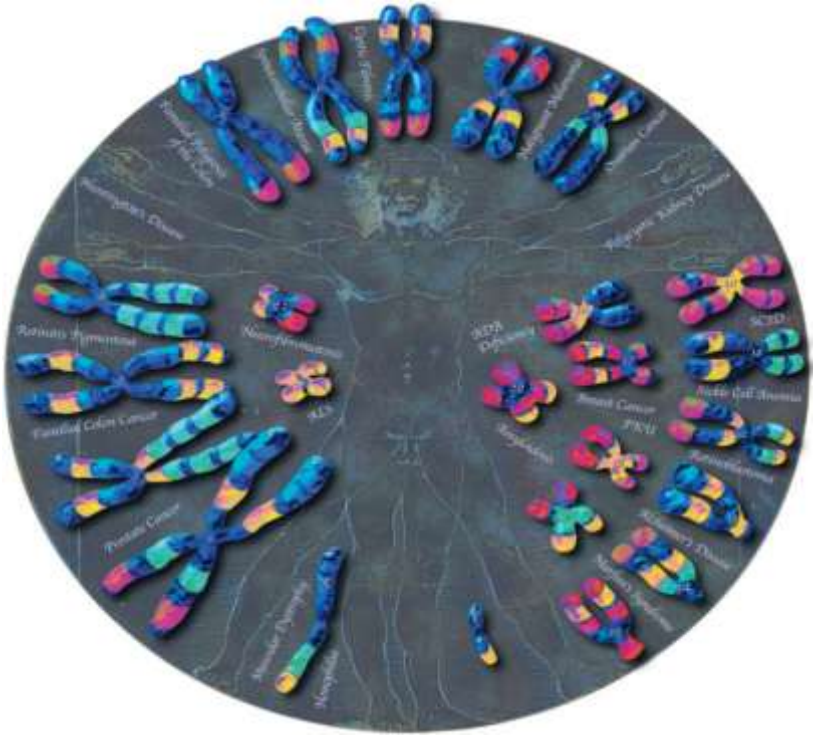
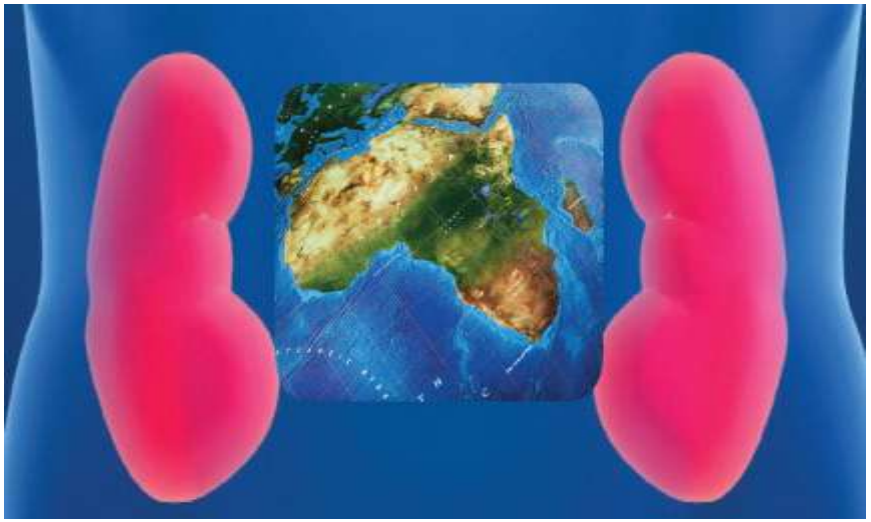
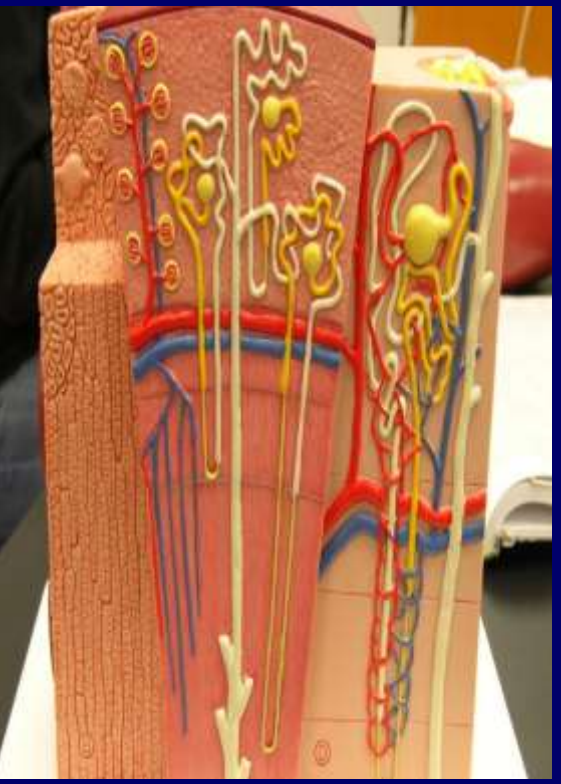
PART I

Mapping Genes in Kidney Health and Disease

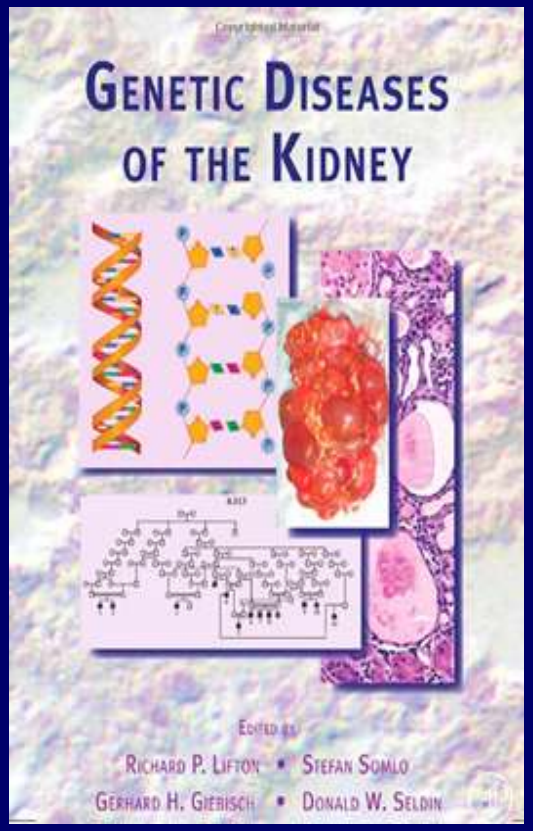




Convergence of Physiology and Genetics



- Clearances studies
- Isolated Kidney
- Micropuncture
- Isolated tubules and vesicles
- Expression Cloning
- Genetically engineered animal models
- Human Genetics



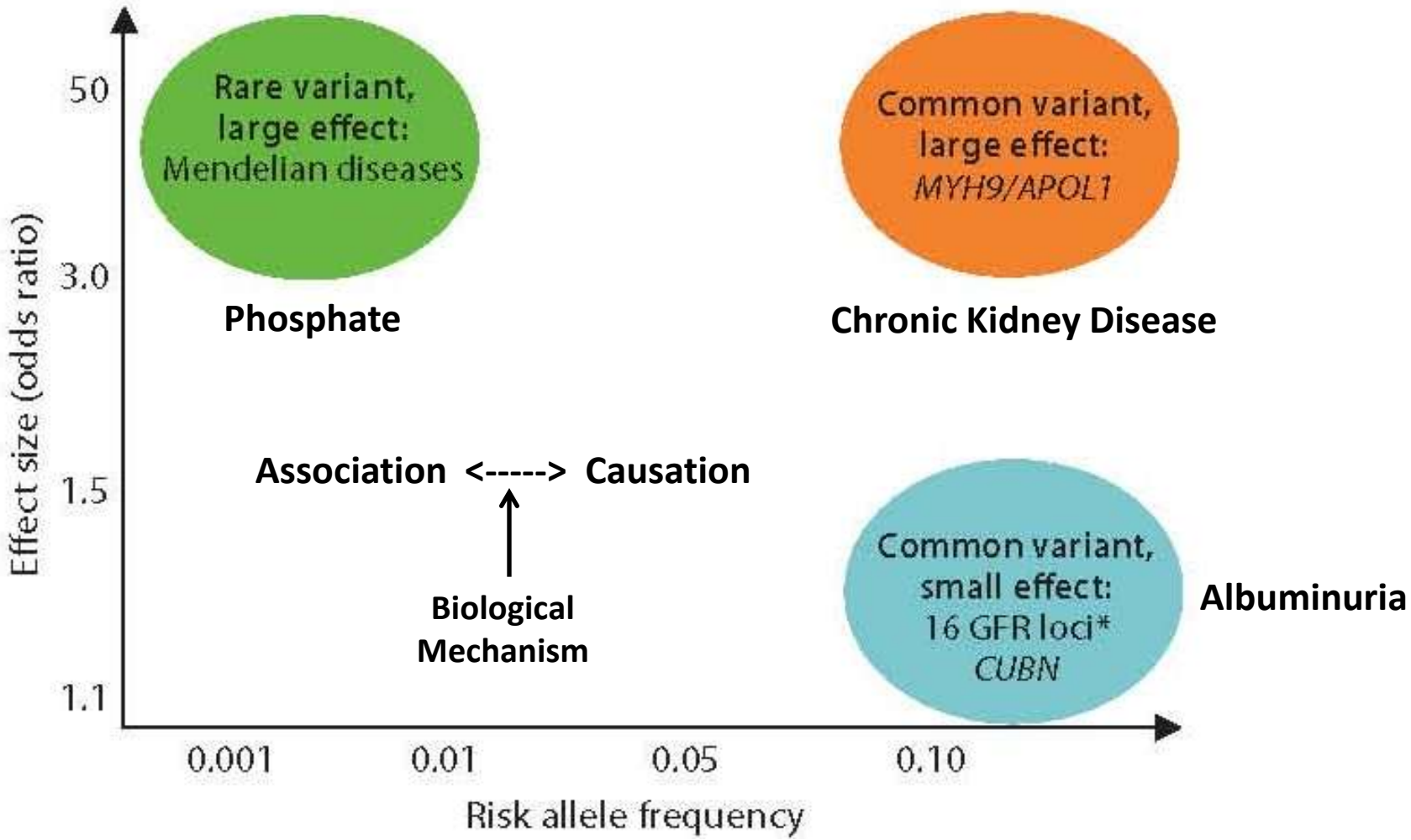
CLASSIFICATION OF INHERITED DISEASES

Monogenic – Mendelian Inheritance patterns

Complex disease and phenotypes

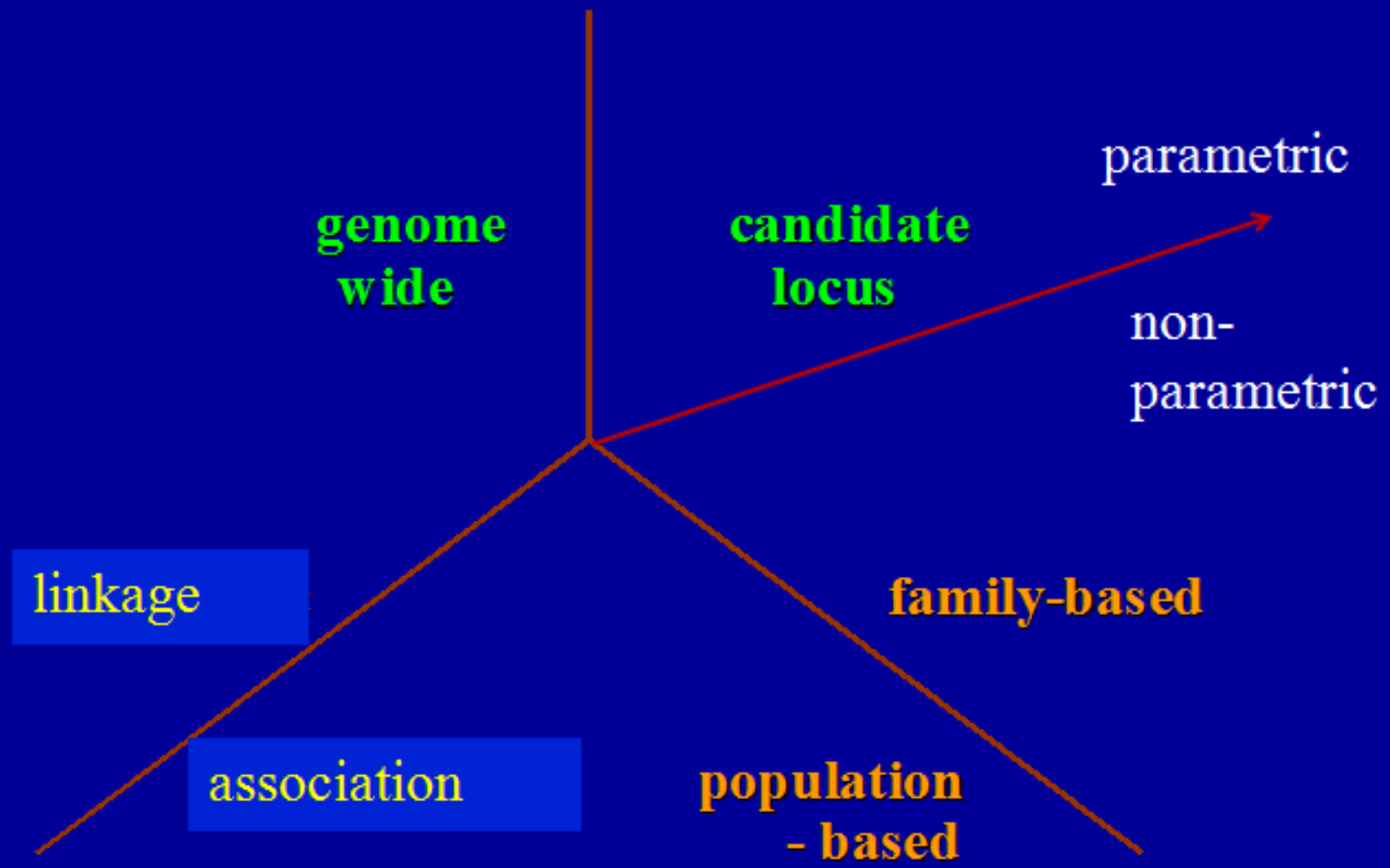
Polygenic – non-Mendelian Inheritance patterns

Relation of effect size and risk allele frequency of DNA sequence variants associated with kidney function and disease risk phenotypes



Adapted from Kidney and Blood Pressure Research 2011:34:225-234

Disease Gene Mapping



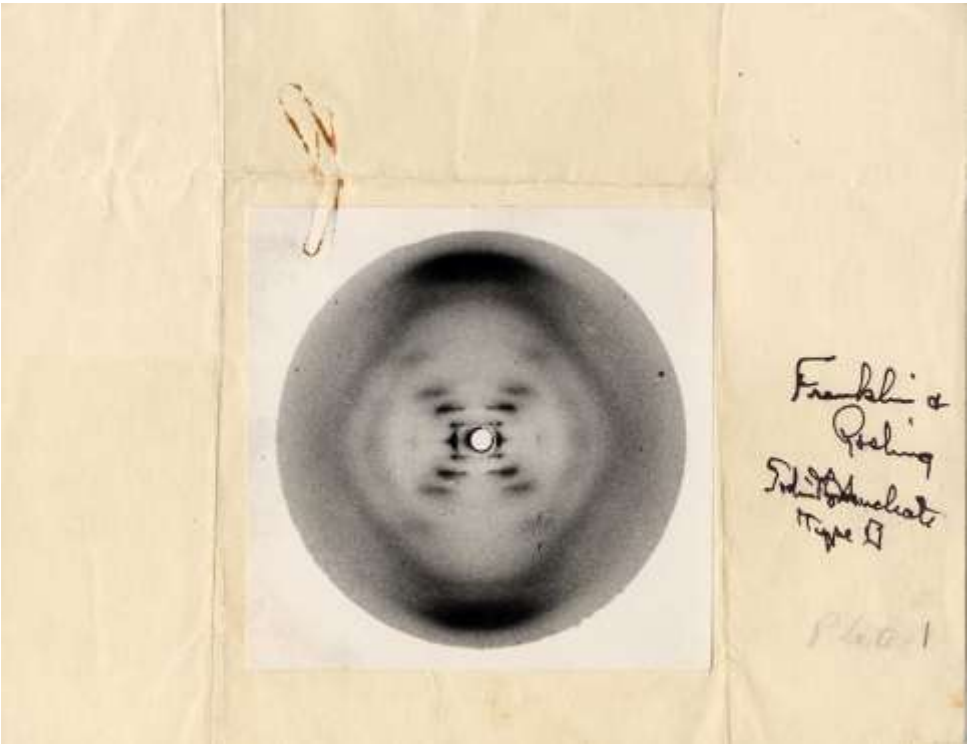
the power *of* DNA:

Biological Sciences

Genetic Ancestry:

- **Genetic Anthroplogy: Human Origins**
- **Genetic Genealogy: Populations, Communities and Families**

Medical Genetics

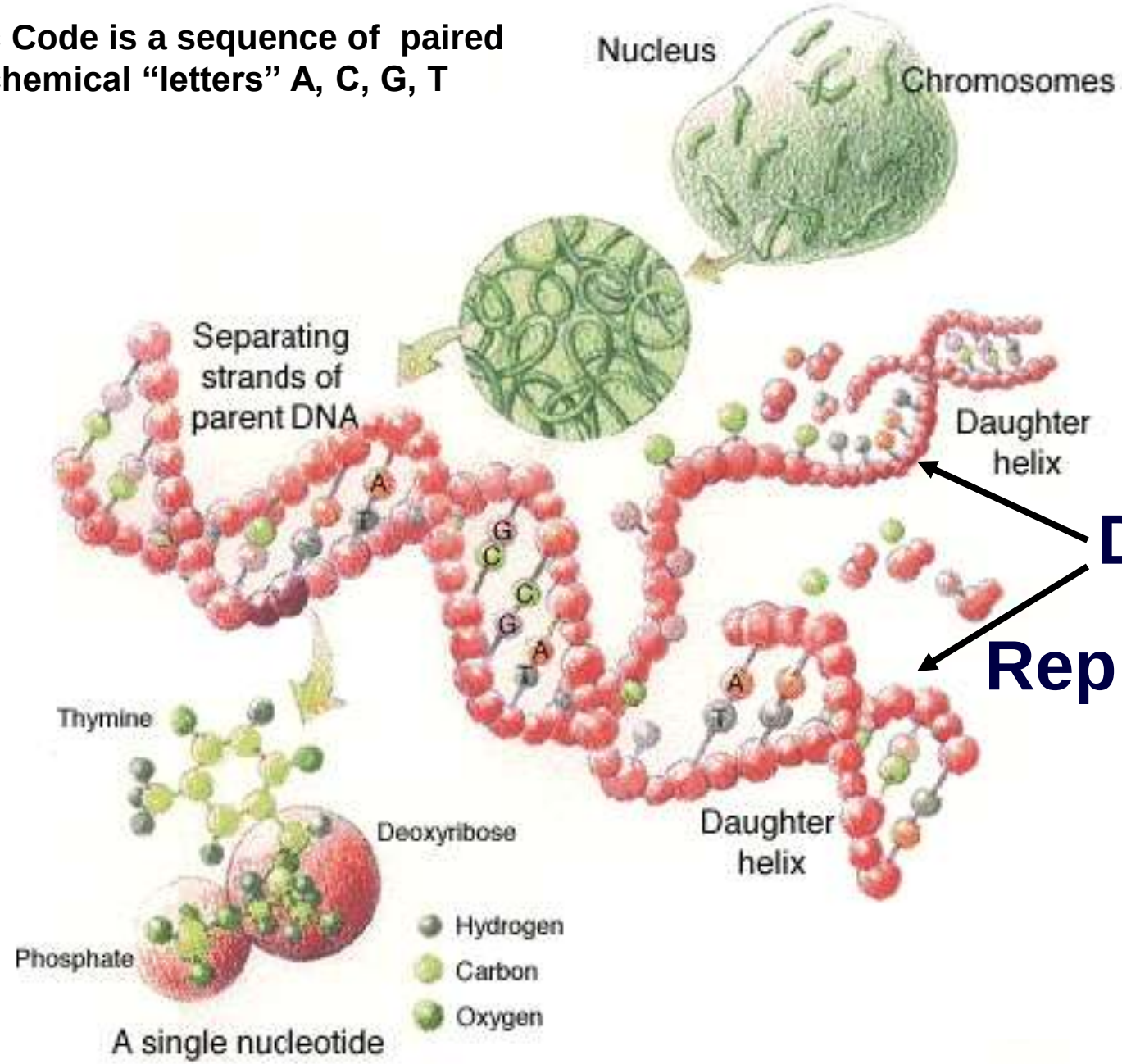


X-Ray Diffraction Study of DNA Structure

Rosalind Franklin

The Double Helix

Genetic Code is a sequence of paired biochemical "letters" A, C, G, T



DNA

Replication

DNA Replication

Precise → no errors

boring world full of identical copies of same sequence

Errors → richness of living diversity

price: illness and variable drug response

Mutations are "errors" in DNA copy editing

Mutations

Most mutations are "silent": Do not cause differences in phenotype or disease (polymorphisms), but they can be detected as **markers** by DNA analysis

- Some mutations cause differences in phenotype, and serve as the basis for the diversity of life forms on earth

- Few mutations cause disease

Markers are used in ***Population Genetics*** and to map disease causing mutations

What are Populations in Genetics and how is Population Genetic Structure Measured and used in Health and Disease

- **A "population" in human genetics can be designated as a group of people who share common ancestry patterns at a "genome wide" or "genetic locus" level**

- **"Population genetic architecture" can be measured using *DNA diversity markers***

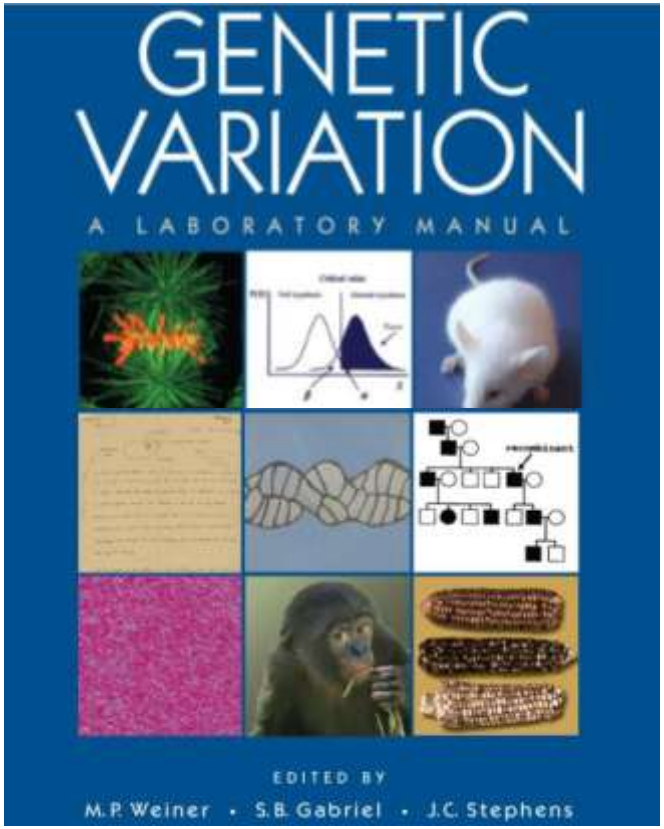
Classification of DNA Diversity Markers

Biological Type

SNPs, **INDELS, STRs, CNVs**, other

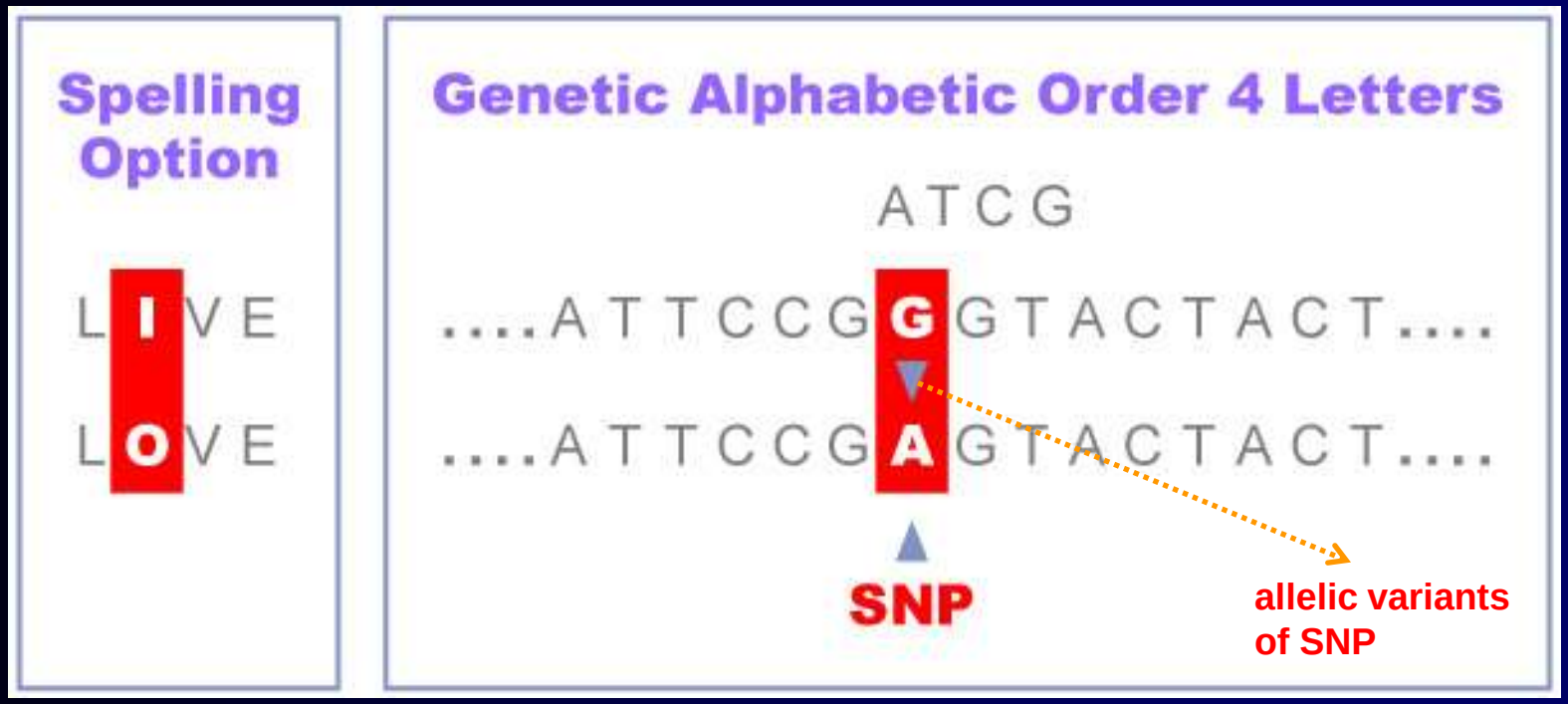
Evolutionary History

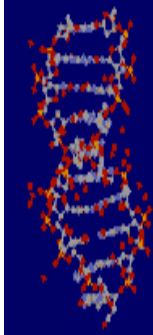
Genomic Location



Choice and combination depends on scientific, historical, genealogical, clinical or forensic question of interest

SNP=Single Nucleotide Polymorphism*





*considered a SNP (rather than private mutation) when minor allele frequency (MAF) > 1 %

*generally occur only once in the course of human evolutionary history (also INDELS), and therefore also termed "unique event polymorphisms" and mark BRANCHES on a genealogic tree

Some 15 million SNPs total:
3 million differences between individuals

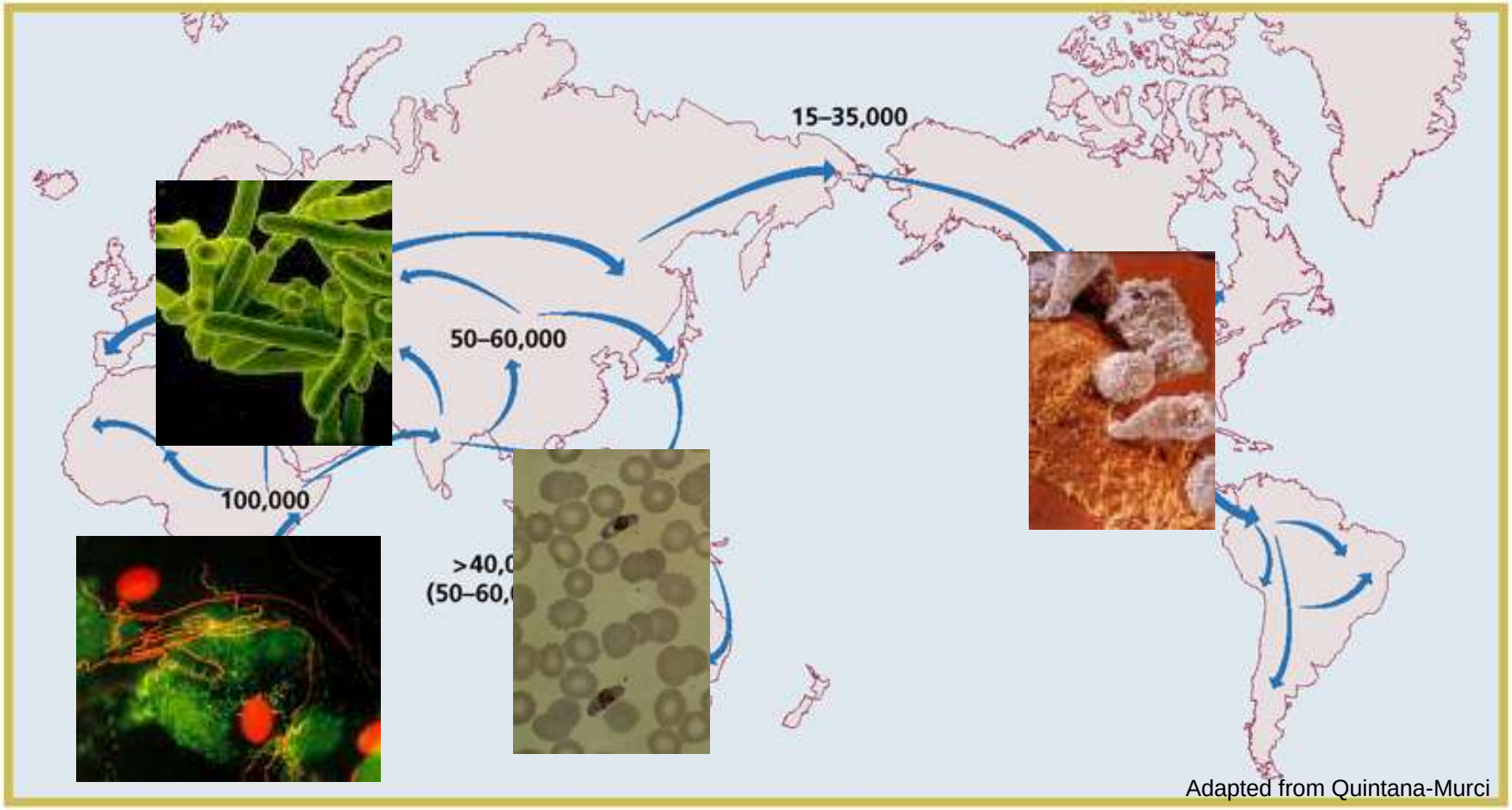
~95% of these differences have no

- **Smaller percentages encode phenotypic differences**
- **An even smaller percentage cause or predispose to disease or variable drug response**
- **Population frequency may be “neutral’ or affected by “selection”**

↓

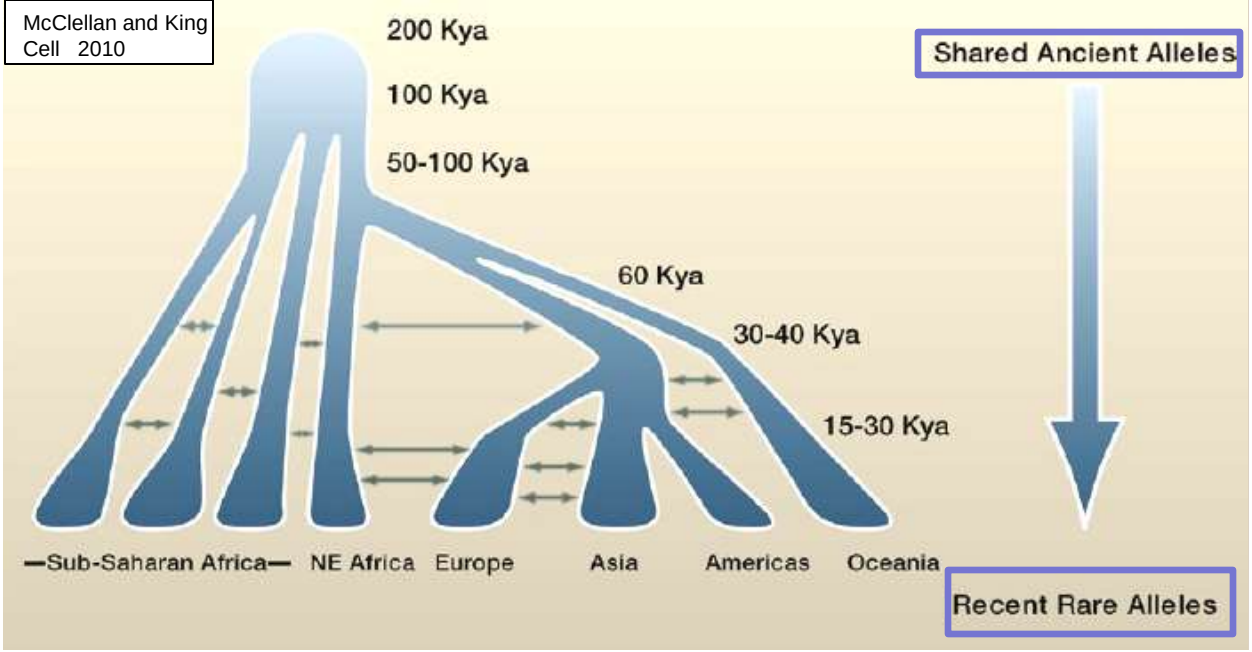
Useful to infer human origins and migrations and also in gene mapping

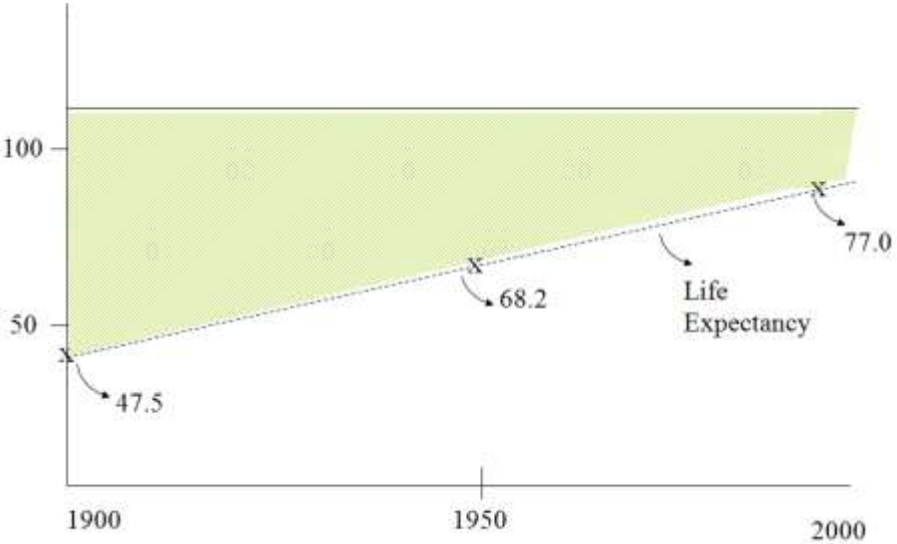
Demographic factors affect *genome wide* allele frequencies:
founder , bottleneck, expansion, migration, admixture
Selection: differential effect on allele frequencies at *specific genomic regions*

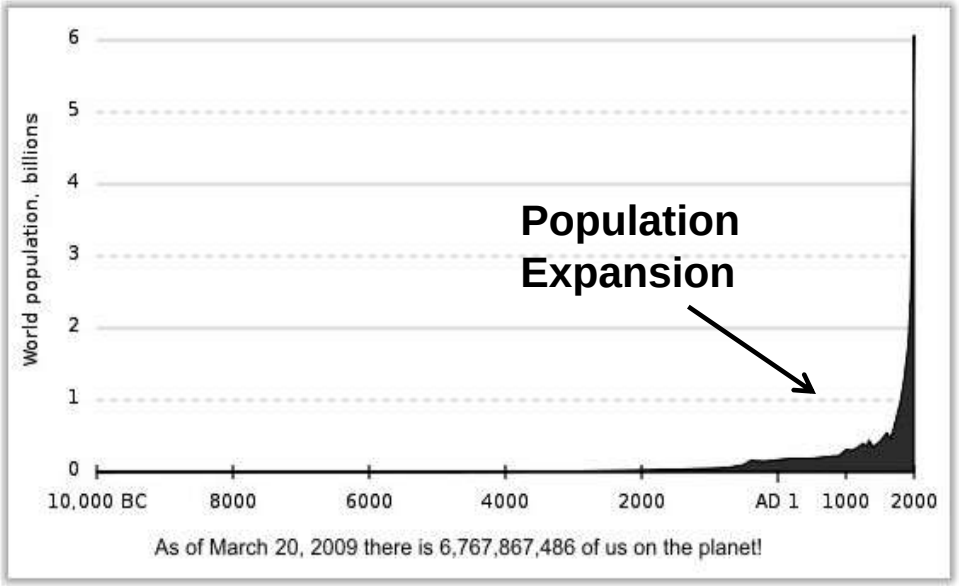


Genetic variants conferring an advantage and better adaptation will be selected and rise to high frequencies (together with neighbouring variants)

Evolutionary Medicine



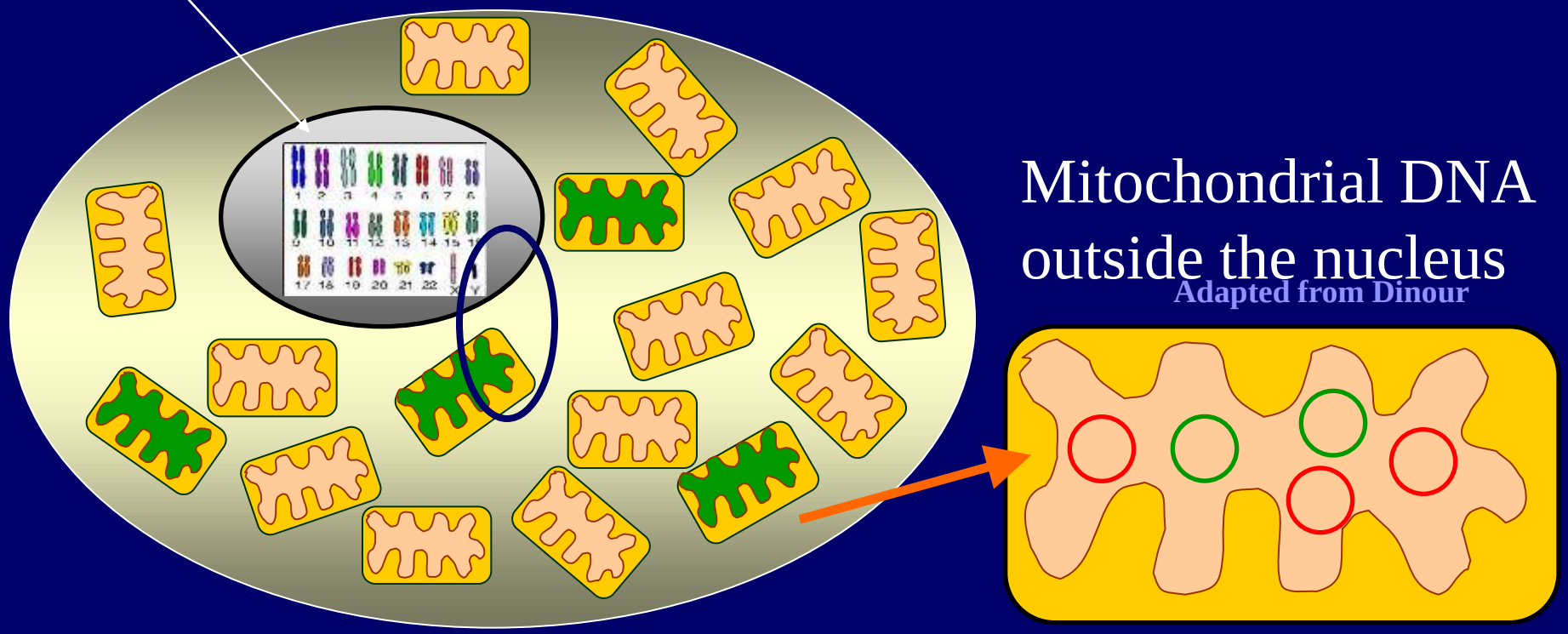


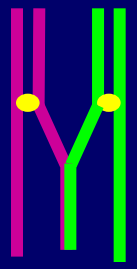


Common variants which passed evolutionary filter may *now* be relevant to common disease due to increased life expectancy (or change in adaptive forces: diet, drugs, bugs)

Very many *new* private rare variants may *now* contribute to common disease and have not passed evolutionary filter

DNA Markers are Located Throughout the Genome



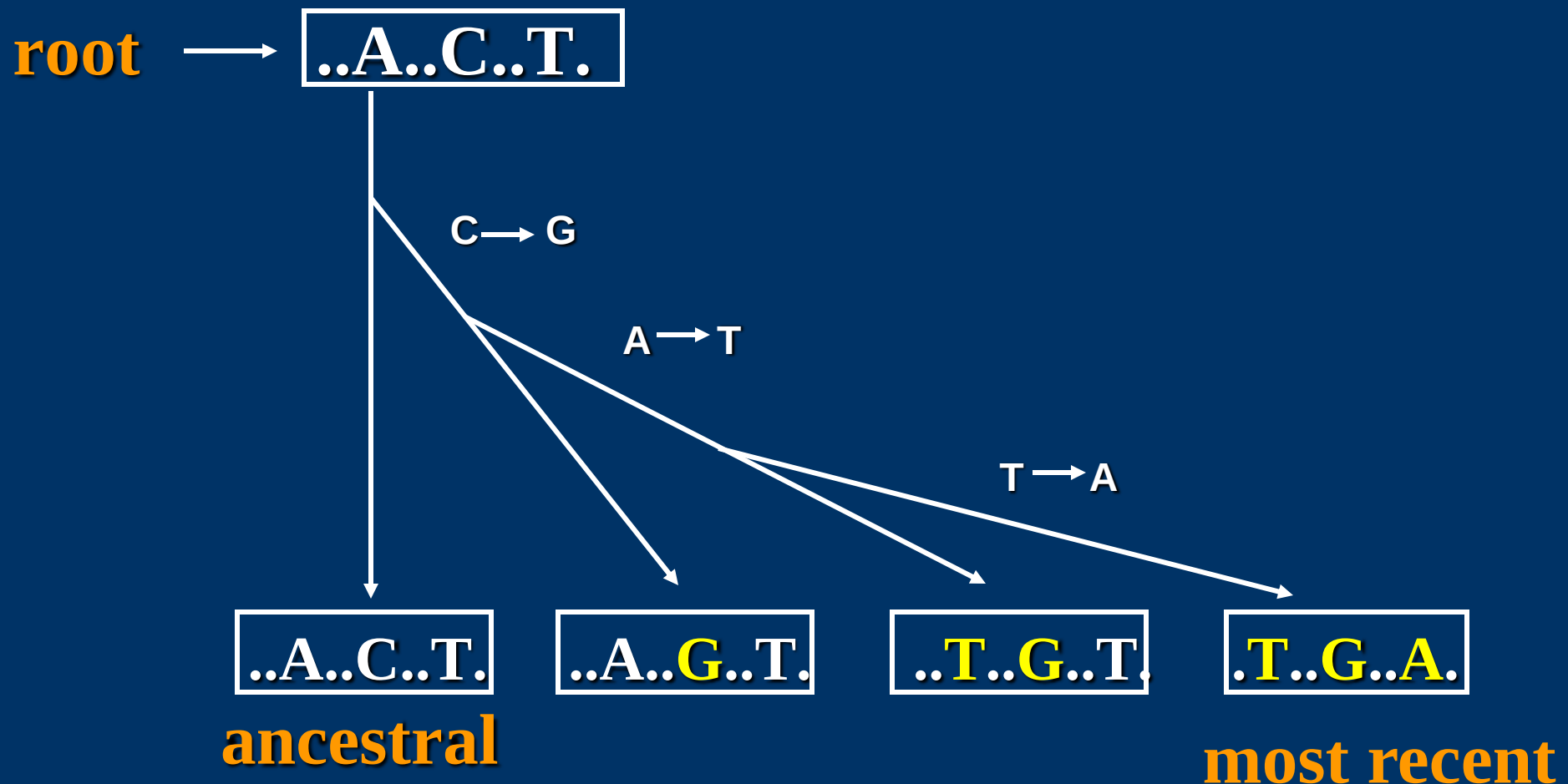


All genomic regions under meiotic recombination except:

- **Most of Y chromosome in males**
- **Mitochondrial DNA**

Markers can be clustered into *Haplotypes*

example: haplotype tree generated by 3 *NON-RECOMBINING* SNPs

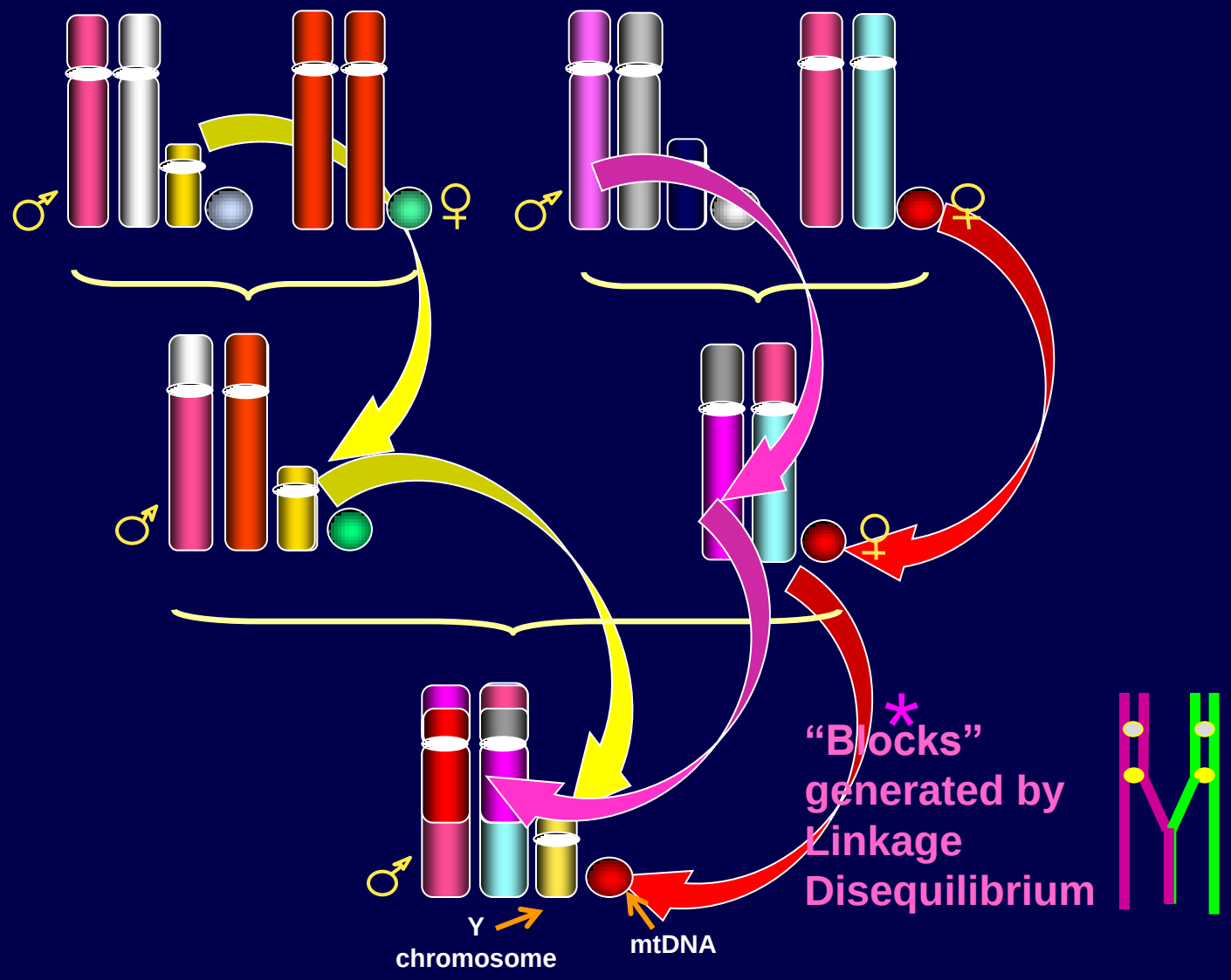


max # haplotypes = # markers + 1

Throughout the Genome there are Population Specific Blocks* of Markers which provide a Genome-Wide view and are especially useful in Disease Gene Mapping

Markers on the Y-Chromosome trace Paternal Lineages

Markers on mitochondrial DNA trace Maternal Lineages



Linkage Disequilibrium (LD)

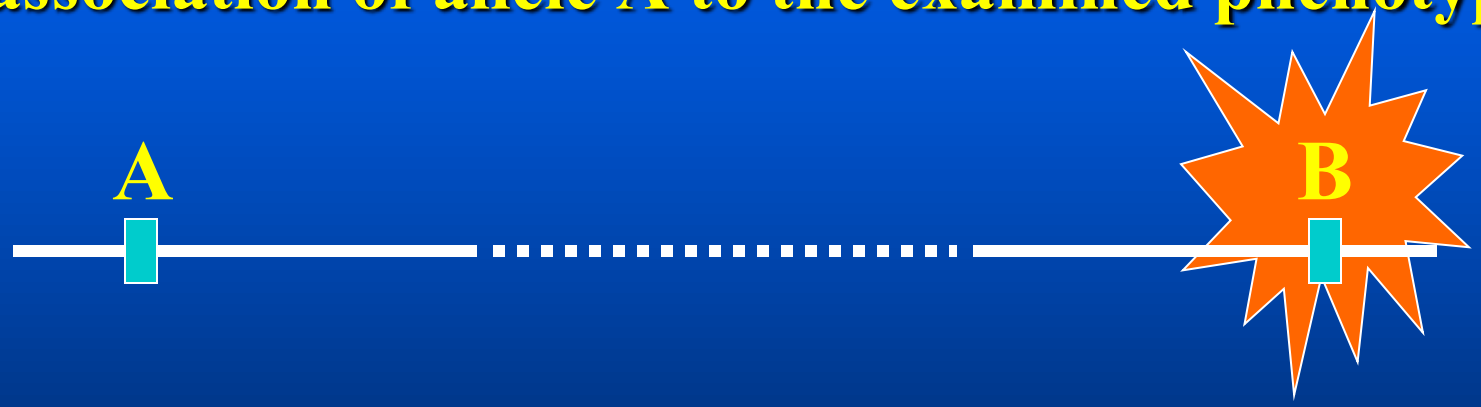
Linkage Disequilibrium (LD) is the nonrandom association (at the population level) of two alleles on the same chromosome:

In equilibrium: $P_{AB} = P_A * P_B$

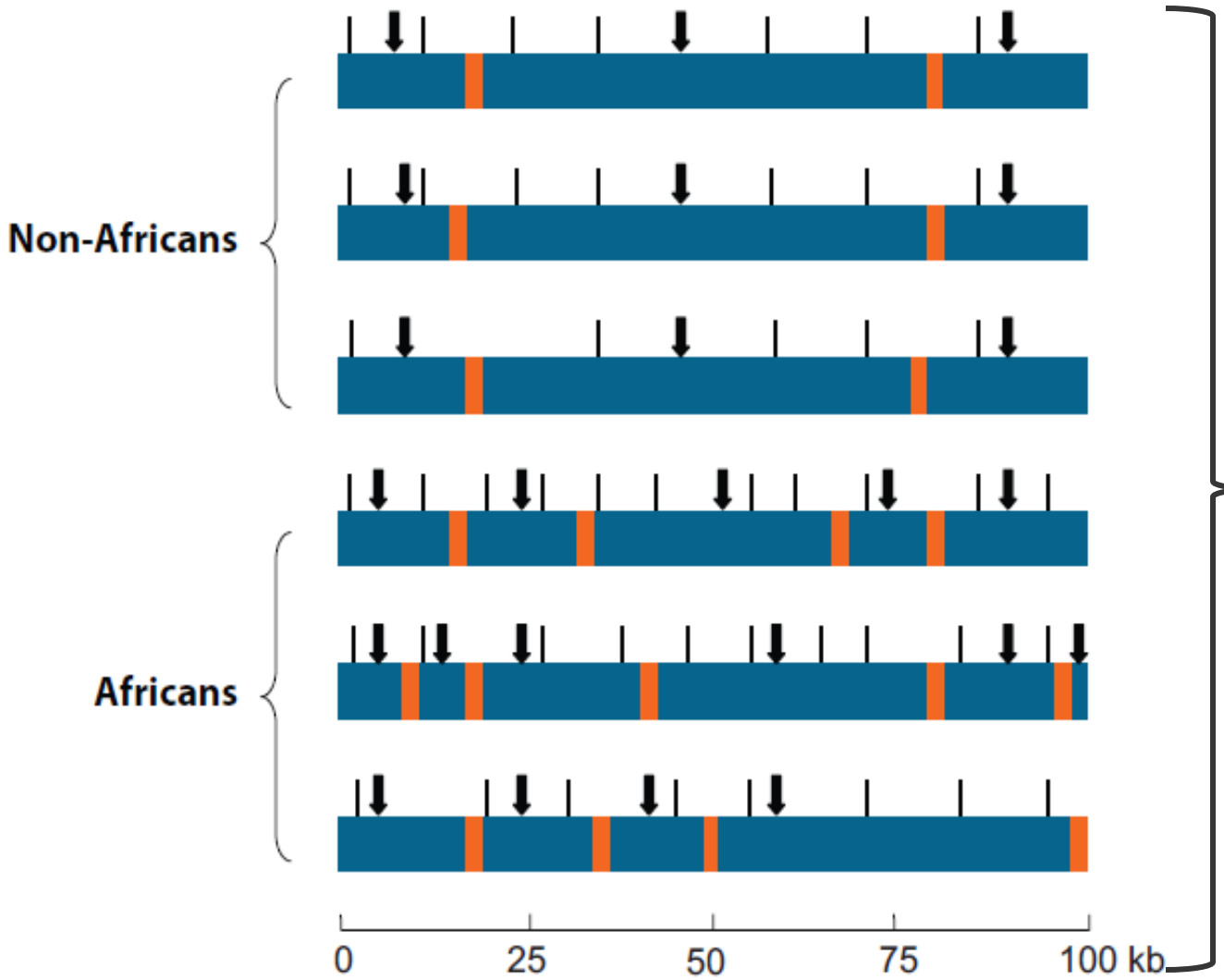
In disequilibrium: $P_{AB} > P_A * P_B$

Linkage Disequilibrium (LD)

If allele A is found in significant LD with a disease risk causative allele (B), we should detect association of allele A to the examined phenotype



In "conventional" genome wide association (GWAS), only markers (A) in sufficient physical proximity to causative locus B to mitigate recombination will be associated with the health or disease phenotype of interest



Blocks of Linkage Disequilibrium

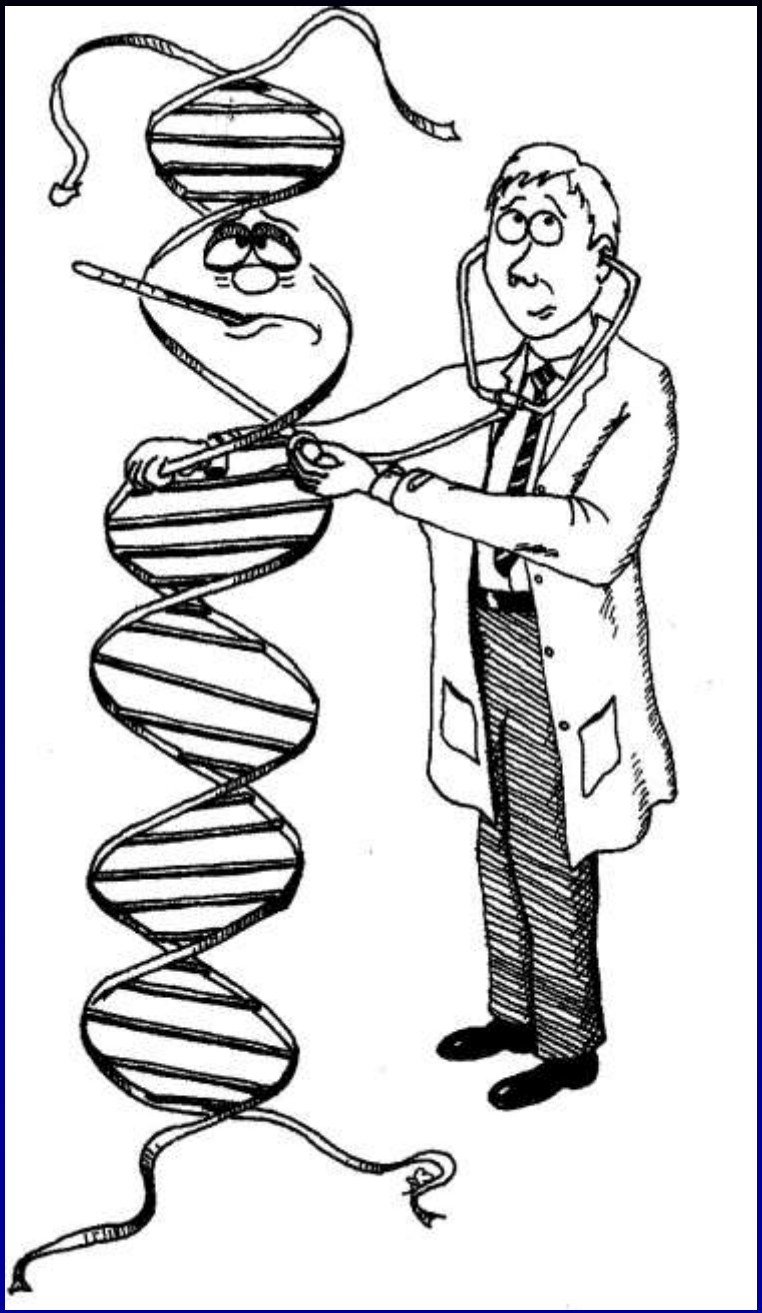
Size and Pattern

Genome Wide:
Demographic History

Genetic Locus:
Evolutionary Selection

Adapted Campbell and Tishkoff 2008

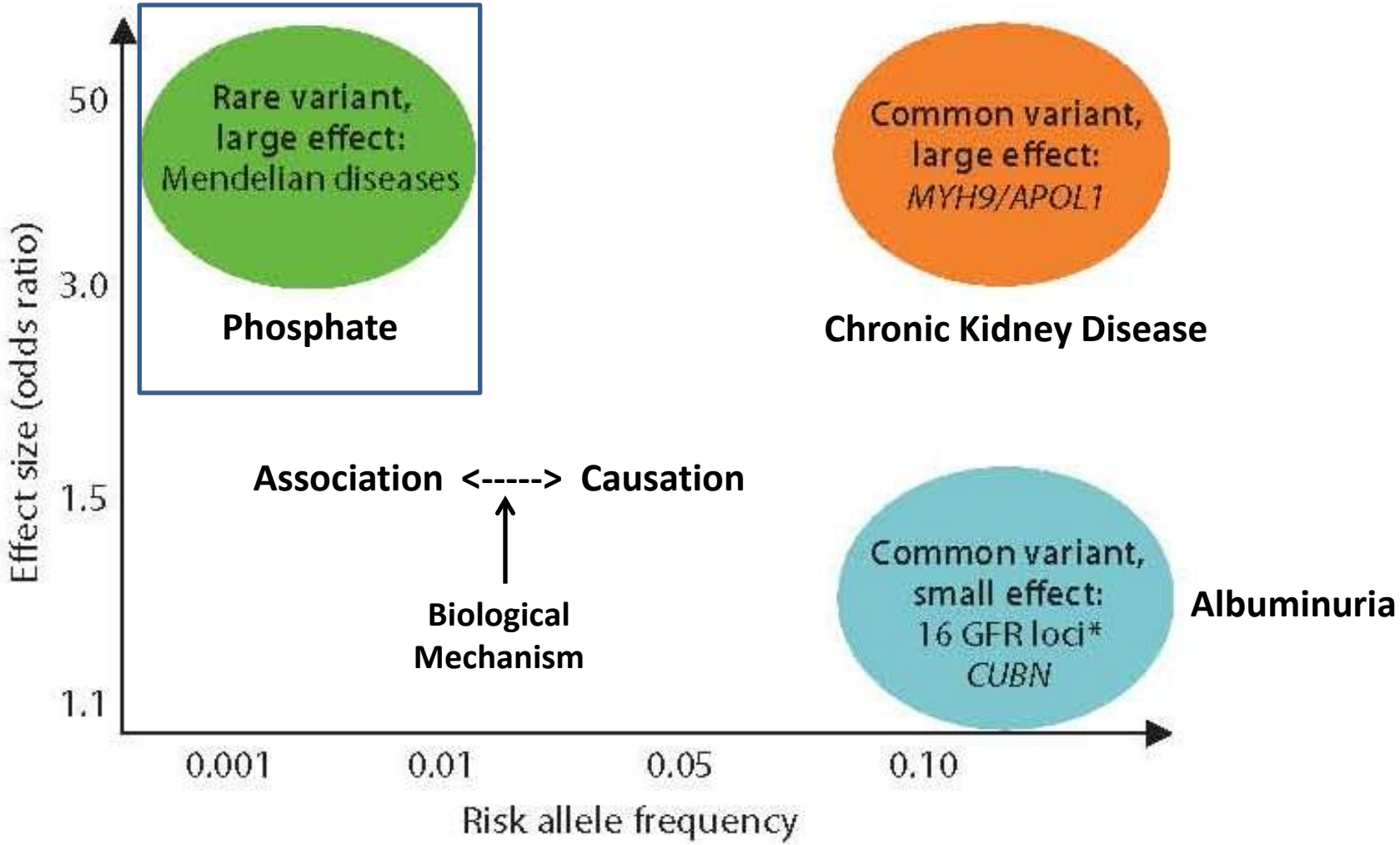
The molecular pathology of genetic kidney diseases



Defining the abnormal phenotype:

“phenomics”

Relation of effect size and risk allele frequency of DNA sequence variants associated with kidney function and disease risk phenotypes



Adapted from Kidney and Blood Pressure Research 2011:34:225-234

N Engl J Med 1988; 319:845-849

Elevated Serum 1,25-Dihydroxyvitamin D Concentrations in Siblings with Primary Fanconi's Syndrome

Martin Tieder, M.D., Raphael Arie, M.D., David Modai, M.D., Ruth Samuel, M.D., Joshua Weissgarten, M.D., and Uri A. Liberman, M.D., Ph.D.

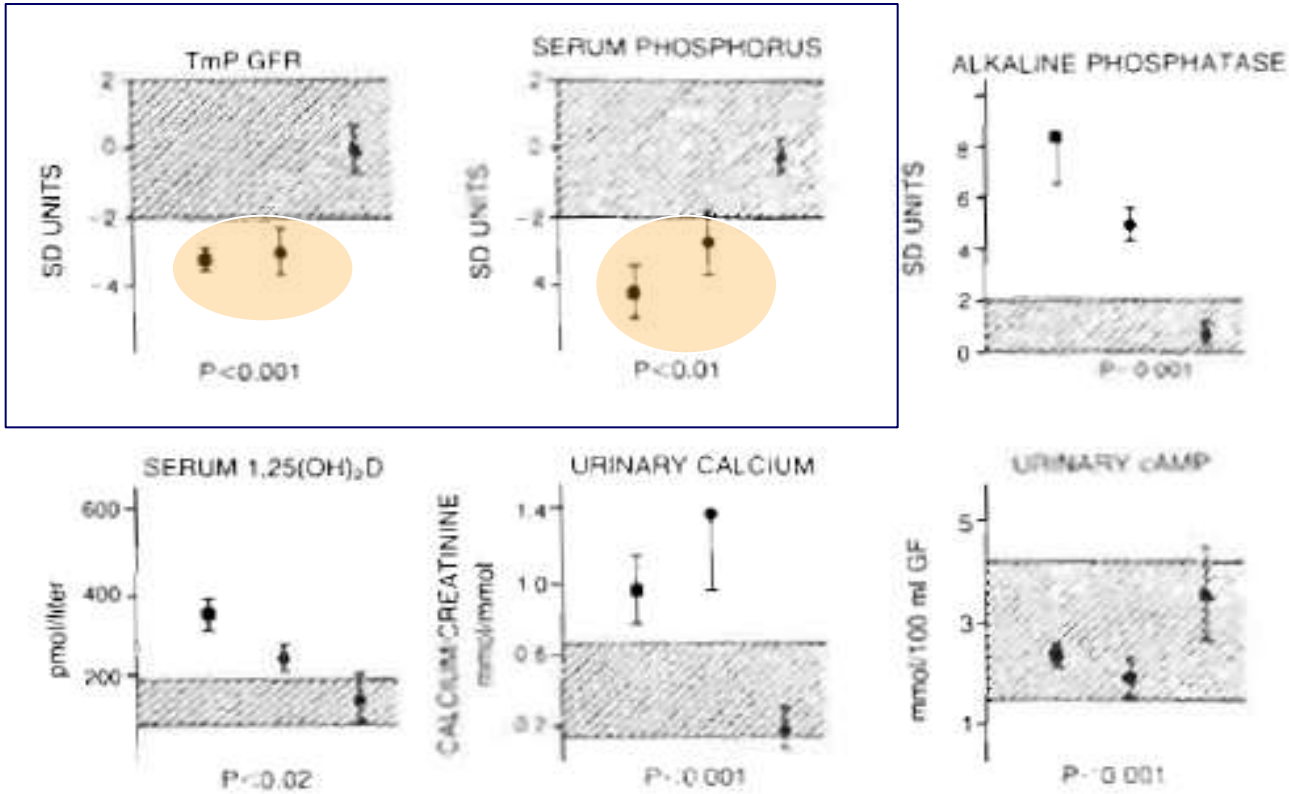
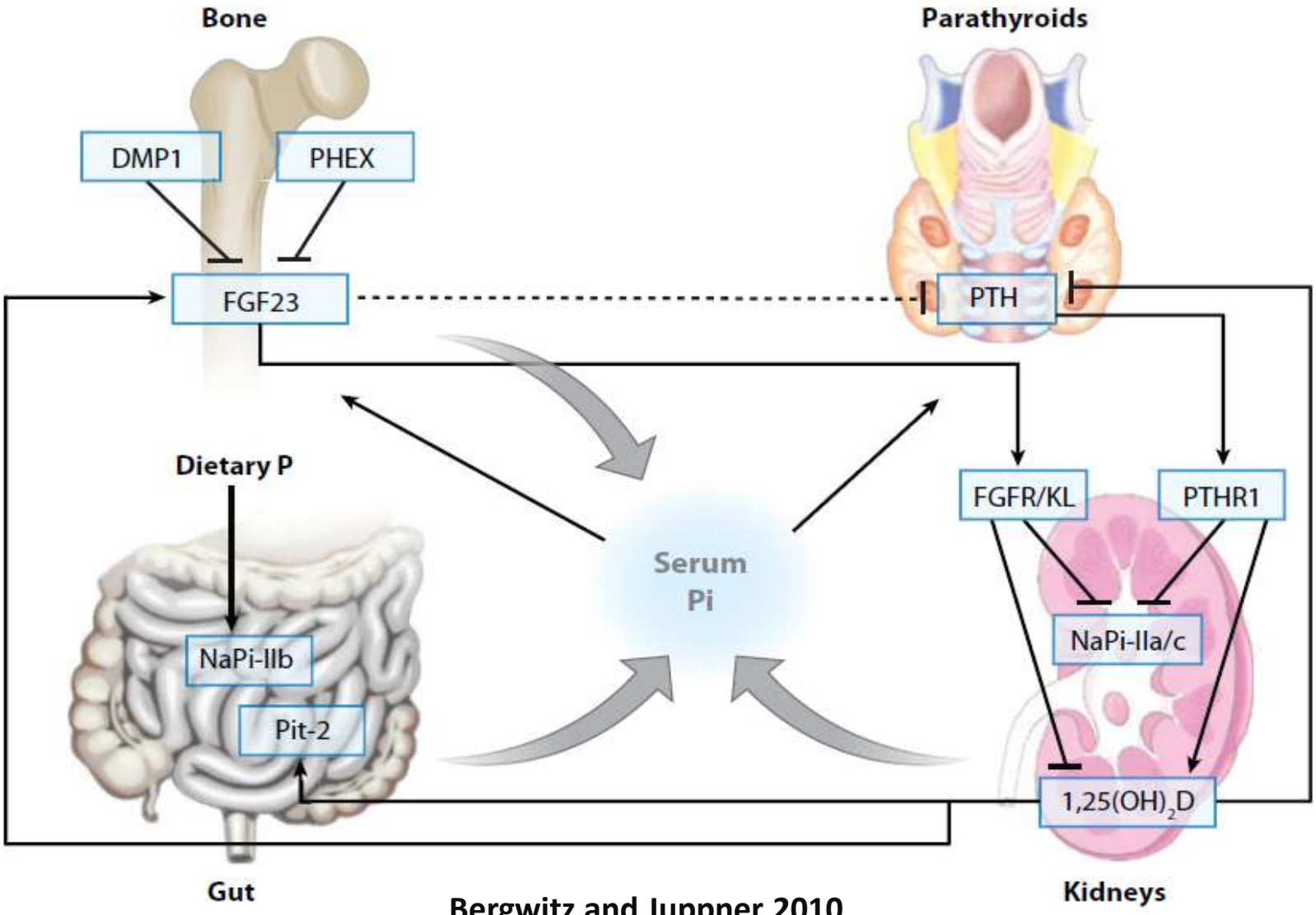


Figure 1. Pretreatment Biochemical Data on Two Patients with Fanconi's Syndrome and Their Seven Normal Immediate Relatives.

With this many hormones, regulators and transporters: almost all possible gain or loss of function aberrations compatible with life can be created in mice or found in humans



Bergwitz and Juppner 2010

Human genetic disorders of phosphate homeostasis

What's missing?

Disorder	Abbreviation	Inheritance	Gene	Mechanism	OMIM
Hypophosphatemic disorders					
X-linked hypophosphatemia	XLH	X-linked	*PHEX*	FGF23-dependent	#307800
Autosomal dominant hypophosphatemic rickets	ADHR	AD	*FGF23*	FGF23-dependent	#193100
Autosomal dominant hypophosphatemic rickets	ADHR	AD	*KL*	FGF23-dependent	#612089
Autosomal recessive hypophosphatemia	ARHP	AR	*DMP1*	FGF23-dependent	#241520
Hereditary hypophosphatemic rickets with hypercalciuria	HHRH	AR	*SLC34A3*	Proximal tubular phosphate wasting, FGF23-independent	#241530
Vitamin-resistant rickets type 1	VDDR1	AR	*CYP27B1*	1,25$(OH)_2$D deficiency, FGF23-independent	#264700
Vitamin-resistant rickets type 2	VDDR2	AR	*VDR*	1,25$(OH)_2$D-resistance, FGF23-independent	#277440
Familial hypocalciuric hypercalcemia/neonatal severe hyperparathyroidism	FHH NSHPT	AD/AR	*CaR*	PTH-excess, FGF23-independent	#145980 #239200
Jansen disease		AD	*PTHR1*	Const. active PTHR1; FGF23-dependent	#156400

Annu. Rev. Med. 2010. 61:91–104

Bergwitz and Juppner

Of Men and Mice: Who Is in Control of Renal Phosphate Reabsorption?

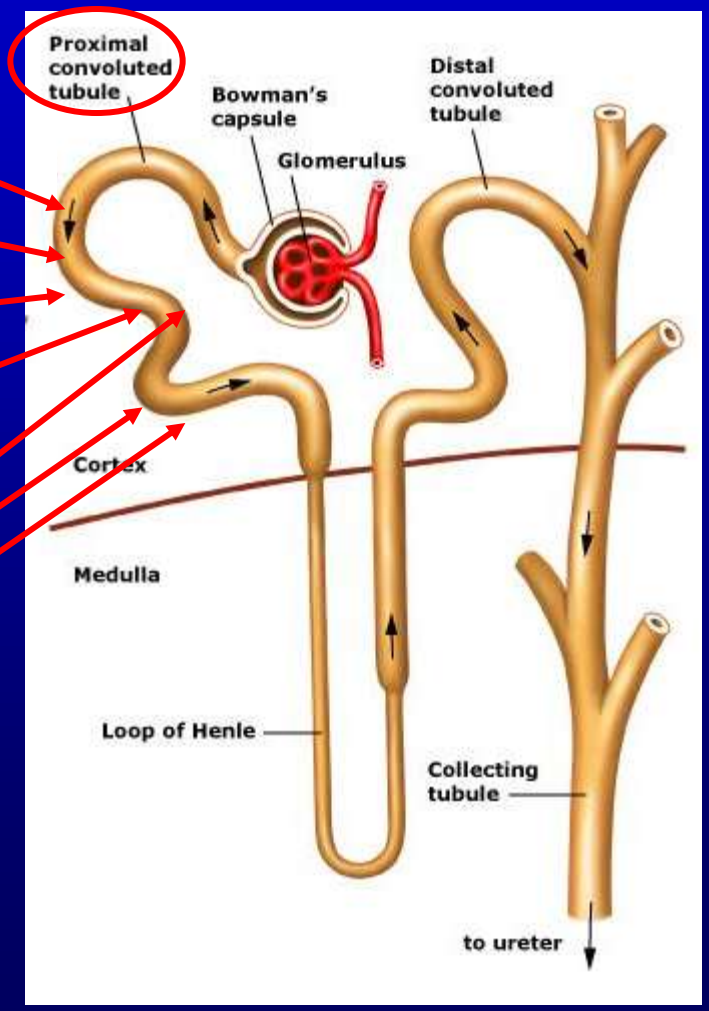
Carsten A. Wagner, Jürg Biber, and Heini Murer

Thus, final proof for the role of NaPi-IIa in human kidney must come from genetic studies identifying patients with hyperphosphaturia and functionally relevant mutations in NaPi-IIa. Although previous reports suggested gene variants in NaPi-IIa seem to be relatively common, they may represent not more than functionally irrelevant polymorphisms. Conversely, NaPi-IIa may play only a minor role in human kidney, and its defects could be fully compensated by the putative major human isoform NaPi-IIc.

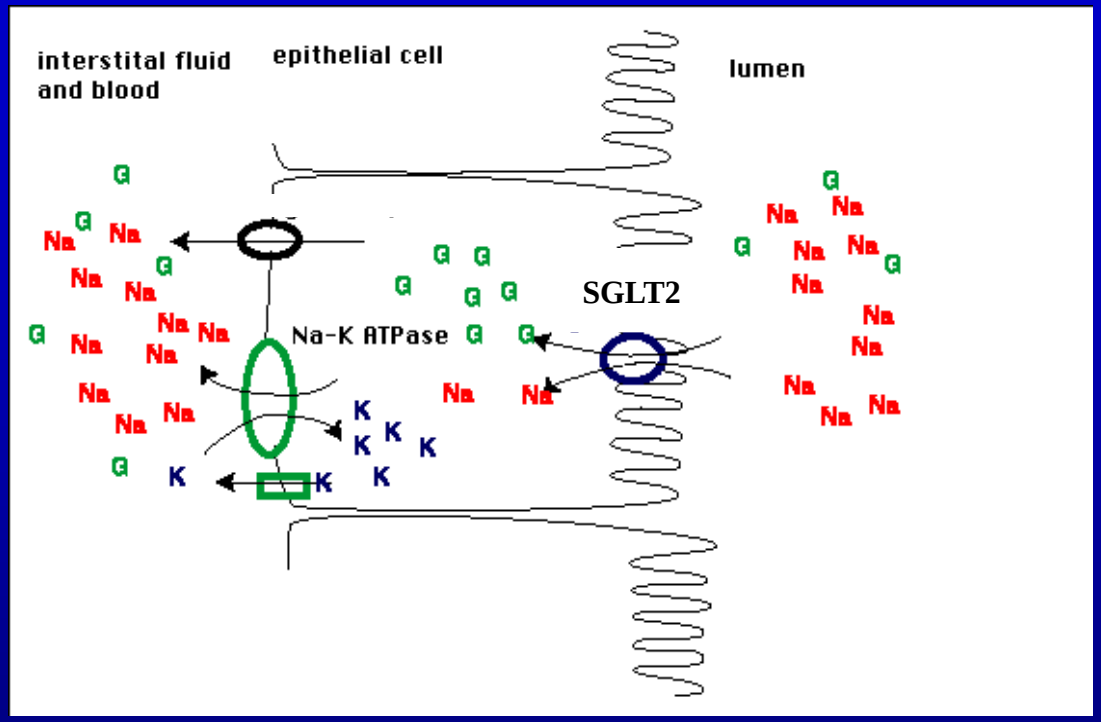
The Proximal Tubule

Reabsorbs the bulk of filtered water and solutes including > 80% of filtered phosphate load

- Sodium
- Glucose
- Amino-acids
- Phosphate
- Bicarbonate
- Uric acid
- Water



Mechanisms of solute reabsorption by the proximal tubule



- *Hediger et al. 1987* SLC5A1 (SGLT1) by expression and then SLC5A2 (SGLT2) and others by homology)
- *Santer et al. 2003, Magen et al. 2005* – validate SGLT2 as major glucose transporter in proximal tubule in families with FRG (OMIM 233100)
- Ly et al. 2011 "Sweet Pee Mouse" – great glycemic control; higher mortality

Note: no generalized proximal tubulopathy in FRG humans

Three Families of Na-Pi Cotransporters

	I	IIa	IIb	IIc	III
Protein	NaPi-I	NaPiIIa	NaPiIIb	NaPiIIc	PiT-1 (Glvr-1) PiT-2 (Ram-1)
Gene	SLC17	SLC34A1	SLC34A2	SLC34A3	SLC20
Tissue Expression	renal cortex brain, liver	renal cortex (prox. tubule)	small intestine lung	renal cortex (prox. tubule)	many cells **Functional at pH<6**
Substrates	anions	Pi	Pi	Pi	Pi
Pi affinity	?~1mM	0.1- 0.2mM	0.05mM	0.07mM	0.025mM
Na^+- Pi coupling	>1	3	3	2	3
Effect of increased pH	none	↑	↓	↑	↓

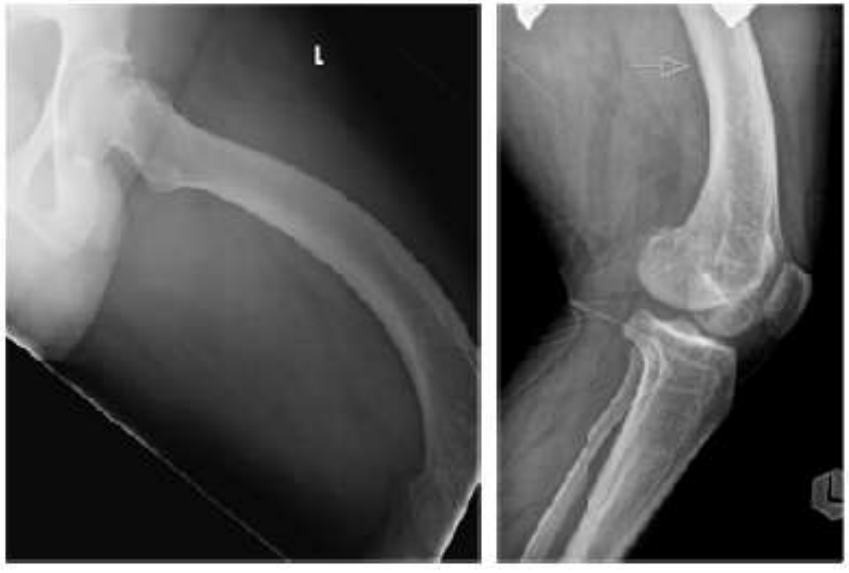
Twenty Year Follow-up

Proximal Tubule Abnormalities

Urine	Patient III-5	Patient III-6	Reference Range
Glucose (mg/dl)	100	300	0*
TRP (%)	49	66	85-95
TmP/GFR (mg/dL)	1.00	1.25	2.5-4.2
Uric acid/dL GFR (mg/dl)	0.8	0.75	<0.57
Urinary amino acids	Aminoaciduria	Aminoaciduria	-----
β_2-microglobulin (ng/mL)	30,940	15,387	<250

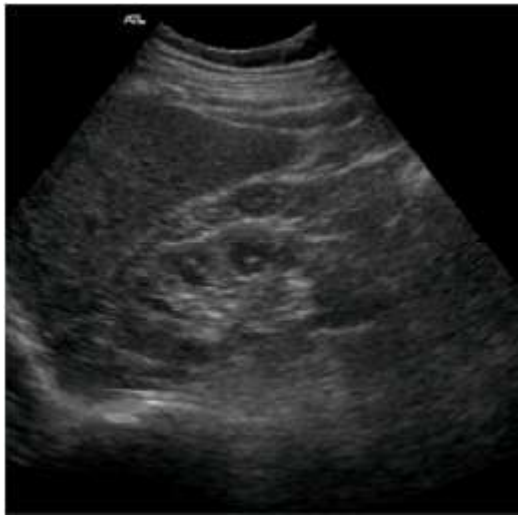
Radiographs of Left Femur and Knee

- Osteopenia
- Severe bowing
- Diaphyseal pseudofracture

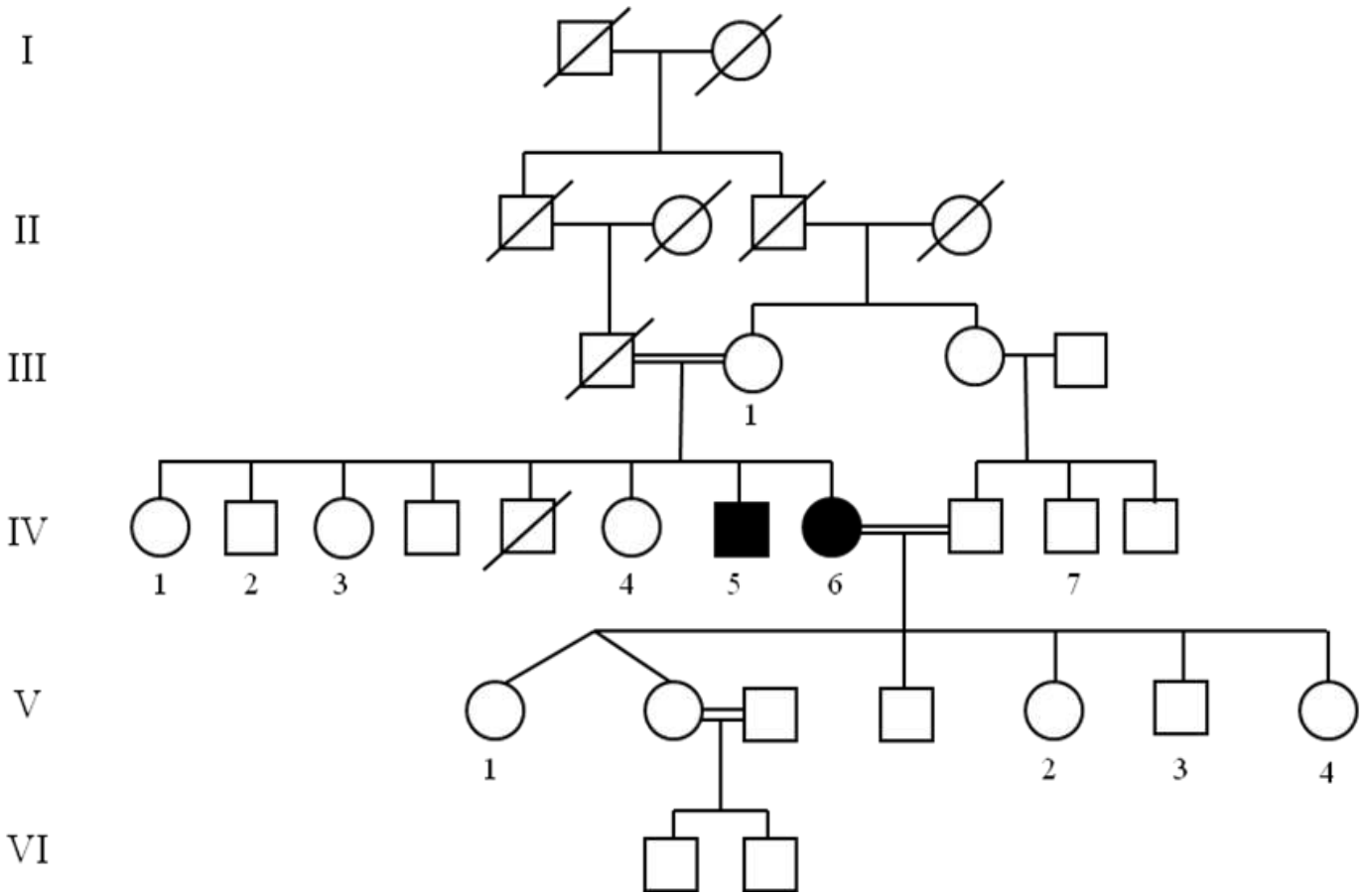


BMD z scores
Lumbar: -7.1,-6.8
Femoral Neck: -5.2, -6.1

Renal Ultrasound – no nephrocalcinosis



Pedigree of Proximal Tubulopathy Family

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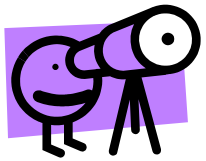
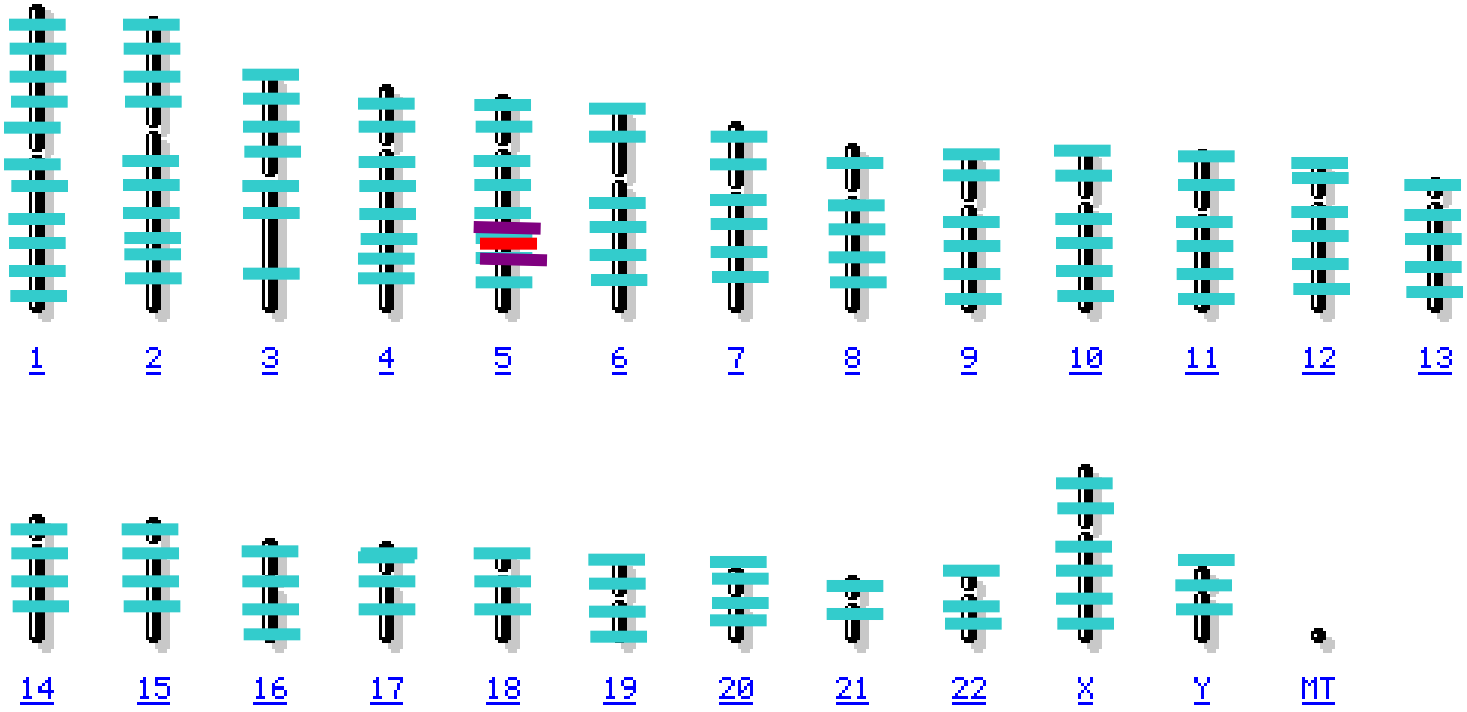
Finding a Needle in a Haystack,

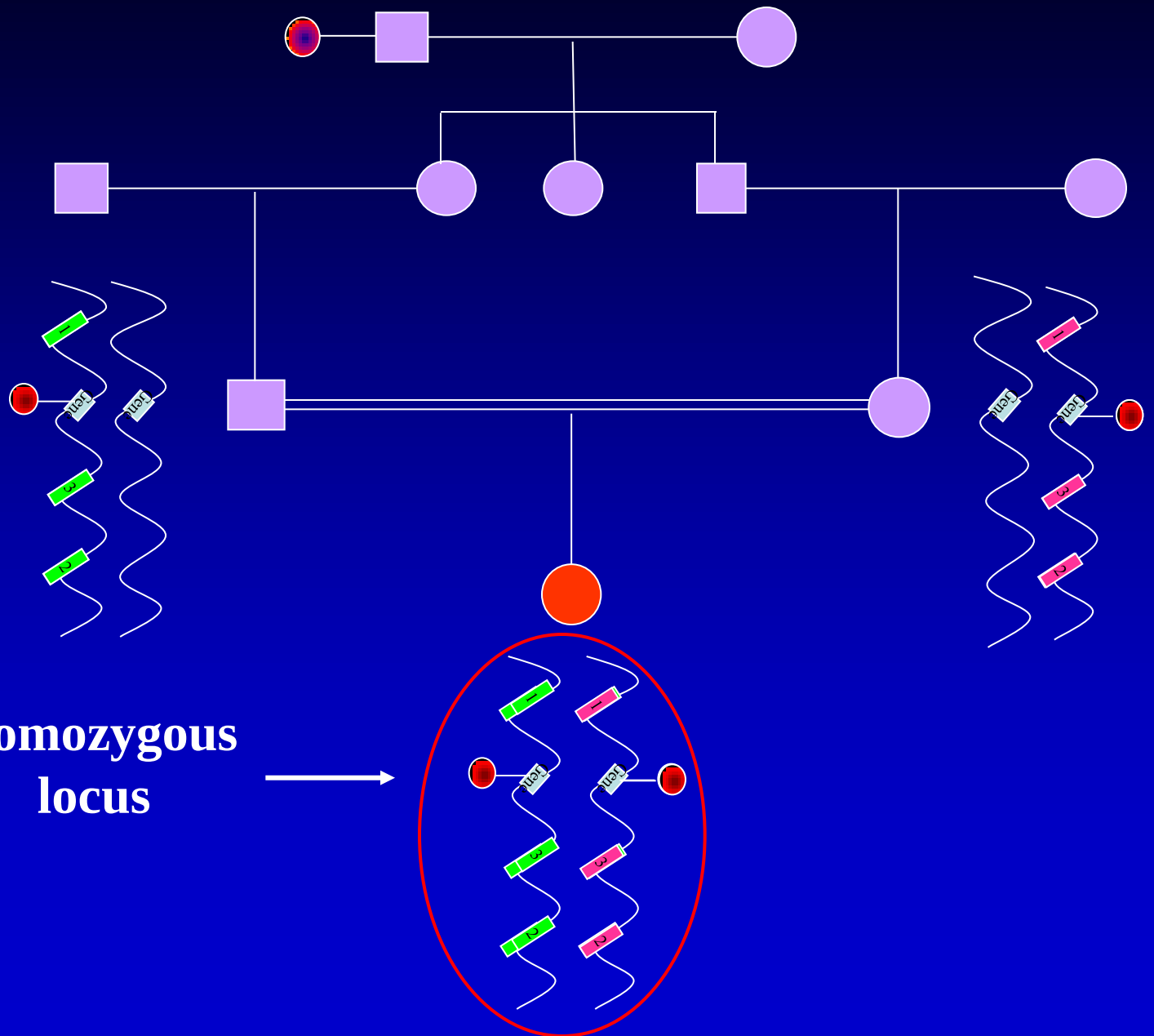


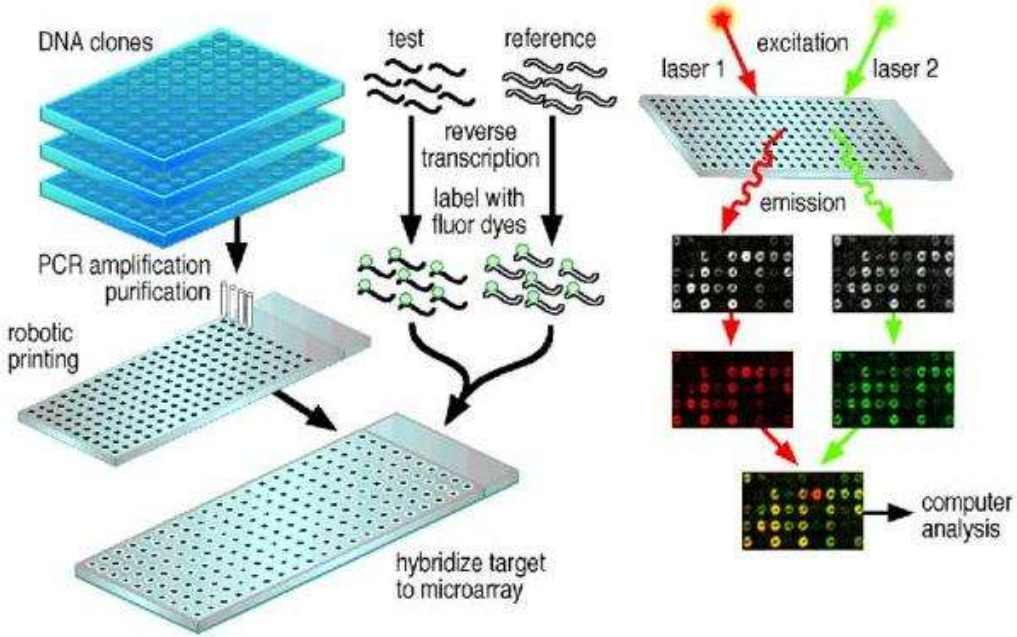


Disease-causing gene
Polymorphic marker

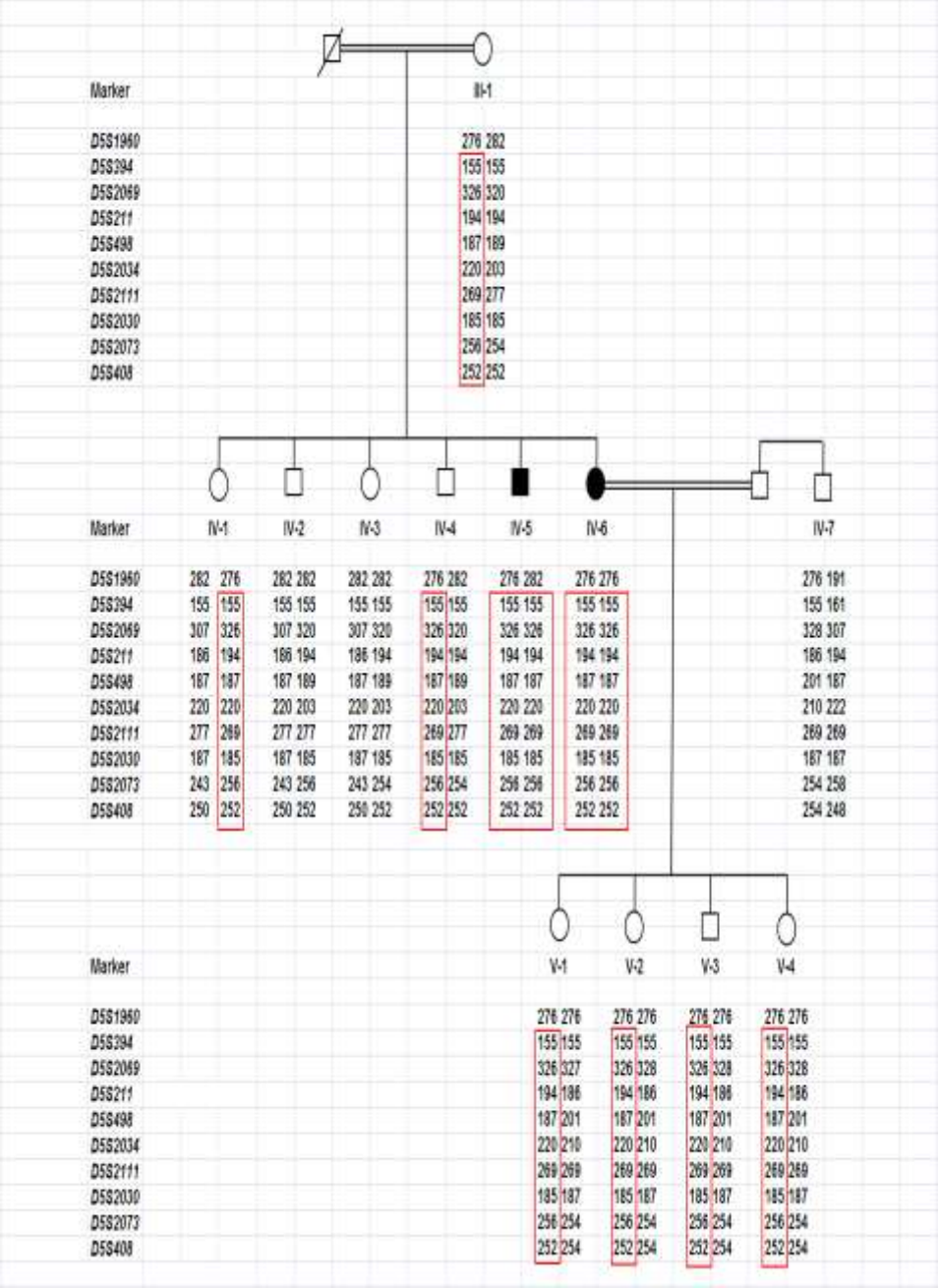
Homozygosity Mapping (whole genome)

Homozygosity mapping





250K SNP-marker microarray → three regions of extended shared homozygosity between affected individuals not shared with healthy siblings



- **Fine mapping with STRs at all three regions excluded 2/3 based on absence of segregation of STR markers with disease phenotype**

- **Linkage (LOD 2.4) to 5q35.1-q35.3 confirmed by genotyping 10STRs along region of homozygosity → 191 genes (NCBI MapViewers) including SLC34A1 encoding NaPiIIa**

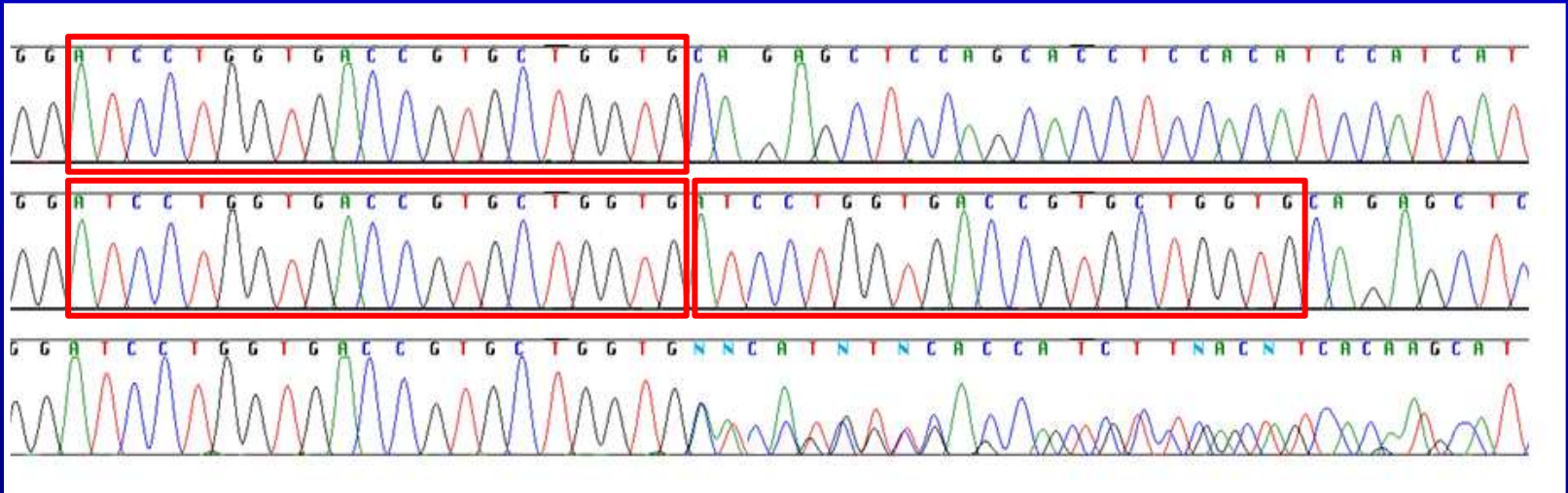
Sequencing of *SLC34A1*

Exon 5

Control

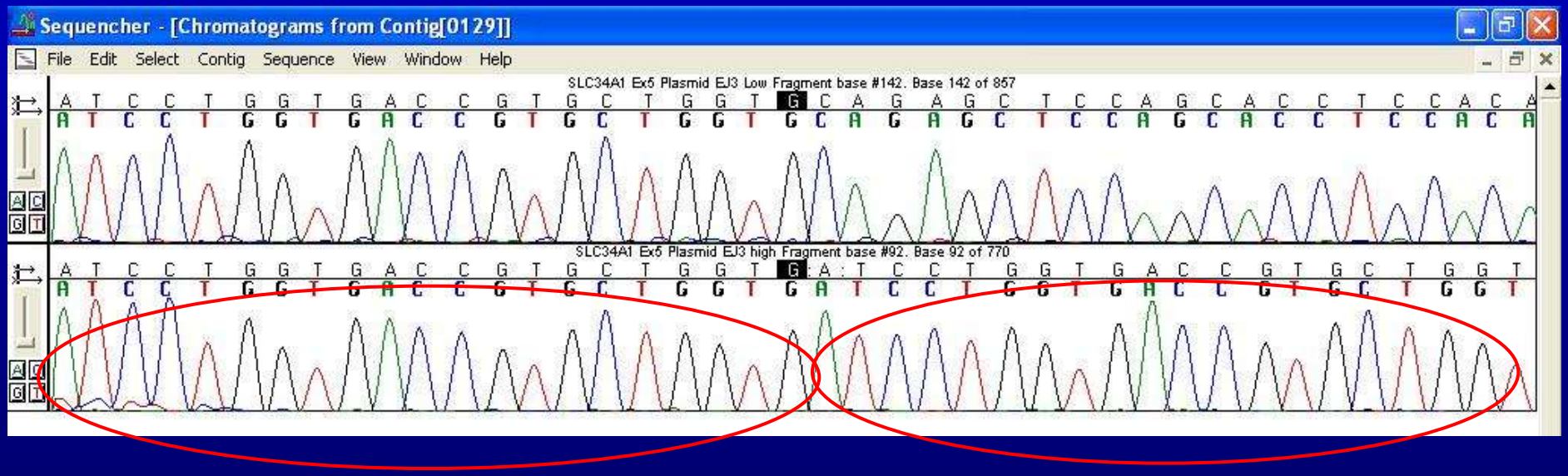
Patient

Mother



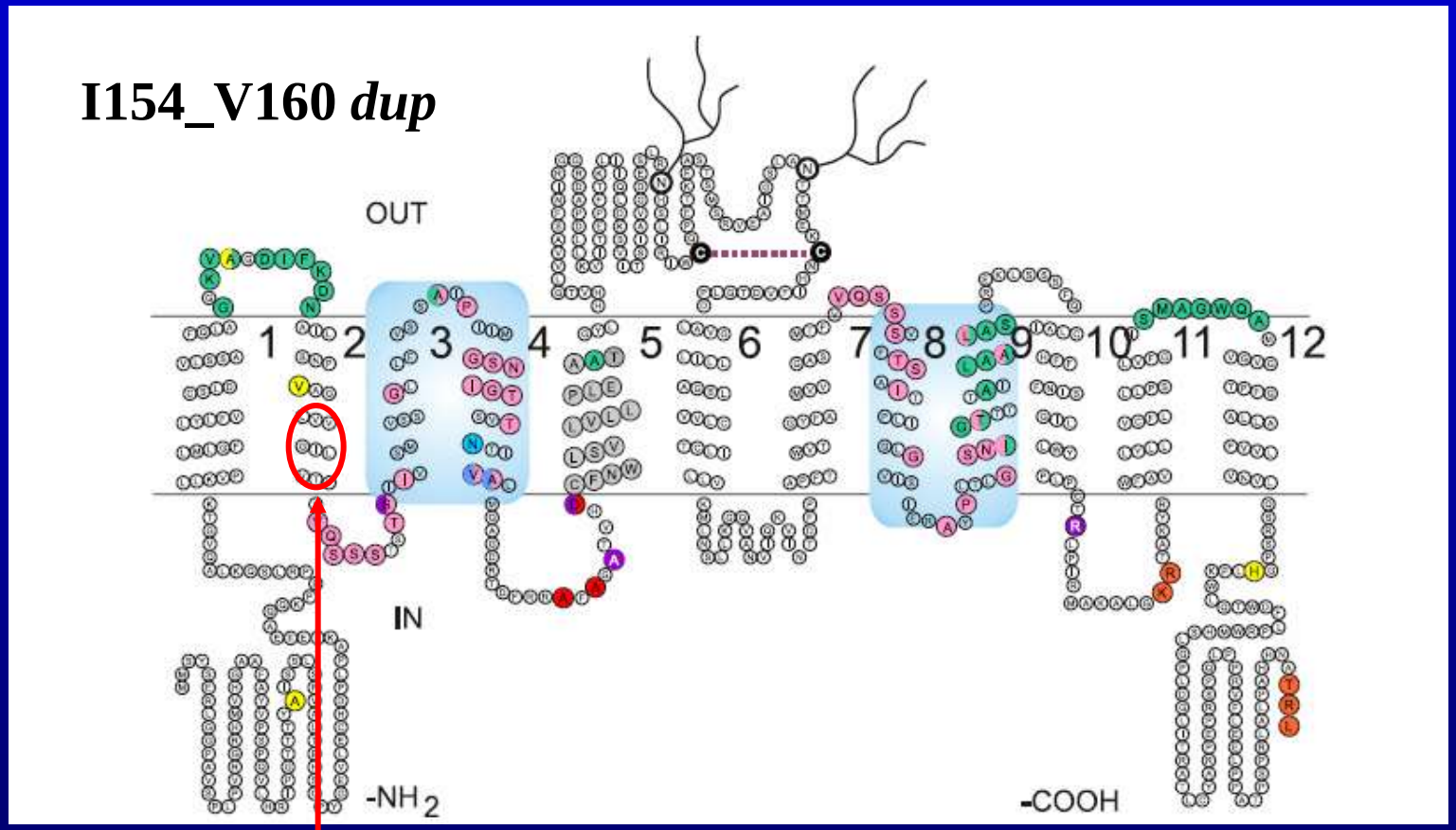
Cloning of PCR product of Exon 5 in heterozygous mother

WT



Mutant

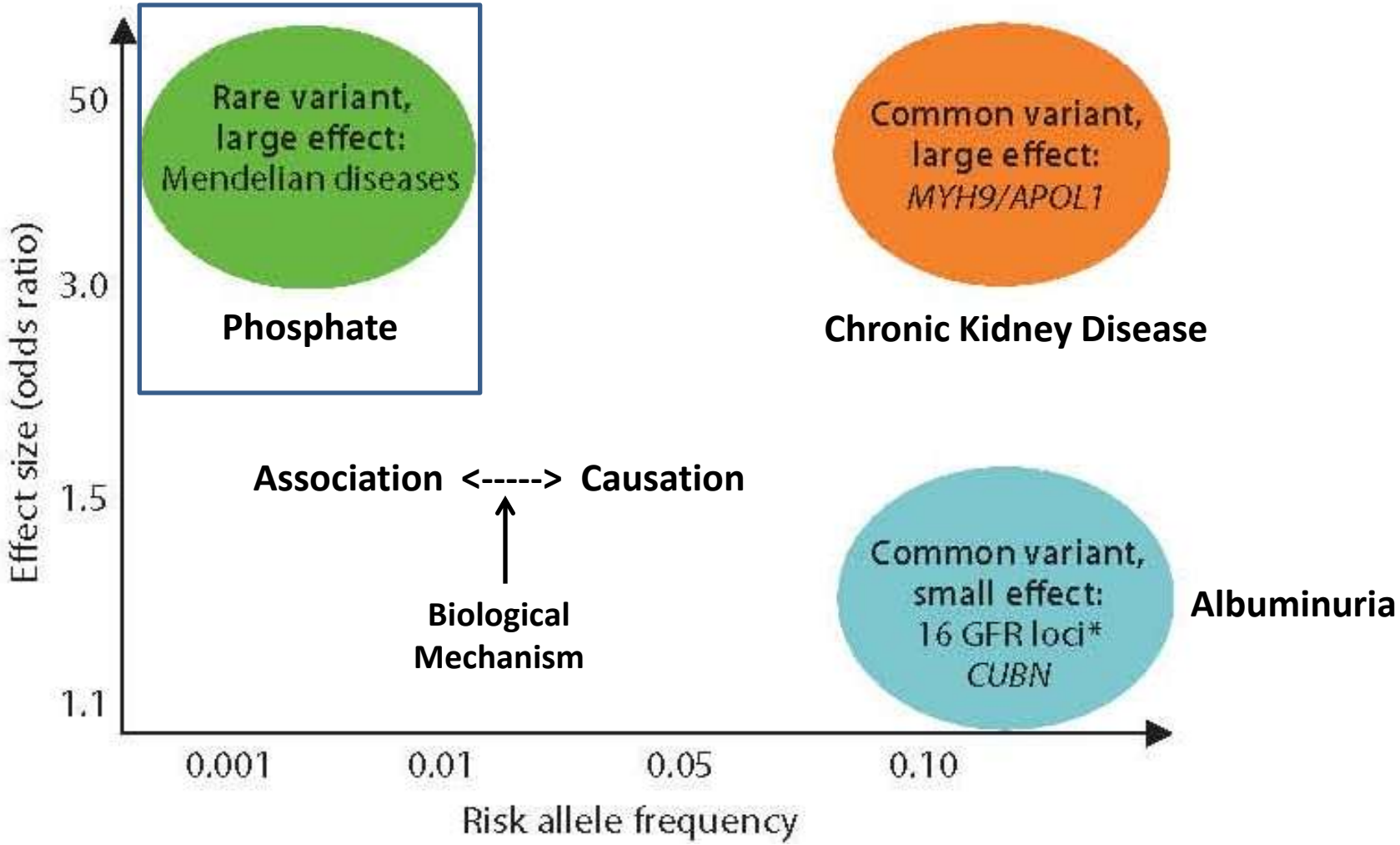
NaPi-IIa cotransporter proein



Region of duplication

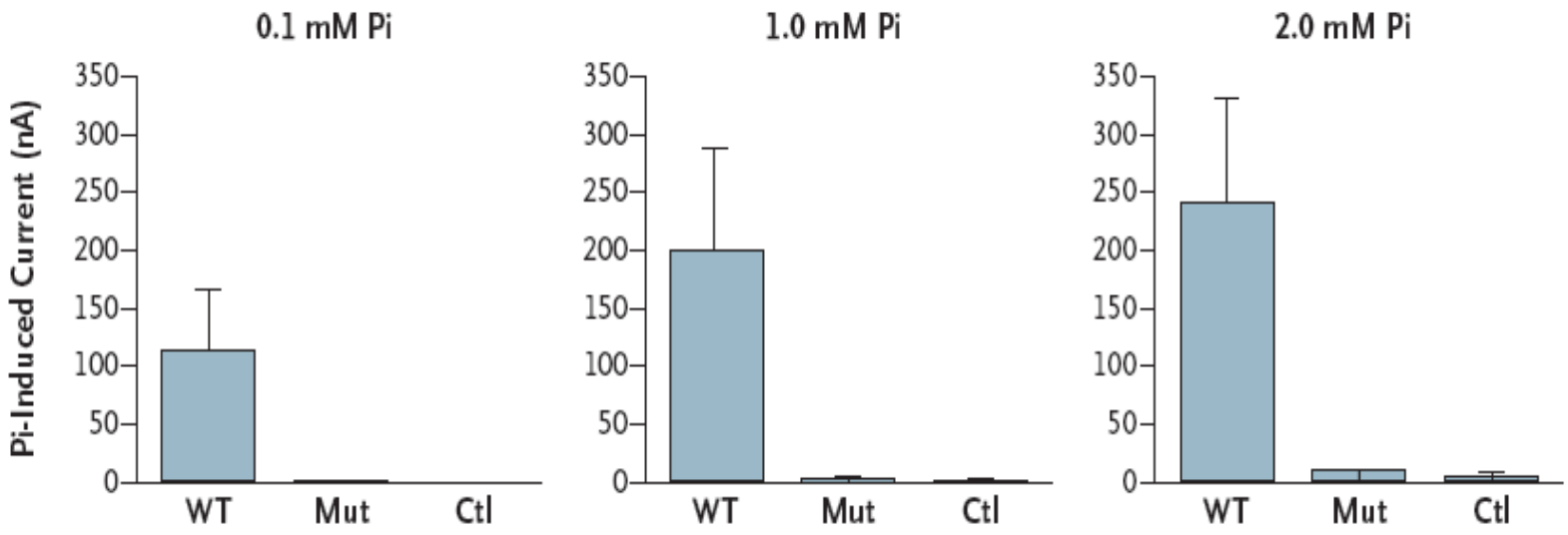
Ile, Leu, Val, Thr, Val, Leu, Val at p.154-160

Relation of effect size and risk allele frequency of DNA sequence variants associated with kidney function and disease risk phenotypes



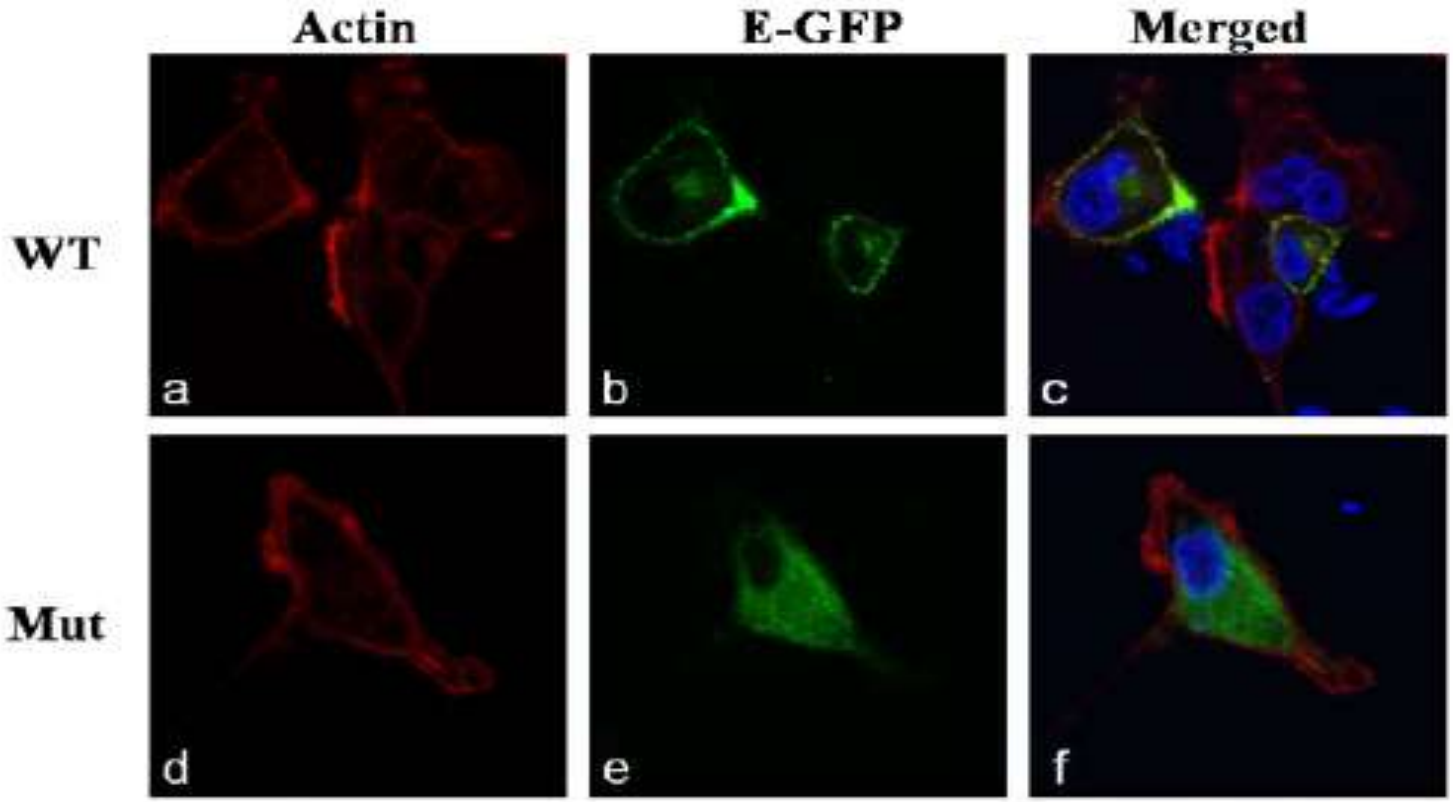
Adapted from Kidney and Blood Pressure Research 2011:34:225-234

Xenopus Oocyte cRNA Studies



- **Wild Type and Mutant cRNA generated in pSPORT for Oocyte injection**
- **Voltage clamped Pi-induced current measurement shows absent current with injection of mutant (no pre-equilibration current, and no dominnant negative effect when mixing 4-fold mutant to WT)**

Mutant NaPi-IIa Mislocalizes and Fails to Reach or be Retained at Plasma Membrane of OK cells



Laser scanning confocal microscopy of OK cells transfected with WT (a-c) or mutant (d-f) EGFP-tagged NaPi-IIa (green), actin conjugated with phalloidin Alexa Fluor 660 (red), and nuclei stained with DAPI (blue)

Questions

Specific:

- Why no RTA? What is the mechanism of proximal tubulopathy with NaPi-IIa disruption?

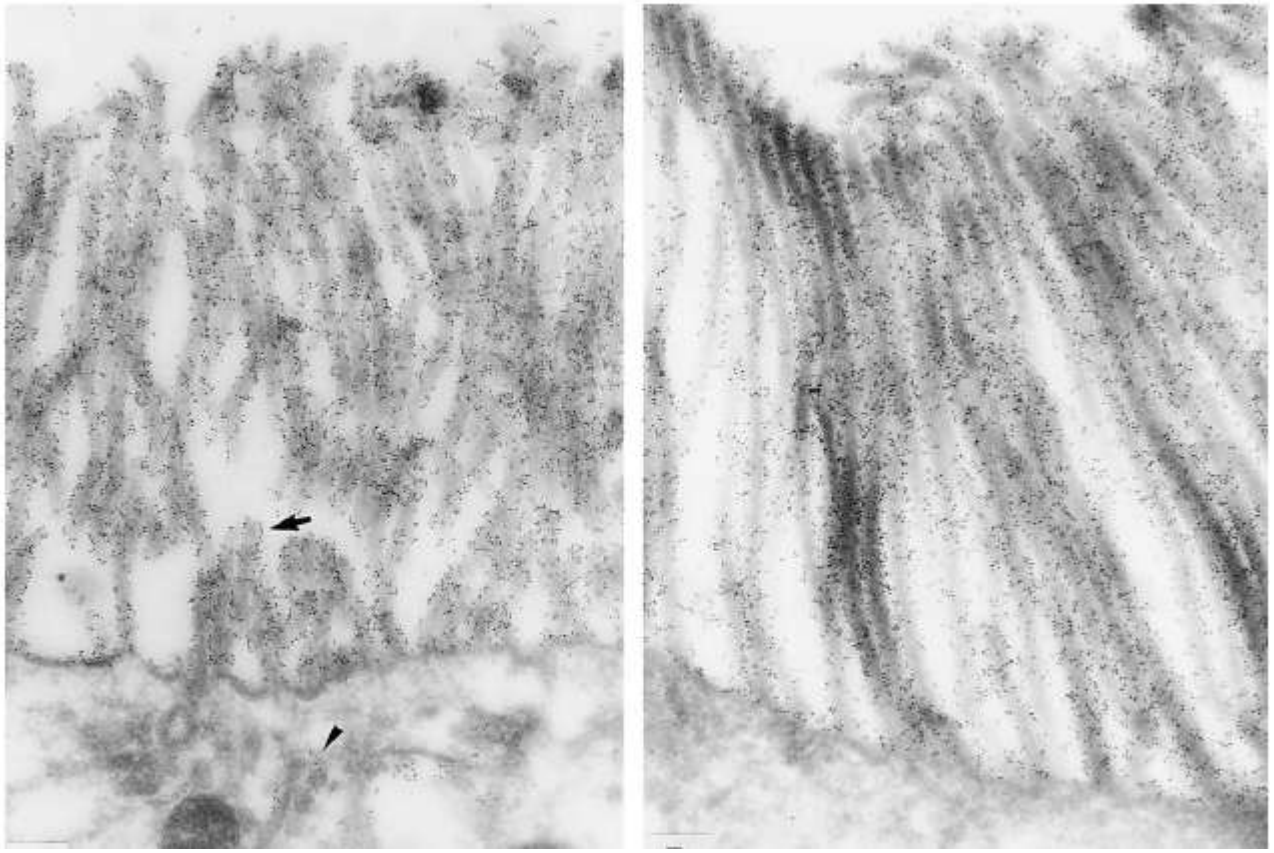
- Is this proof that NaPi-Iia is the major human transporter for renal PI reabsorption?

- Why are there only two patients reported to date (among many with phosphaturia phenotypes)

- Will other NaPi-IIa mutations also cause proximal tubulopathy?

NHE and NHERF Localization in Proximal Tubule

Sabolic´ et al



NHE3 highly abundant in S3

NaPiIIa diminishes in abundance from S1 to S3

Consequences of Misfolded Protein

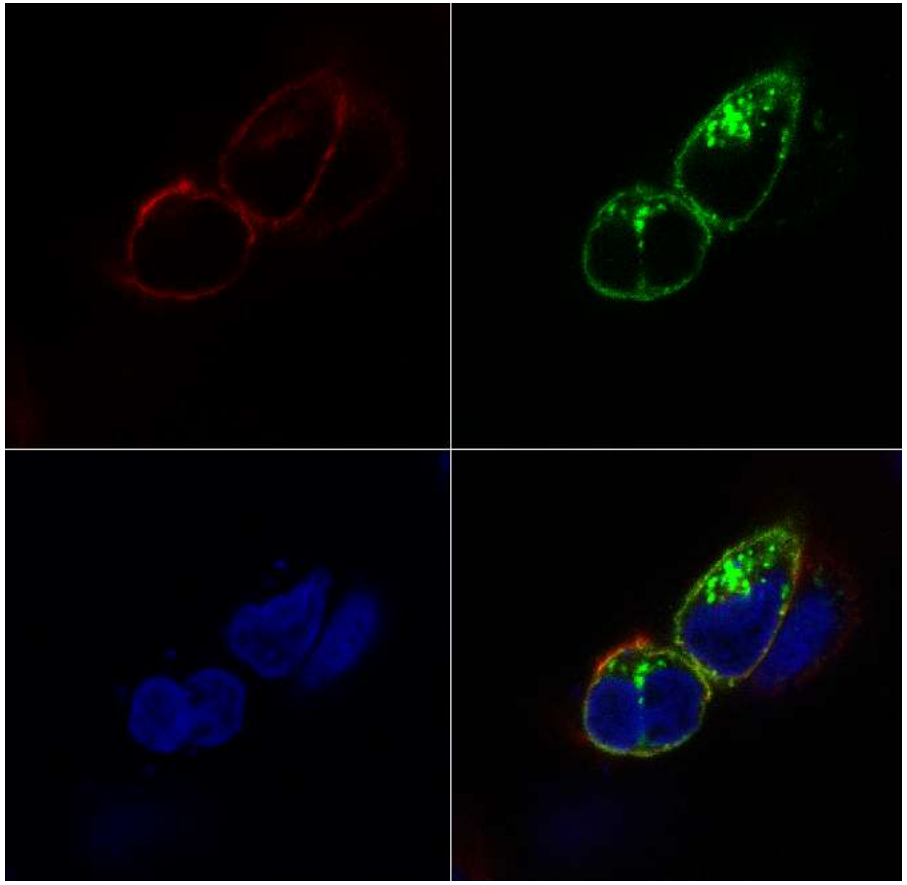
- **Misfolded Protein causes ER stress to epithelial cells in which mutant is expressed**
- **Possibility of rescue by pharmacologic or chemical chaperones**

DaniellaMagen

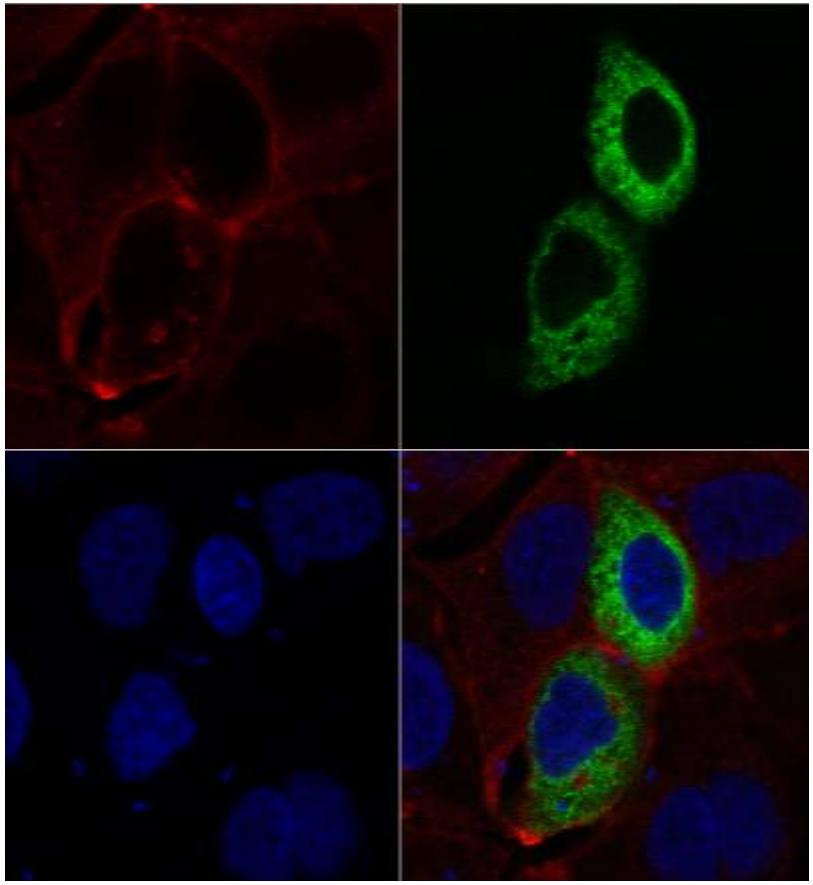
Ron Kopito's lab

Phenotypic Specificity Related to Endogenous Cell Specific Expression of Mutant Gene Product in Affected Patients (with possible developmental, dietary, hormonal, pharmacological response of proximal tubulopathy *per se*)

Caco-WT



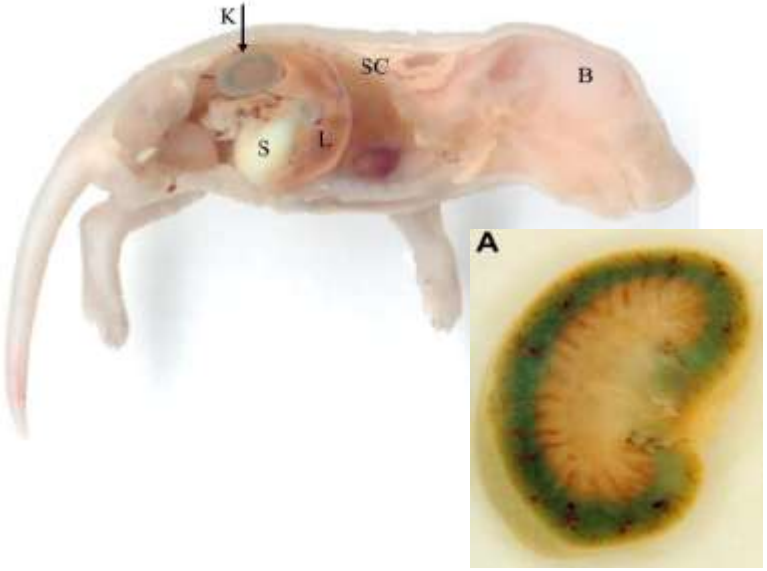
Caco-MUT

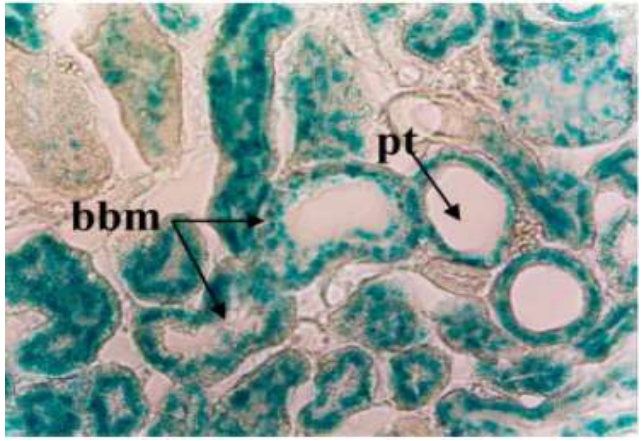


Am J Physiol Renal Physiol 292: F1617–F1625, 2007.
First published February 6, 2007; doi:10.1152/ajprenal.00412.2006.

A murine transgenic model for transcriptional regulation of the Na/Pi-IIa major renal phosphate cotransporter

Tzur Rosenberg,[1]* Catherine Shachaf,[1,3]* Maty Tzukerman,[1] and Karl Skorecki[1,2]





Working Biological Model: This NaPi2a mutant disrupts epithelial cell integrity – therefore general proximal tubulopathy and gain of injury model

Questions

General:

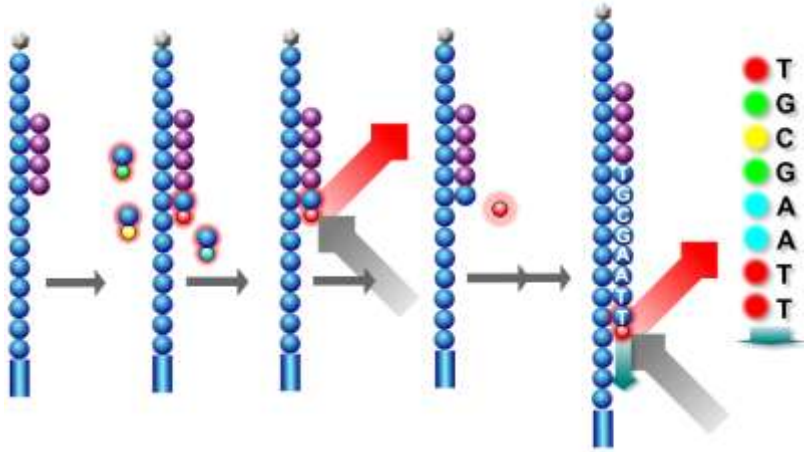
- ❖ **Will High Throughput Technologies (e.g. whole exome capture) supplant marker-based mapping?**

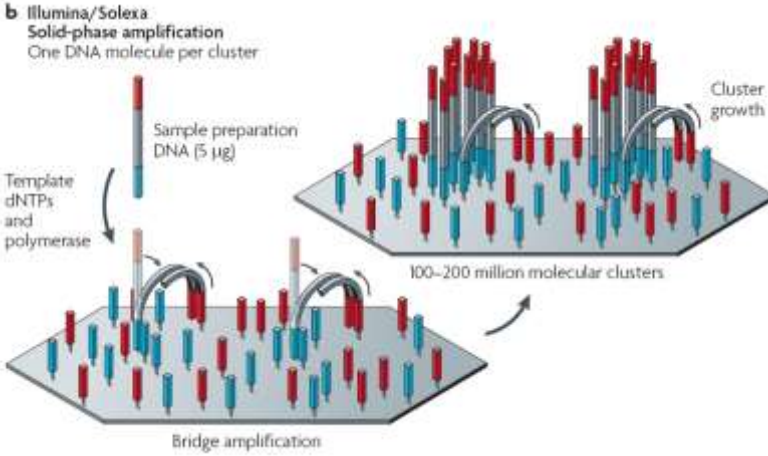
- ❖ **Relation of rare monogenic disease genes to common health and disease phenotypes**

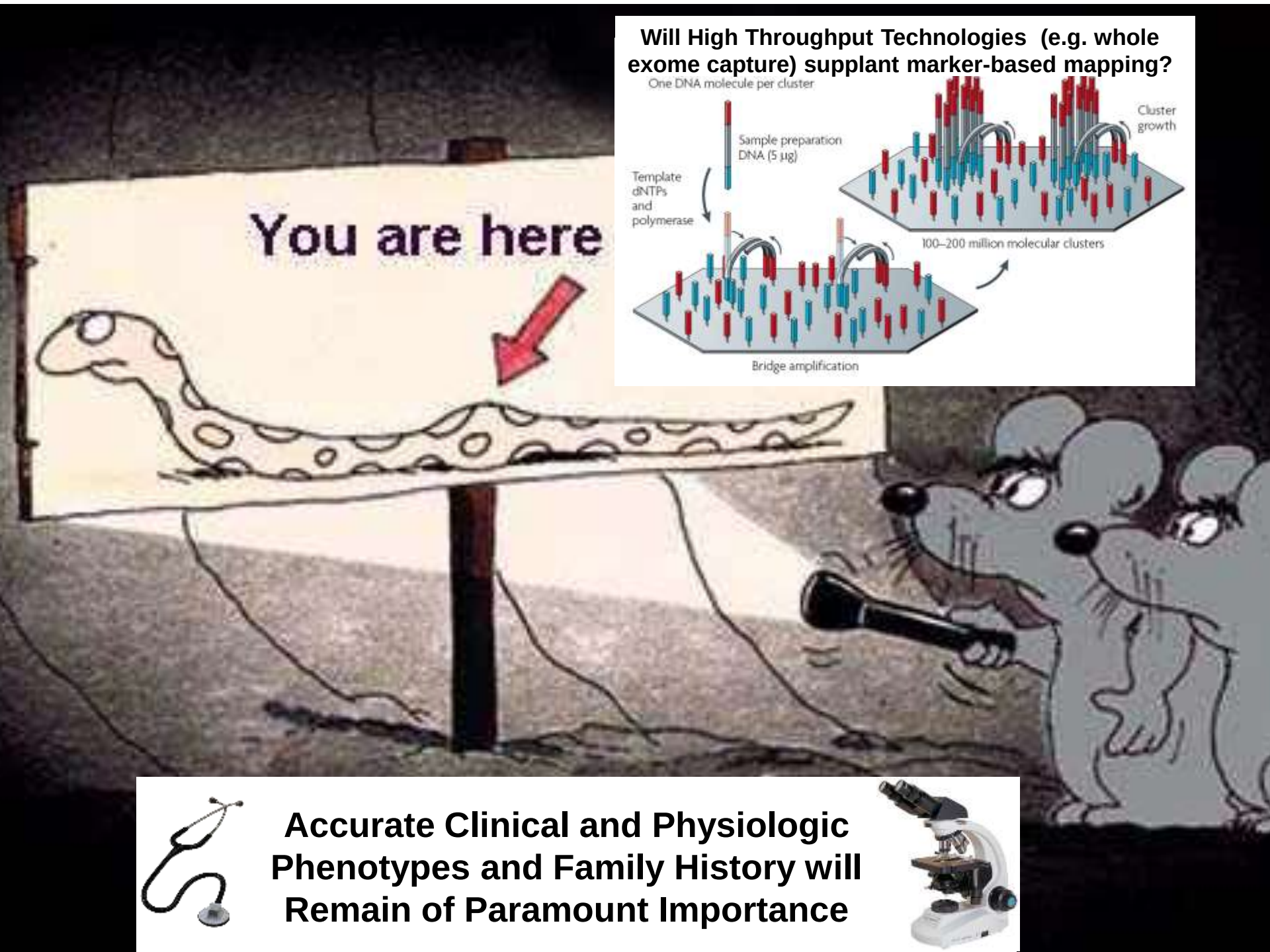
Step A – Regions of Homozygosity or Compound Heterozygosity (using Illumina 5M SNP CHIPS)

Step B – Whole Exome Capture and Sequencing (HiSeq 2000) in Informative Family Members (affected and non-carrier sib; trio etc.)

Sequencing by synthesis






You are here
Will High Throughput Technologies (e.g. whole exome capture) supplant marker-based mapping?
One DNA molecule per cluster
Sample preparation DNA (5 µg)
Template dNTPs and polymerase
Cluster growth
100–200 million molecular clusters
Bridge amplification
Accurate Clinical and Physiologic Phenotypes and Family History will Remain of Paramount Importance

Questions

General:

- ❖ **Will High Throughput Technologies (e.g. whole exome capture) supplant marker-based mapping?**

- ❖ **Relation of rare monogenic disease genes to common health and disease phenotypes**

Phosphate in Health and Disease

Cellular Processes

- nucleic acids
- membrane phospholipids
- protein posttranslational modification
- ATP energetics
- other

Extracellular Processes

- bone mineral composition
- growth plate chondrocyte growth and apoptosis
- plasma and urine buffering
- urolithiasis
- extraosseous calcification

The primacy of phosphate retention in:

- CKD-MBD pathogenesis
- CKD pathogenesis
- Cardiovascular Health and Disease (at all levels of GFR)

Control of Phosphate Excretion in Uremic Man

The Journal of Clinical Investigation Volume 47 1968 1865

E. SLATOPOLSKY, A. M. ROBSON, I. ELKAN, and N. S. BRICKER

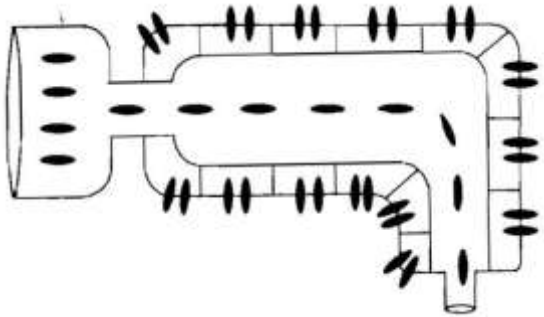
From the Renal Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

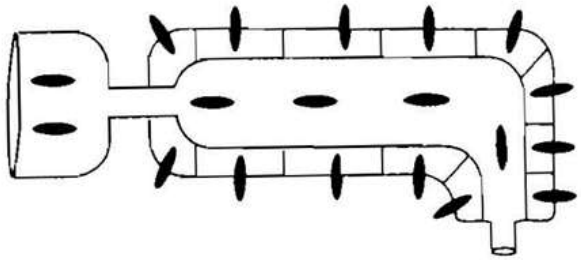
NEJM 286:20, 1093, 1972

ON THE PATHOGENESIS OF THE UREMIC STATE

An Exposition of the "Trade-off Hypothesis"

NEAL S. BRICKER, M.D.

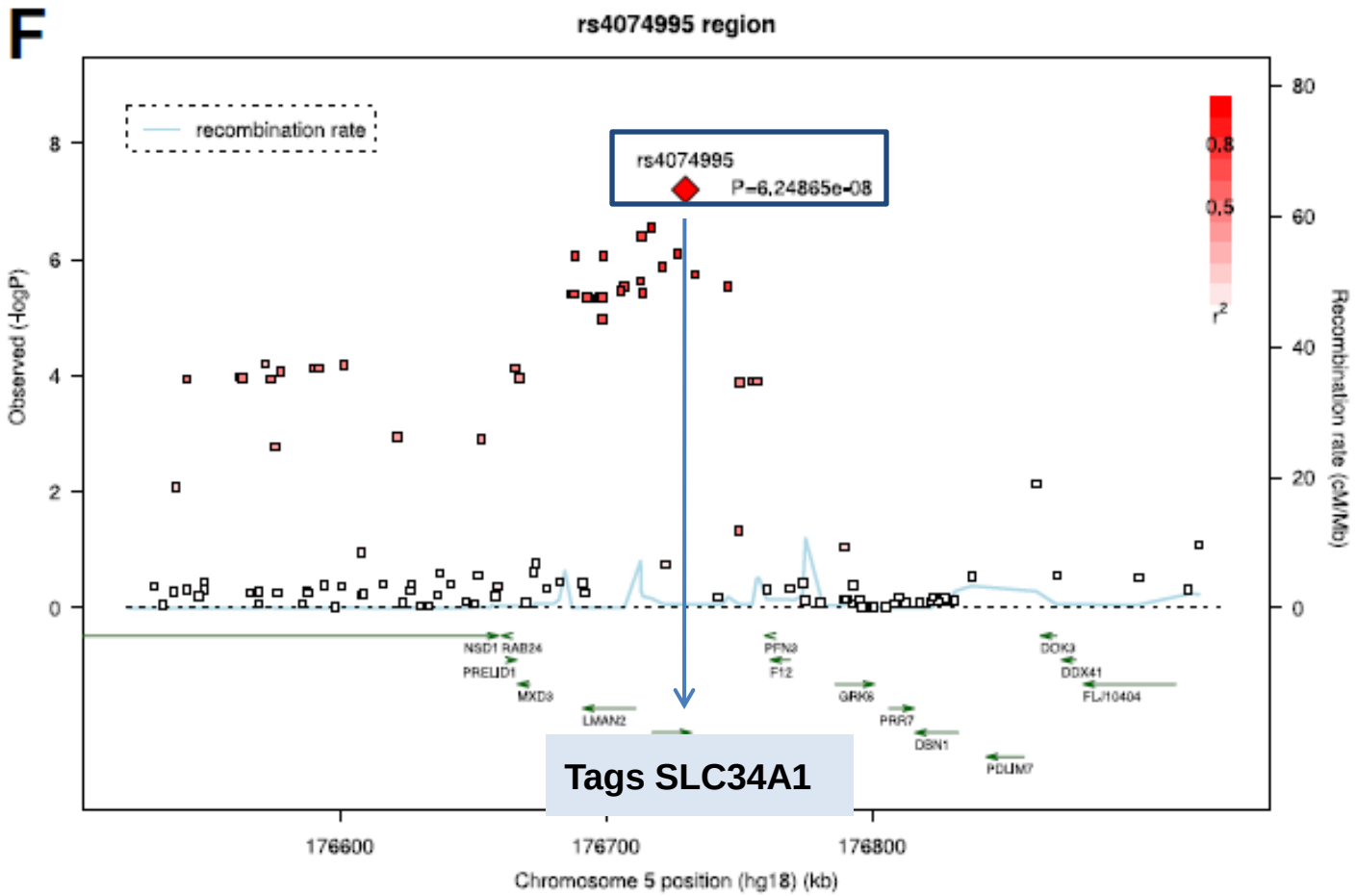


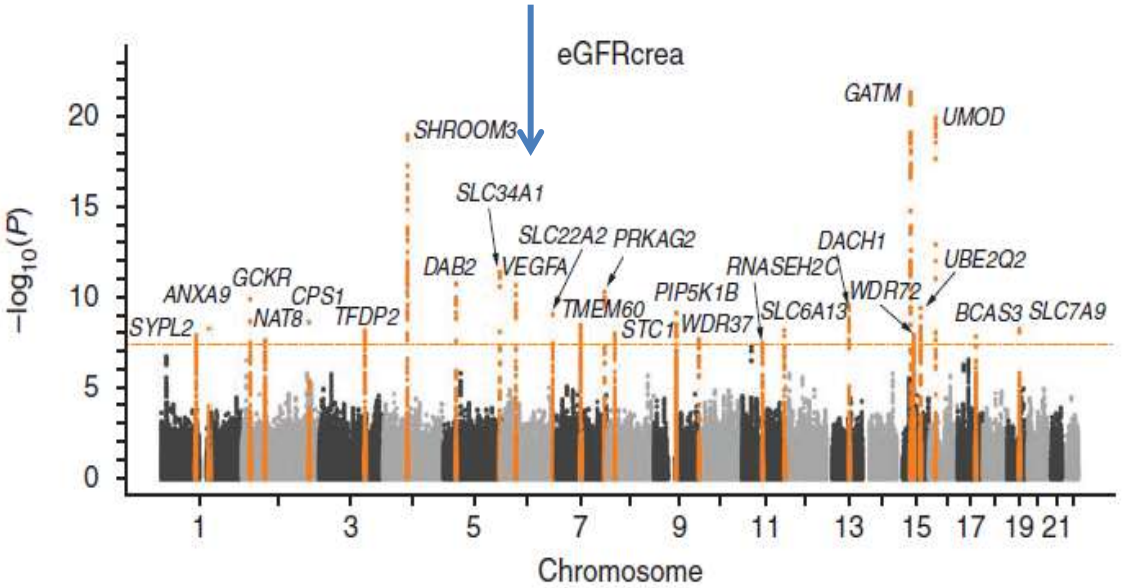


Does Mapping of Mutations in Genes Causing Rare Monogenic Disease Shed Light on Common Health and Disease Phenotypes (e.g. Stones, Bones, CKD)

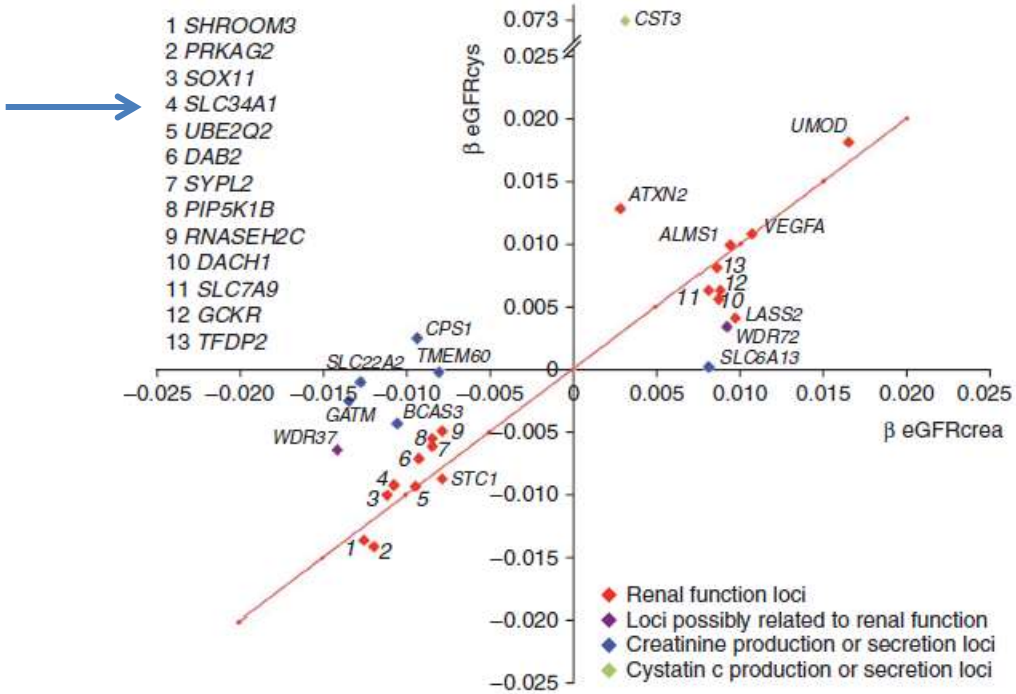
Common Genetic Variants Associate with Serum Phosphorus Concentration

Kestenbaum et al. JASN 2010





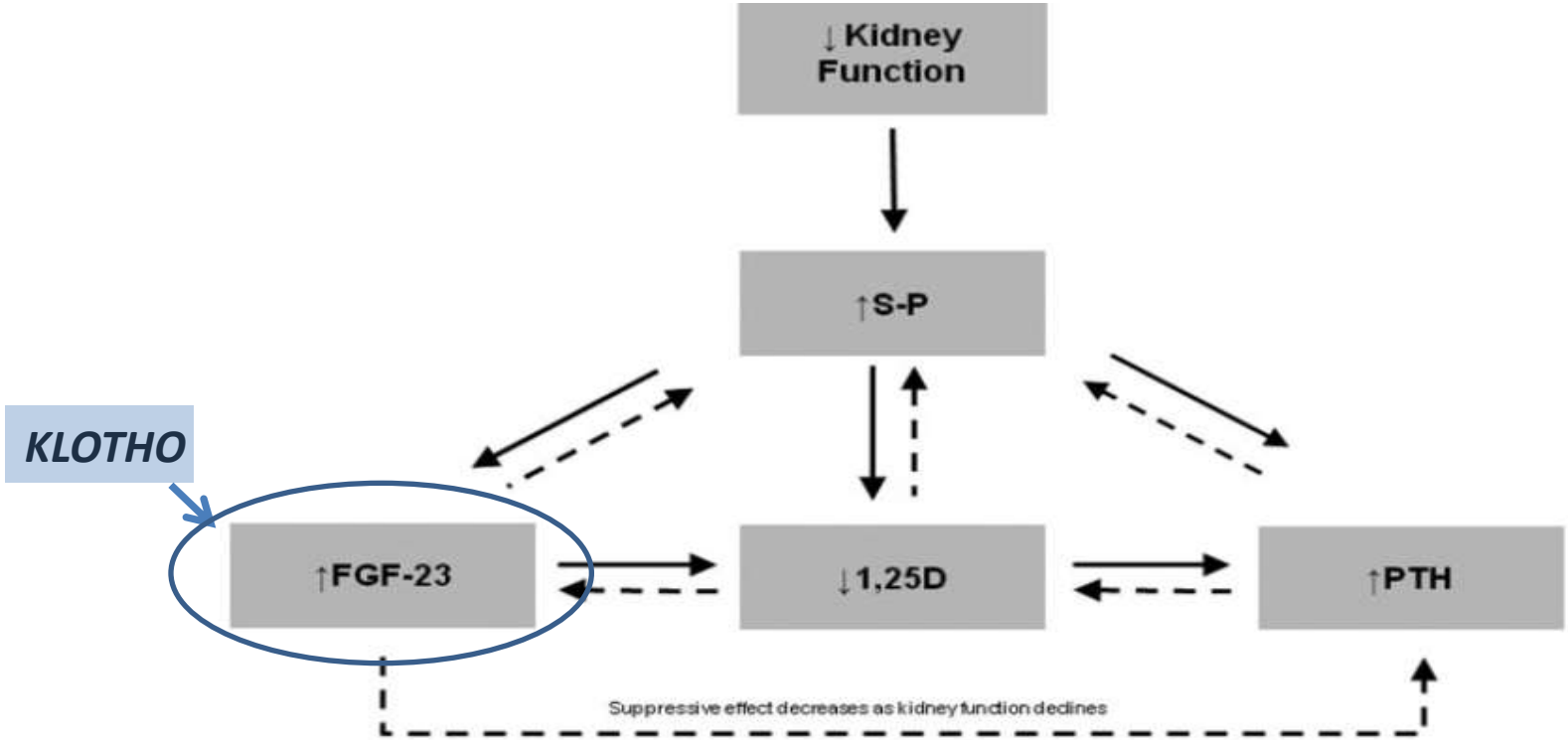
CKD



Kottgen et. al 2010

Martin and Gonzales 2011

Prevention and Control of Phosphate Retention/ Hyperphosphatemia in CKD-MBD: What Is Normal, When to Start, and How to Treat?



CLASSIFICATION OF INHERITED DISEASES

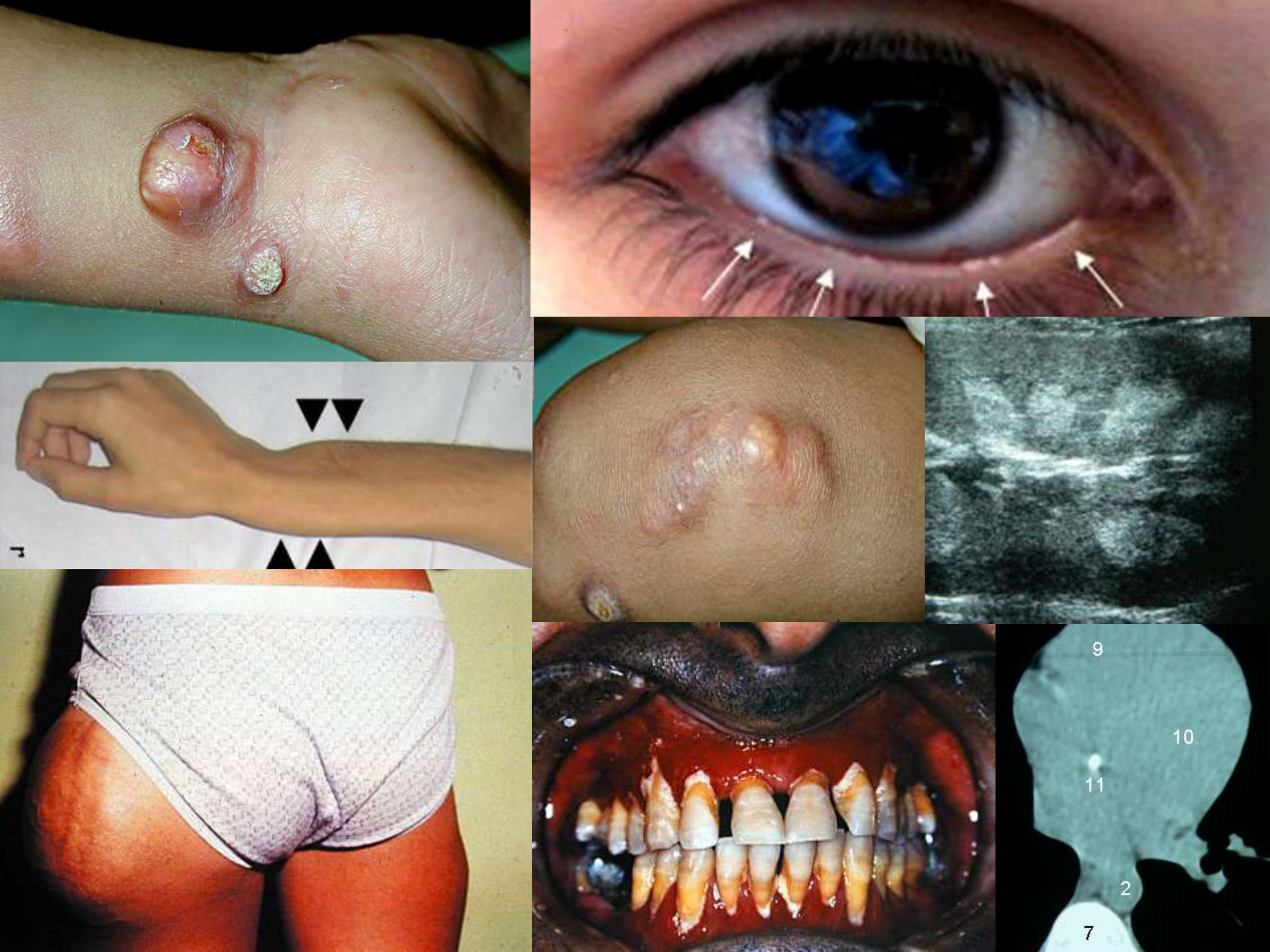
Monogenic – Mendelian Inheritance patterns

Complex disease and phenotypes

Polygenic – non-Mendelian Inheritance patterns

Defining the abnormal phenotype:

“phenomics”


9
10
11
2
7

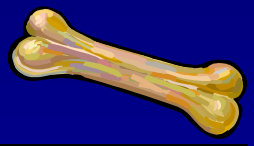
Hyperostosis-hyperphosphatemia syndrome (HHS)

- Autosomal recessive
- Repeated attacks of painful swellings of the long bones
- Radiological evidence of periosteal reaction and cortical hyperostosis.
- Metabolic abnormalities as in HFTC
- Reports of coexistence of FTC and HHS

GALNT3 MUTATION

- one mutation
- two different phenotypes

MODIFIER TRAIT



HFTC

HHS

"Harris, when I said 'any questions' I was using only a figure of speech."