes and gene-nutrient interactions



Gene X Gene

Gene X Environment (diet)

Environmental (diet) Factors

Genetic Factors

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Research question??

- What are the genes that affect nutrient metabolism?

Or

- How do our nutrient or diet intake affect the expression of a gene?

Nutritional Epidemiological approaches

- Correlation studies
- Special exposure groups
- Migrant studies
- Case control and cohort studies
- Controlled trials

Willett W. Overview of Nutritional Epidemiology
gDOI:10.1093/acprof:oso/9780199754038.003.0001

Genetic Epidemiological approaches

- Case studies
- Cross sectional studies
- Cohort
- Case-control
- Family-based, twin and trio studies
- Clinical trials

Defining the phenotype and genotype

- Phenotype
  - Is it properly defined?
  - Is it genetically controlled?
  - Is it likely to have effects mediated by a given environmental factor?

- Genotype
  - Does it show evidence for linkage, association, or interaction?
  - Are the SNPs in promoter, intron or exon?
  - What do we know about the SNP?

Biological samples and other data

- Nutrient data
- DNA (from blood, saliva and other tissues)- genotyping or sequencing, epigenetics
- RNA (from blood or tissues) – transcriptomic profiles
- Blood (serum or plasma) –biochemical variables, metabolomics, proteomics, lipidomics, etc
- Urine – biochemical variables, metabolomics
- Data such as age, sex, BMI, etc
- Medical history
- Environmental factors

How can variants affect phenotypes?

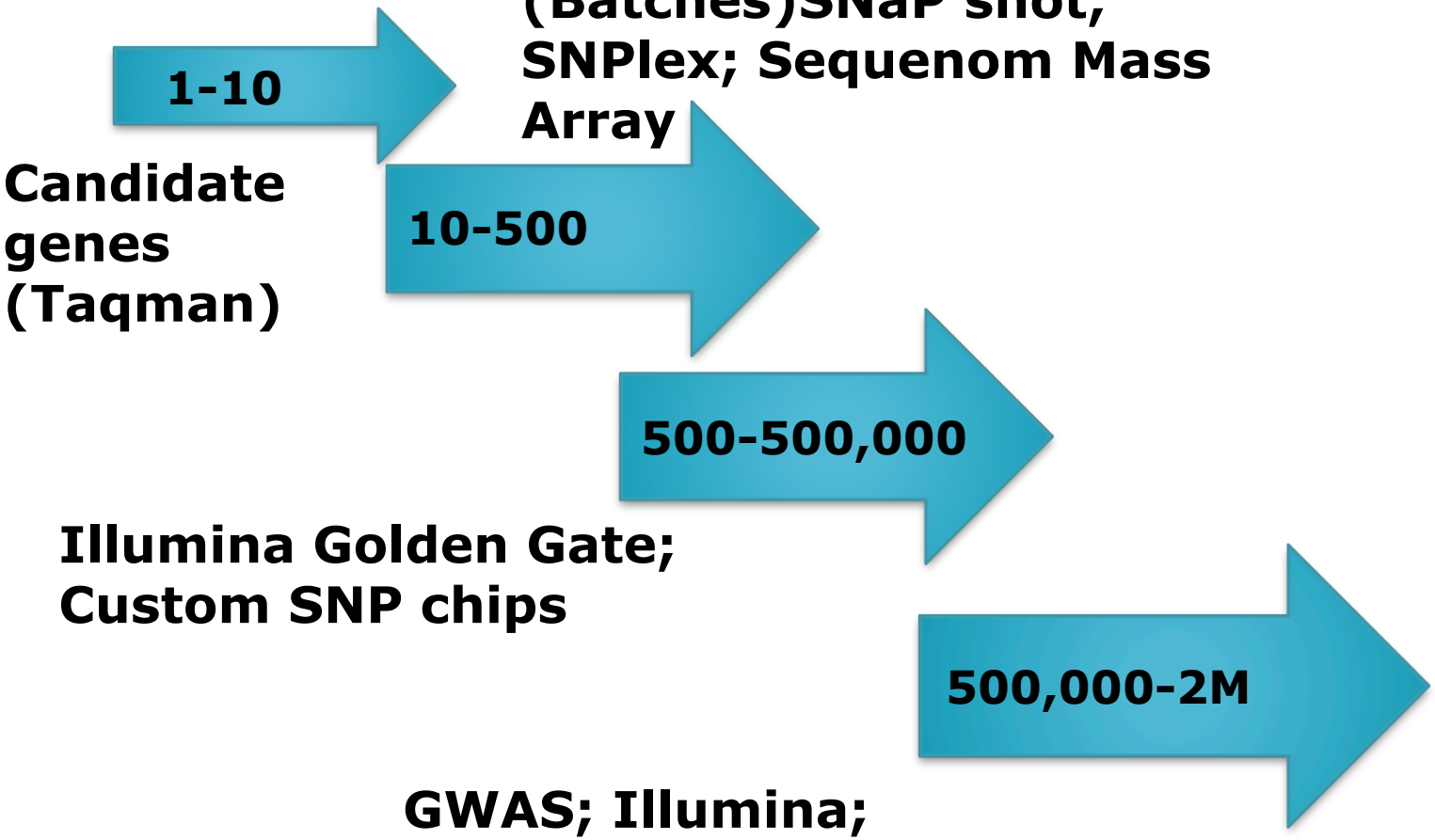
- silent; most variants have no effect
- Altered protein sequence – nonsynonymous, nonsense, splice, stop loss
- Altered RNA processing
- Altered RNA expression (regulatory)
- Other

Approaches to genotyping

- Candidate genes:
- genotype only markers in genes potentially related to the trait

- Genome screen:
- genotype anonymous markers spanning the genome at regular intervals

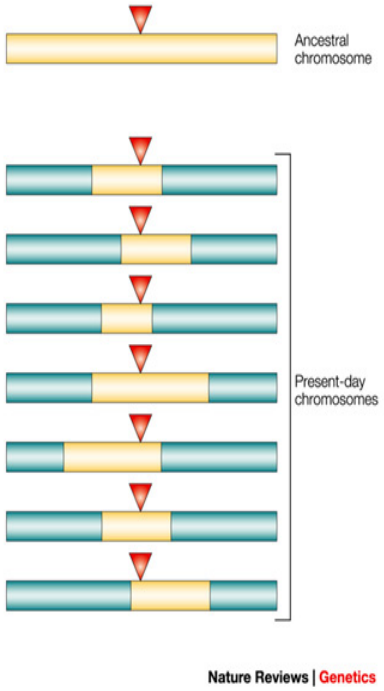
Genotyping



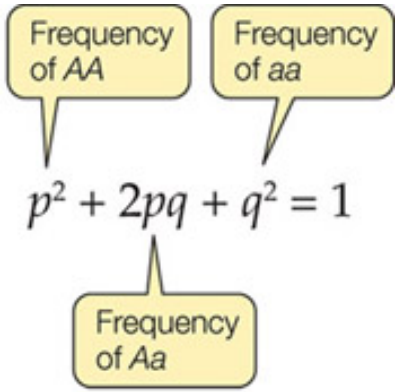
Adapted from Edenberg and Liu. Cold Spring Harbor Laboratory Press; 2009

Terminology

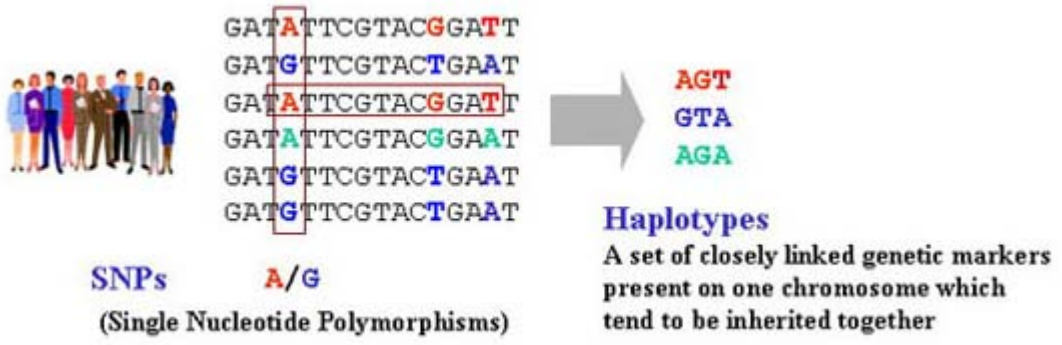
Linkage Disequilibrium



Hardy-Weinberg Principle



Haplotype



Ardlie, Kruglyak & Seielstad
Nature Reviews Genetics, 2002; 3, 299-309

Genome-wide Approaches

LINKAGE

Design:Family
Phenotypes:Quantitative /Qualitative Phenotypes
Markers:STR or SNP
Information:Segregation (IBD)

ASSOCIATION

Design:Case-Control / Family
Phenotypes:Quantitative /Qualitative Phenotypes
Markers:STR or SNP
Information:Linkage Disequilibrium (IBS)

Modeling in genetic epidemiology

- Homozygote (AA) –
- 2 copies of major allele ('common')

- Heterozygote (Aa) –
- 1 copy of major allele and 1 of minor allele

- Homozygote (aa) –
- 2 copies of minor allele ('variant')

Modeling in genetic epidemiology

- the mode of inheritance
  - 1. Additive
  - 2. Dominant
  - 3. Recessive

- With family data/ pedigrees – assess mode of inheritance

- BUT….Can't be done in studies of unrelated individuals, complex with common traits
- Statistical power is reduced if you specify the wrong model

Recoding for alternative models

	Additive	Dominant	Recessive
AA	0	0	0
AG	1	1	0
GG	2	1	1

- Dominant model combines AG+GG
  - Only need one copy of rare allele for disease
  - Used when frequency of GG is low
- Recessive model combines AA & AG
  - Have to have 2 copies of rare allele (G) for disease
  - Rarely used

Power calculation

- http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-9-36 (PGA-Power calculator for case-control genetic association studies)
- http://www.biostat.ucsf.edu/sampsize.html
- http://homepage.stat.uiowa.edu/~rlenth/Power/
- http://biomath.info/power/
- http://pngu.mgh.harvard.edu/~purcell/gpc/

Genetic association tools

- http://goldenhelix.com/products/SNP_Variation/index.html
- http://genemapping.org/online material/online-resources
- http://www.broadinstitute.org/scientific-community/software?criteria=Genetic%20Analysis
- http://bmcresnotes.biomedcentral.com/articles/10.1186/1756-0500-4-158
- http://www.biostat.wustl.edu/genetics/geneticssoft/SoftwareList.htm

Genetic association tools

- http://www.stats.ox.ac.uk/~marchini/software/gwas/gwas.html
- http://www.disgenet.org/web/DisGeNET/menu;jsessionid=16q535dpjpour10rfwtudqdjt4
- http://biostats.usc.edu/software
- http://pngu.mgh.harvard.edu/~purcell/

XWAS: A Software Toolset for Genetic Data Analysis and Association Studies of the X Chromosome

Feng Gao*, Diana Chang*, Arjun Biddanda*, Li Ma, Yingjie Guo, Zilu Zhou, and Alon Keinan

Websites

- UCSC Genome Browser – https://genome.ucsc.edu/cgi-bin/hgGateway

- NCBI Map Viewer http://www.ncbi.nlm.nih.gov/projects/mapview/

Others

- Online Mendelian Inheritance in Man
  http://www.ncbi.nlm.nih.gov/omim

- Gene
  http://www.ncbi.nlm.nih.gov/gene

- Gene Cards
  http://www.genecards.org/

Others

- HAPMAP

http://hapmap.ncbi.nlm.nih.gov/

- ENCODE

https://genome.ucsc.edu/ENCODE/

Or

http://www.genome.gov/10005107

LocusZoom

- http://locuszoom.sph.umich.edu/locuszoom/
- a tool to plot regional association results from genome-wide association scans or candidate gene studies. This is Version 1.1

Effects of gene-environment interaction on phenotypes

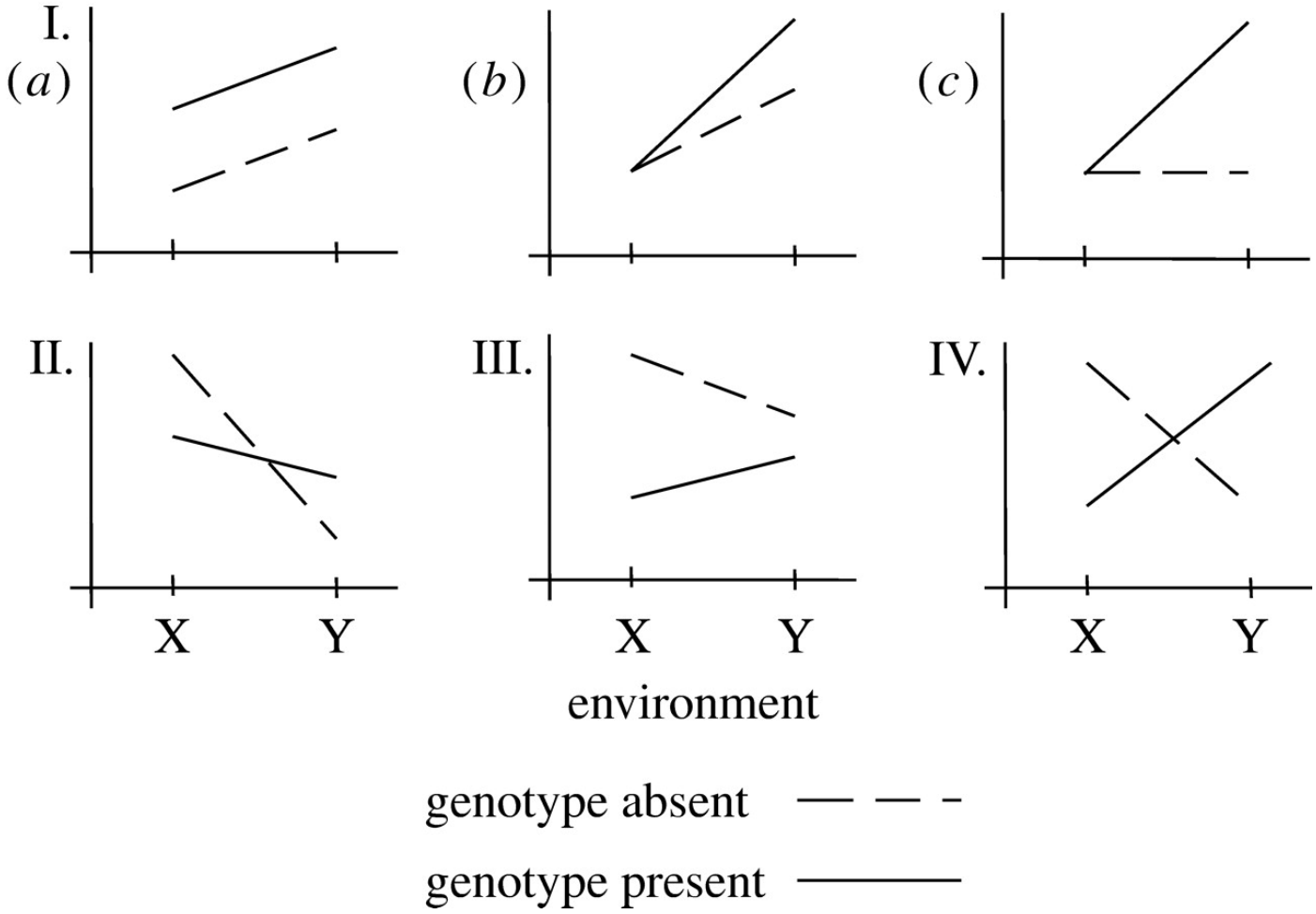
What is Gene-Environment Interaction (GEI)?

- Distinct effects of an environmental factor in individuals with different genotypes

or

- Distinct effects of a genotype in two different environments

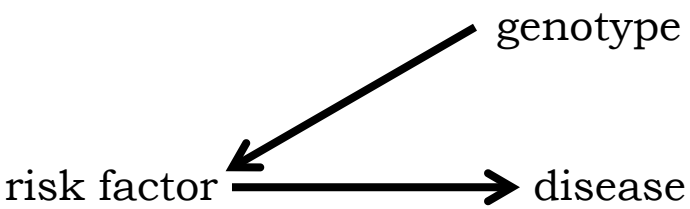
Gene-Environment Interaction



Kraft and Hunter.. 2005. Philosophical Transactions of the Royal Society B.

Gene by nutrient interaction effects on metabolic disease

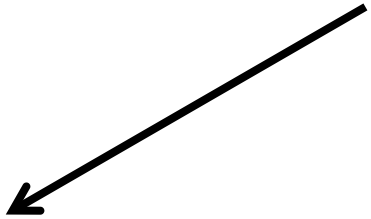
Model I -Phenylketonuria



Model 1

Genotype increases the expression of risk factor

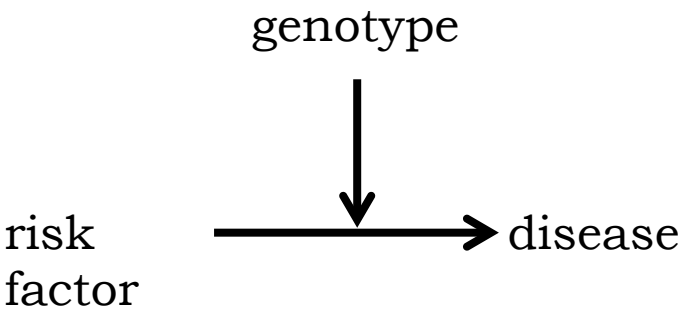
Mutation in phenylalanine hydroxylase



High levels of phenylalanine in blood

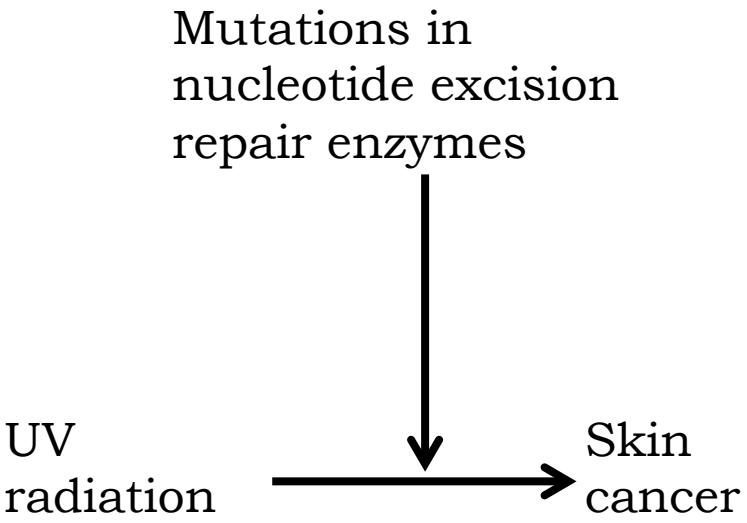
PKU

Model II – Xeroderma Pigmentosum

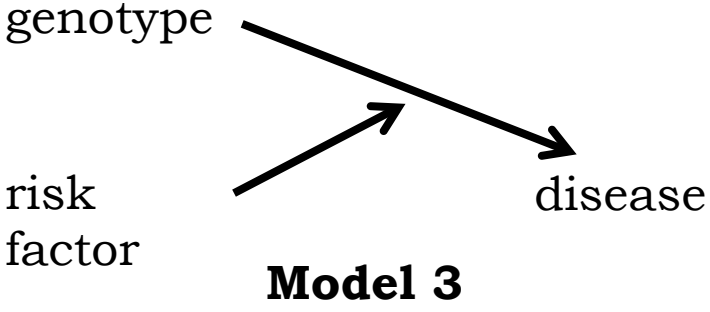


Model 2

Genotype excarbates the effect of the risk factor

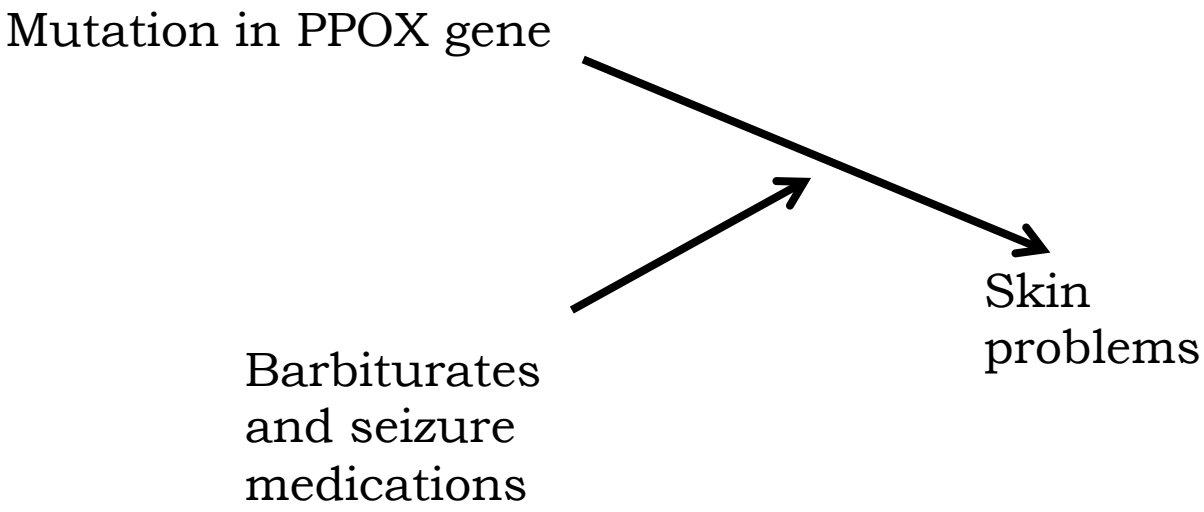


Model III- Porphyria variegate

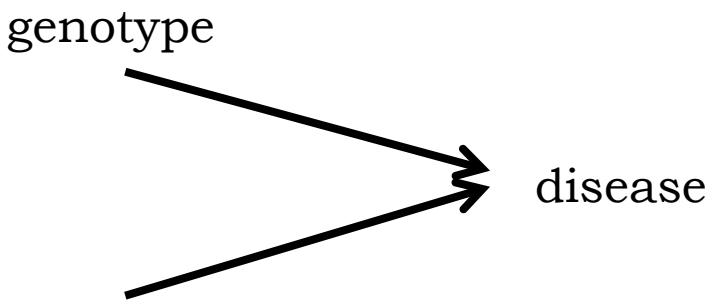


Model 3

The risk factor excarbates the effect of the genotype

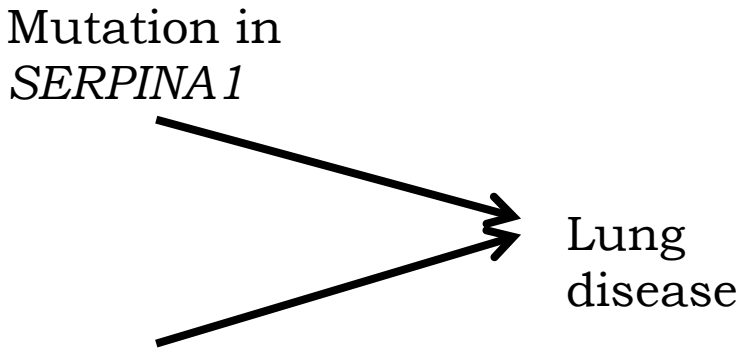


Model IV- alpha-1 antitrypsin deficiency



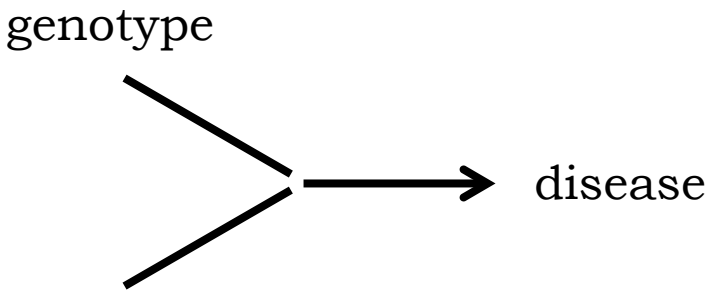
Genotype and risk factor each influence the risk by themselves

Model 4



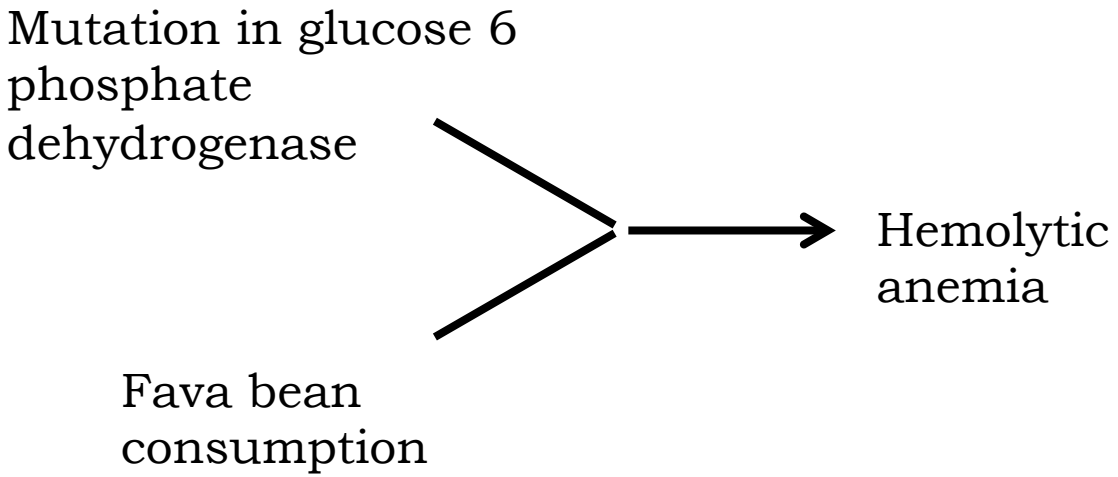
Model 4

Model V-G6PD deficiency



Both genotype and risk factor are required to raise the risk

Model 5



Nutrigenetic differences

- Most of them may have been inherited from our ancestors
- Genetic variation affects food tolerances among populations
- Nutritional environments seem to be the major determinants of human variation evolution
- Populations vary in their requirements for foods and response to diet

SNP by Environment Interaction

Main effects model:

- $T(E) = \beta^{M}_{0i} + \beta^{M}_{1i}E + \beta^{M}_{2i}SNP$

Interaction effects model:

- $T(E) = \beta^{I}_{0i} + \beta^{I}_{1i}E + \beta^{I}_{2i}SNP + \beta^{I}_{3i}SNPxE$
  - $T(E)$ = variation in the phenotype T,
  - β^{M} = coefficients related to main effects,
- β^{M} = coefficients related to interaction effects,
  - E = environmental factor,
- SNP is usually coded as 0,1 and 2 based on the number of rare alleles, and
  - SNP x E= interaction term

Example

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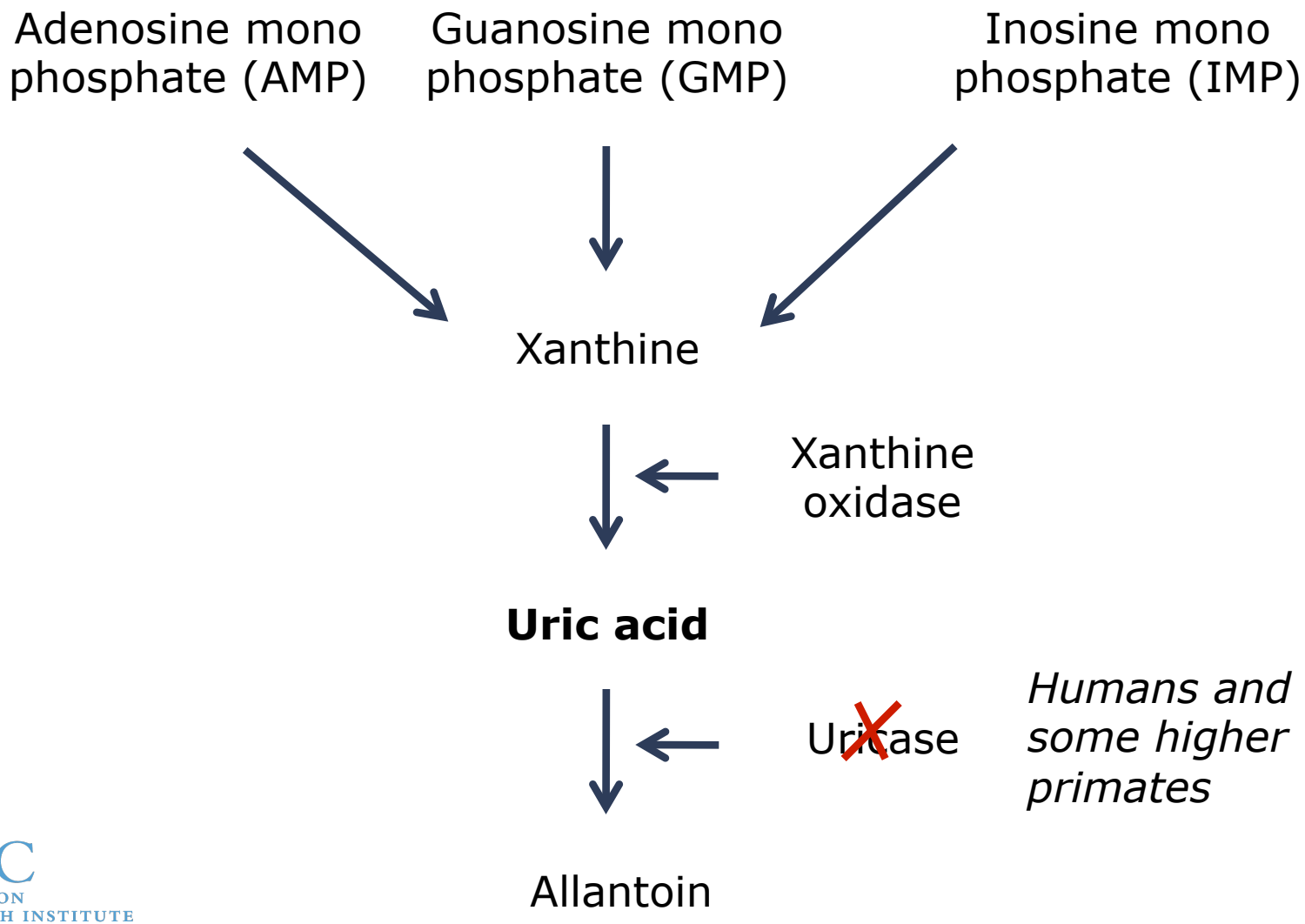
Serum uric acid



Strong Heart Family study (SHFS) [PI: Dr. Shelley Cole]

- Is a genetic study of CVD risk in American Indians

- It is the genetic component of the Strong Heart Study started in 1998

- More than 3800 members from multigenerational families enrolled from three centers located in Arizona, Dakotas and Oklahoma

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Viva La Familia [PI: Dr. Nancy Butte]

- Overweight/obese Hispanic children aged 4-19 years were recruited

- Some unique phenotypes such as calorimetry measurements, physical activity and energy expenditure have been collected

- Genome-wide SNP, exome and metabolomic data available

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Descriptives

	SHFS	VFS
Age	39.50 ± 17	11.0 ± 4
Serum uric acid (mg/dl)	5.1 ± 1.5	5.2 ± 1.7
Hyperuricemia (%)	17	25
Sugars intake (% of total calories)	16.3	22
Heritability (%)	46	45

SLC2A9* SNPs and serum uric acid levels (SHFS)**

	All			Arizona			Dakotas			Oklahoma		
SNP	Risk allele/ frequency	Effect size (%)[a]	P-value	Risk allele/ frequency	Effect size (%)	P-value	Risk allele/ frequency	Effect size (%)	P-value	Risk allele/ frequency	Effect size (%)	P-value
rs16890979	A/0.47	4.6	1.3E−31	A/0.55	3.8	3.8E−11	A/0.42	3.2	1.1E−8	A/0.43	5.1	7.8E−13
Post. Prob[b]		0		0.12			0			0		
rs6832439	A/0.47	4.5	7.7E−31	A/0.55	3.8	2.8E−11	A/0.41	3.3	1.5E−8	A/0.47	4.8	7.2E−12
Post. Prob[b]		0		0.09			0			0		
rs6449213	G/0.28	4.5	1.5E−29	G/0.37	3.0	1.6E−11	G/0.20	3.3	2.8E−9	G/0.27	6.4	1.2E−14
Post. Prob[b]		1.0		0.10			1.0			1.0		
rs13131257	A/0.48	4.5	1.8E−29	A/0.56	4.2	5.8E−12	A/0.42	3.3	5.0E−8	A/0.46	4.1	2.6E−10
Post. Prob[b]		0		0.43			0			0		
rs737267	A/0.49	4.4	2.9E−29	A/0.56	4.1	9.1E−12	A/0.45	3.1	4.5E−8	A/0.48	4.4	1.1E−10
Post. Prob[b]		0		0.21			0			0		
rs10805346	A/0.29	4.1	5.4E−28	A/0.21	4.1	2.4E−11	A/0.39	3.3	1.4E−8	A/0.34	2.7	1.6E−8
Post. Prob[b]		1.0		1.0			1.0			0		
rs12498956	C/0.47	3.4	5.1E−23	C/0.43	3.3	5.9E−10	C/0.49	2.1	1.0E−5	C/0.49	4.1	1.1E−9
Post. Prob[b]		0		0.05			0			0		

[a]Effect size – proportion of residual phenotypic variance that is explained by the SNP.
[b]Post. Prob – posterior probability of a functional effect. Posterior probability of >0.95 is considered as statistically significant evidence of functional effect.
Final model included covariates age, sex, age × sex, body mass index (BMI), eGFR, type 2 diabetes status, self-reported alcohol intake and medications.

Dietary variable	All		Arizona		Dakotas		Oklahoma	
	β (SE)	P value	β (SE)	P value	β (SE)	P value	β (SE)	P value
Alcohol intake	-0.219 (0.03)	5.6×10^{-10}	-0.187 (0.04)	4.1×10^{-6}	-0.175 (0.04)	9.0×10^{-5}	-0.192 (0.04)	3.2×10^{-6}
Protein intake	0.0007 (0.0002)	0.0004	0.0004 (0.0002)	0.16	0.0008 (0.0003)	1.8×10^{-2}	0.0012 (0.0004)	0.008
Simple sugars	0.0003 (0.0002)	0.82	-0.0009 (0.0003)	0.72	0.0014 (0.0003)	0.65	0.0007 (0.0004)	0. 768

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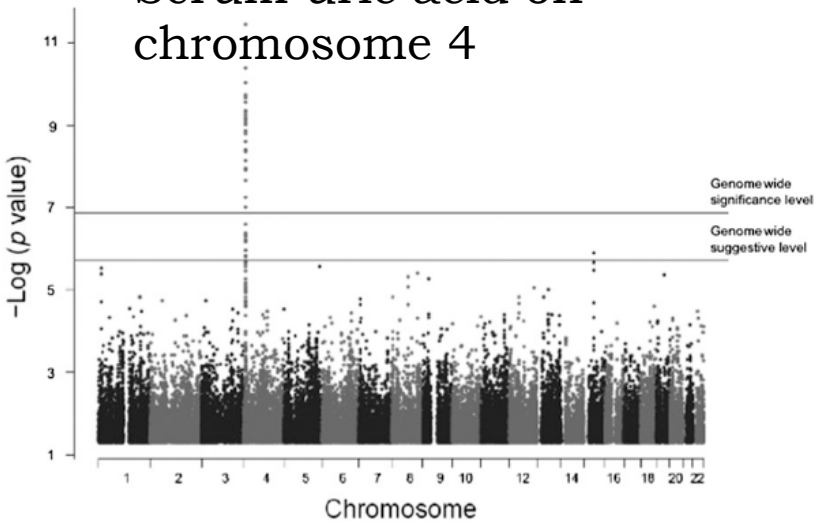
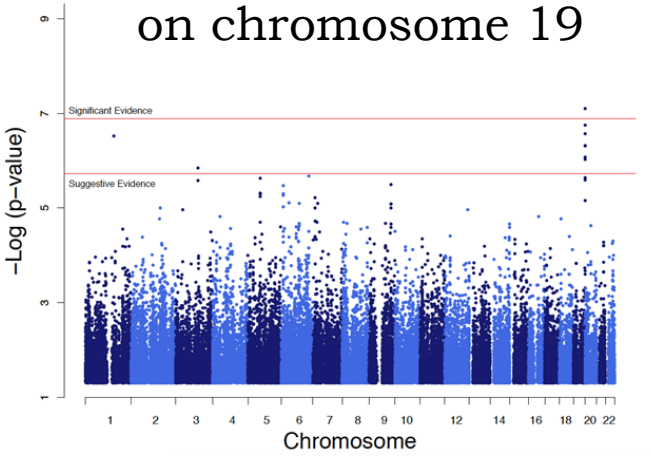
* Solute carrier family 2, member 9
** Voruganti et al., EJHG 2014

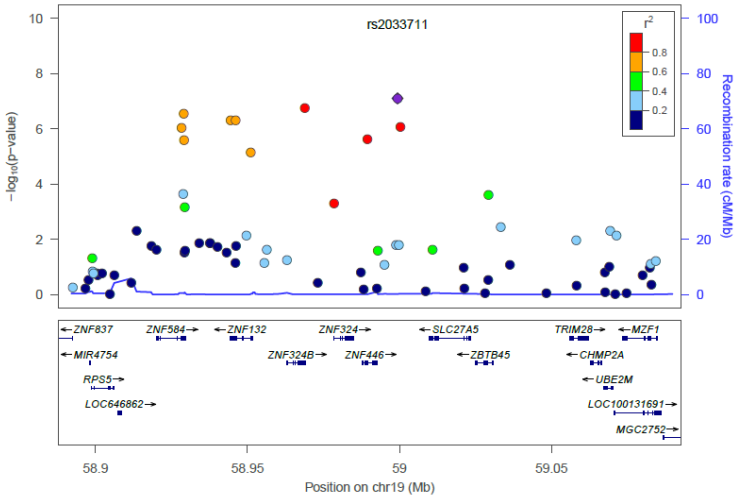
Genetic influence on serum uric acid and clearance

Serum uric acid on chromosome 4



Uric acid clearance on chromosome 19





Locus Zoom plot showing the most significant SNPs on chr 19q13

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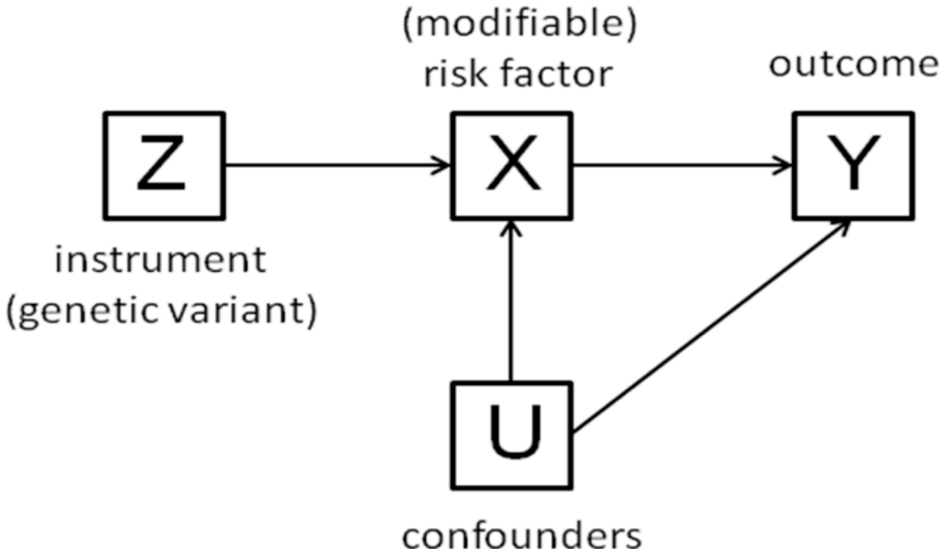
Mendelian randomization



SLC2A9 variants
(Instrument)

Serum uric acid
(risk factor)

Age, sex, age*sex
(Confounders)

Chronic kidney disease
(Outcome)

Association of SUA genetic risk score with kidney function markers

Study	Phenotype	B coefficient	SE	P value
Strong Heart Family study - All	Serum uric acid	0.0561	0.004	**3.5×10^{-38}**
	GFR	-0.0099	0.005	**0.04**
	UACR	-0.0095	0.005	0.05
	Serum creatinine	0.0202	0.005	**5.0×10^{-5}**
	GFR	-0.0165	0.009	0.05
	UACR	0.0085	0.008	0.24
	Serum creatinine	0.0152	0.008	0.07
Dakotas	Serum uric acid	0.0609	0.005	**1.0×10^{-33}**
	GFR	-0.0152	0.005	**3.0×10^{-3}**
	UACR	-0.0096	0.005	0.05
	Serum creatinine	0.0205	0.005	**4.0×10^{-5}**
Oklahoma	Serum uric acid	0.0602	0.005	**1.8×10^{-33}**
	GFR	-0.0144	0.005	**0.004**
	UACR	-0.009	0.005	0.06
	Serum creatinine	0.0198	0.005	**7.0×10^{-5}**
San Antonio Family Heart Study	Serum uric acid	0.0799	0.014	**4.7×10^{-8}**
	GFR	0.0018	0.013	0.89
	UACR	0.0456	0.013	**6.0×10^{-4}**
	Serum creatinine	-0.0106	0.013	0.42
Genetics of Kidney Disease in Zuni Indians	Serum uric acid	0.0587	0.010	**9.8×10^{-9}**
	GFR	-0.017	0.010	0.10
	UACR	0.0064	0.010	0.53
	Serum creatinine	0.0185	0.010	0.07
Genetics of Coronary Artery Disease in Alaska Natives	Serum uric acid	0.0361	0.009	**6.0×10^{-5}**
	GFR	-0.0116	0.013	0.37
	UACR	-0.0204	0.013	0.12
	Serum creatinine	0.0114	0.011	0.30

Dietary Factors affecting serum uric acid levels

Fructose
[Carbonated beverages, most canned products, honey]

High-purine foods and amino acids
[Organ meats such as liver, spleen, heart etc]

Alcohol

ATP depletion

AMP, GMP or IMP

Dehydration

Competition with uric acid for the same transporter (SLC2A9)

Hyperuricemia

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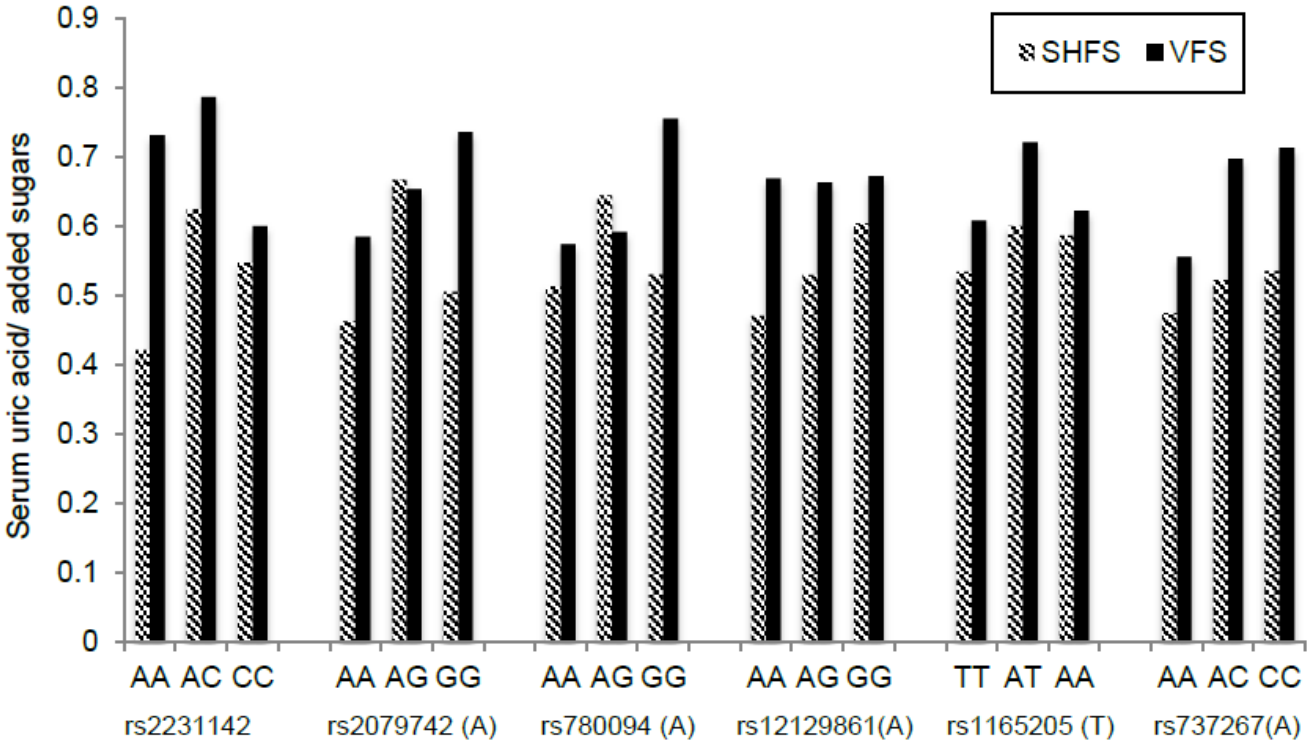
Uric acid and Fructose

- Uric acid is a byproduct of fructose degradation and shares a transporter with fructose (GLUT9/SLC2A9)
- Fructokinase is poorly regulated and phosphorylates fructose rapidly
- Fructose upregulates its transporter GLUT5 as well as fructokinase
- Serum uric acid increases rapidly after ingestion of fructose
- Fructose interferes with uric acid excretion

Genotype-specific differences in SUA/added sugars



Minor allele shown next to the SNP in parantheses; added sugars are shown as percent of calories

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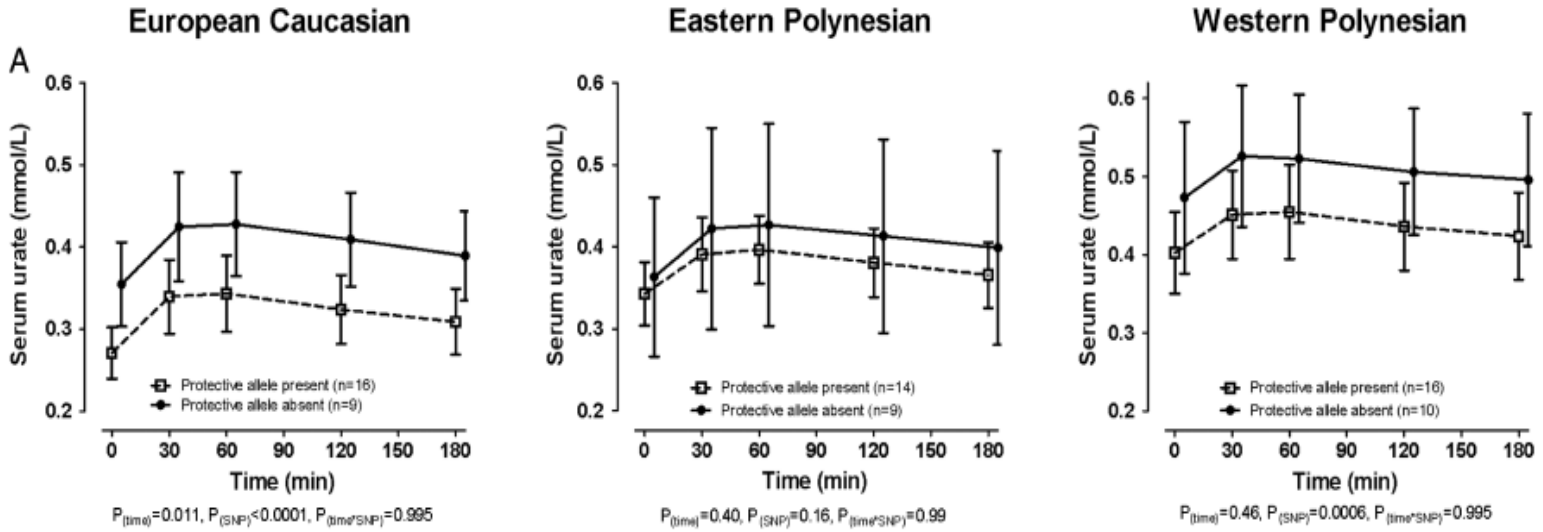
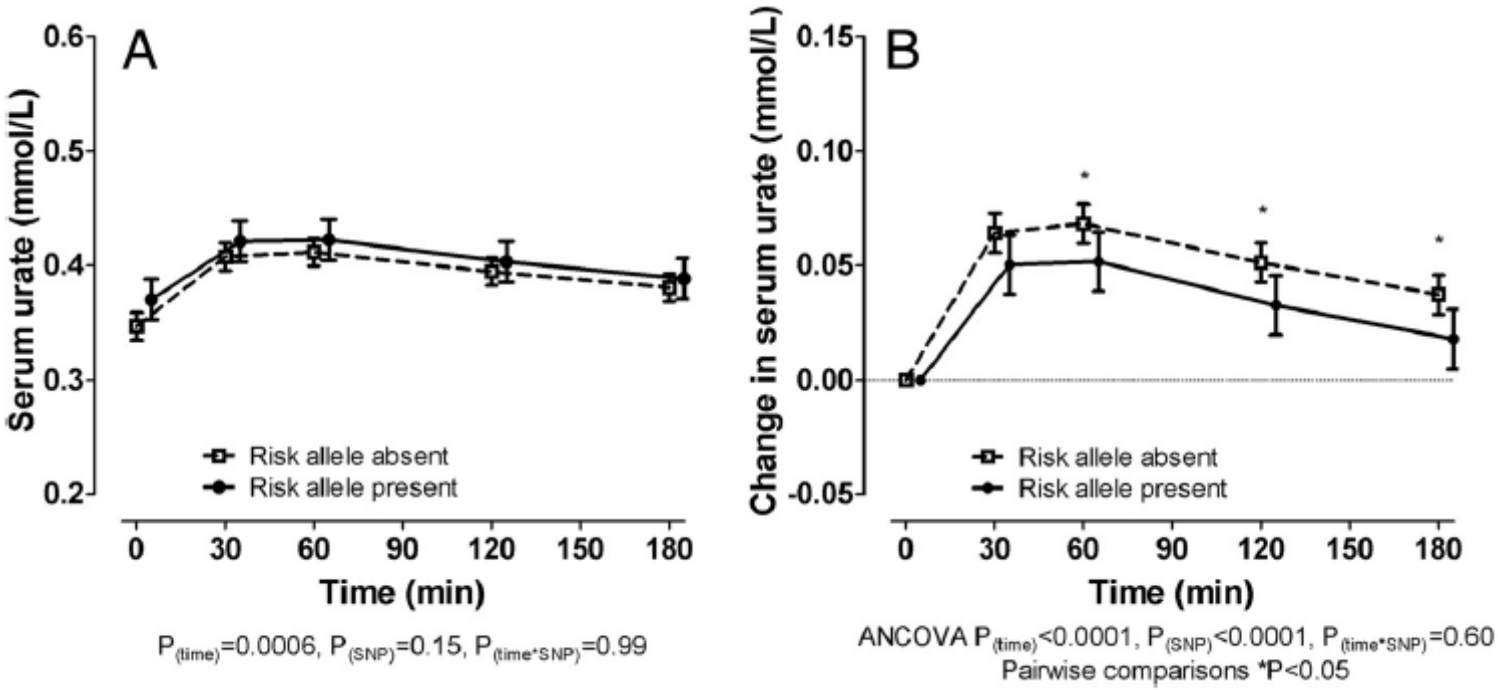
Population-specific effects of *SLC17A1* on serum uric acid concentrations during a fructose load



Dalbeth et al. Ann Rheum Dis. 2014; 73: 313-314

Effect of *ABCG2* genotype on serum uric acid concentrations during a fructose load



Dalbeth et al. Arthritis Research and Therapy. 2014; 16:R34

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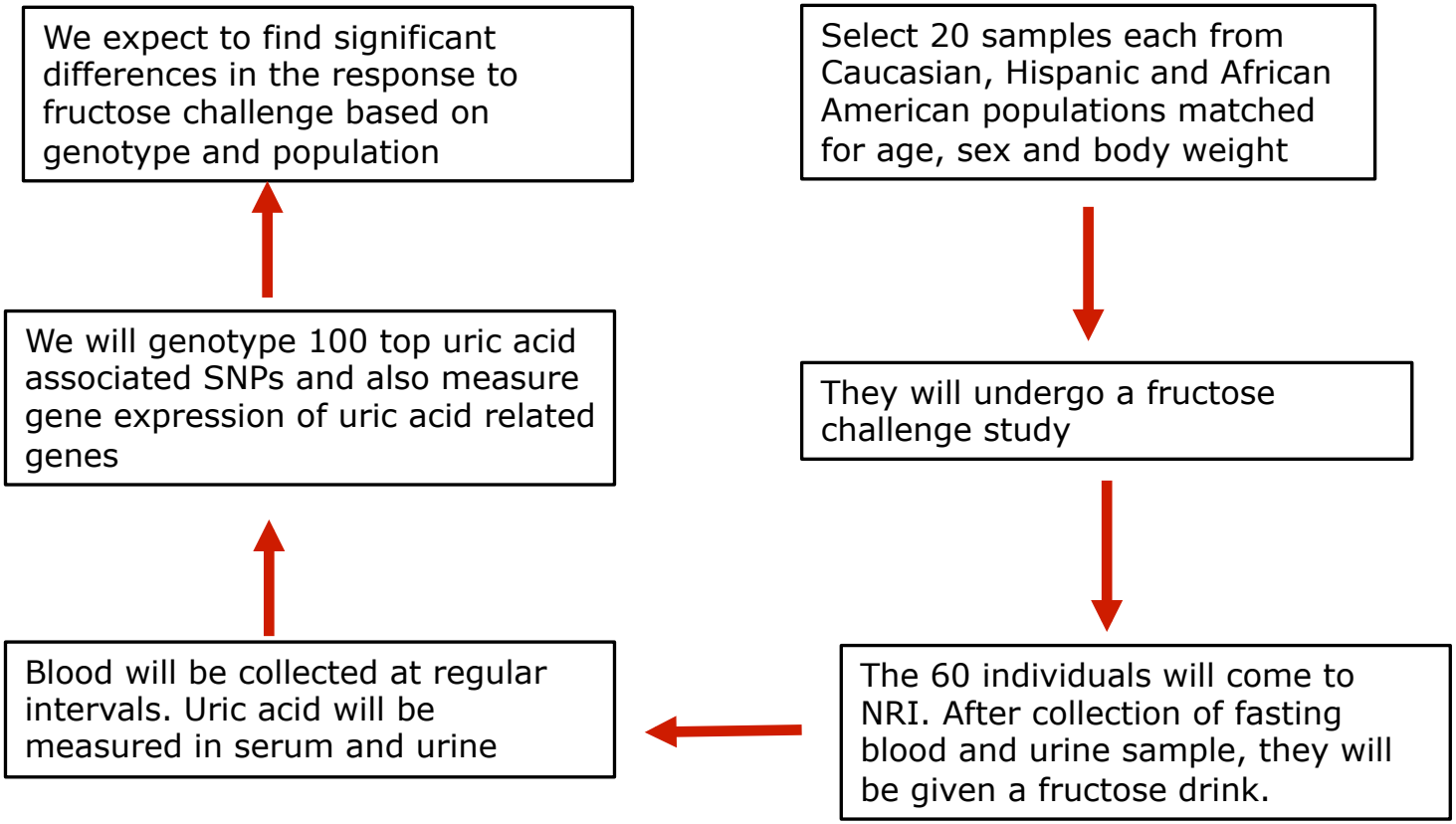
Genotype- and population-specific effects of fructose on uric acid related genes



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- **UNC NRI faculty and staff**
- **NIH Grants**
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- **Participants of all studies**
- **Collaborators**
  Texas Biomedical Research Institute, San Antonio
  Baylor College of Medicine, Houston
  MURDOCK Study, Duke University

- **Voruganti Lab**

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