UNC
CENTER FOR
PHARMACOGENOMICS AND
INDIVIDUALIZED THERAPY

Pre-emptive Pharmacogenetics

Oct 20th, 2015

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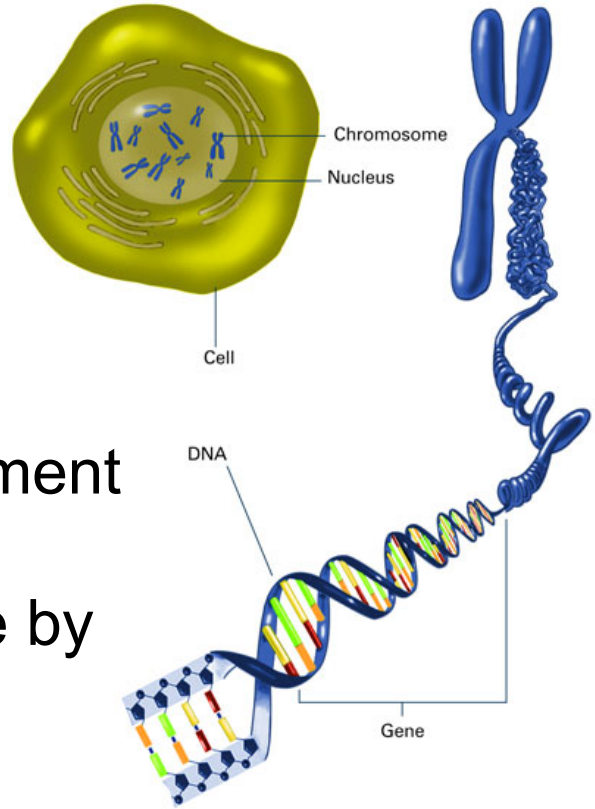
Associate Professor, UNC Eshelman School of Pharmacy

Here's my sequence…

New Yorker - 2000

Variation in the Human Genome

- Our genome contains 3 billion base pairs of DNA
- Between 2 people, there are approximately 3 million base pair differences
- Understanding variation has shown promise for improving disease treatment and outcomes
- Variation can change drug response by affecting pharmacokinetics or pharmacodynamics

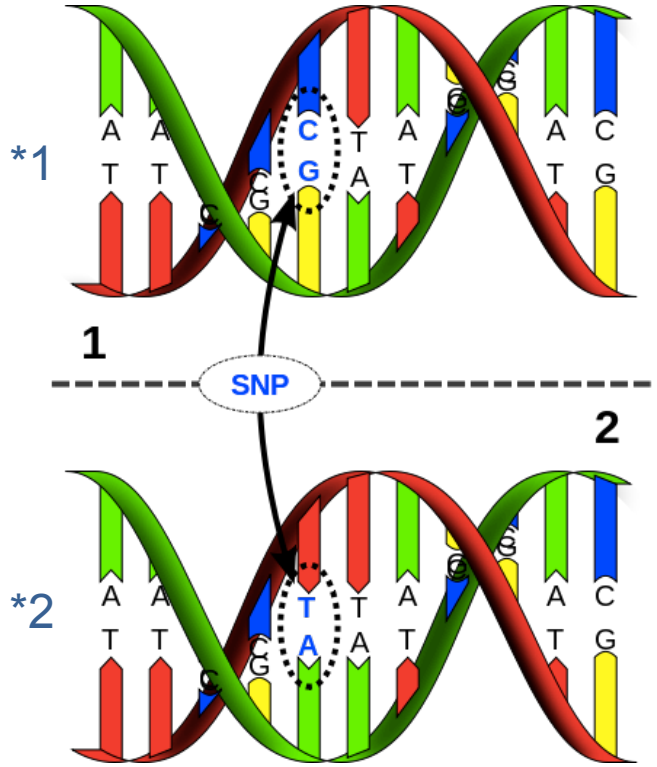


National Institute of General Medical Sciences

Variation in the Human Genome

- Single nucleotide polymorphism (SNP)
  - Most common cause of genetic variation
  - Example: *VKORC1* 1173 C>T
- Other polymorphisms:
  - Insertions
  - Deletions
  - Duplications

Polymorphism
"Poly" *Many*
"Morphe" *Form*

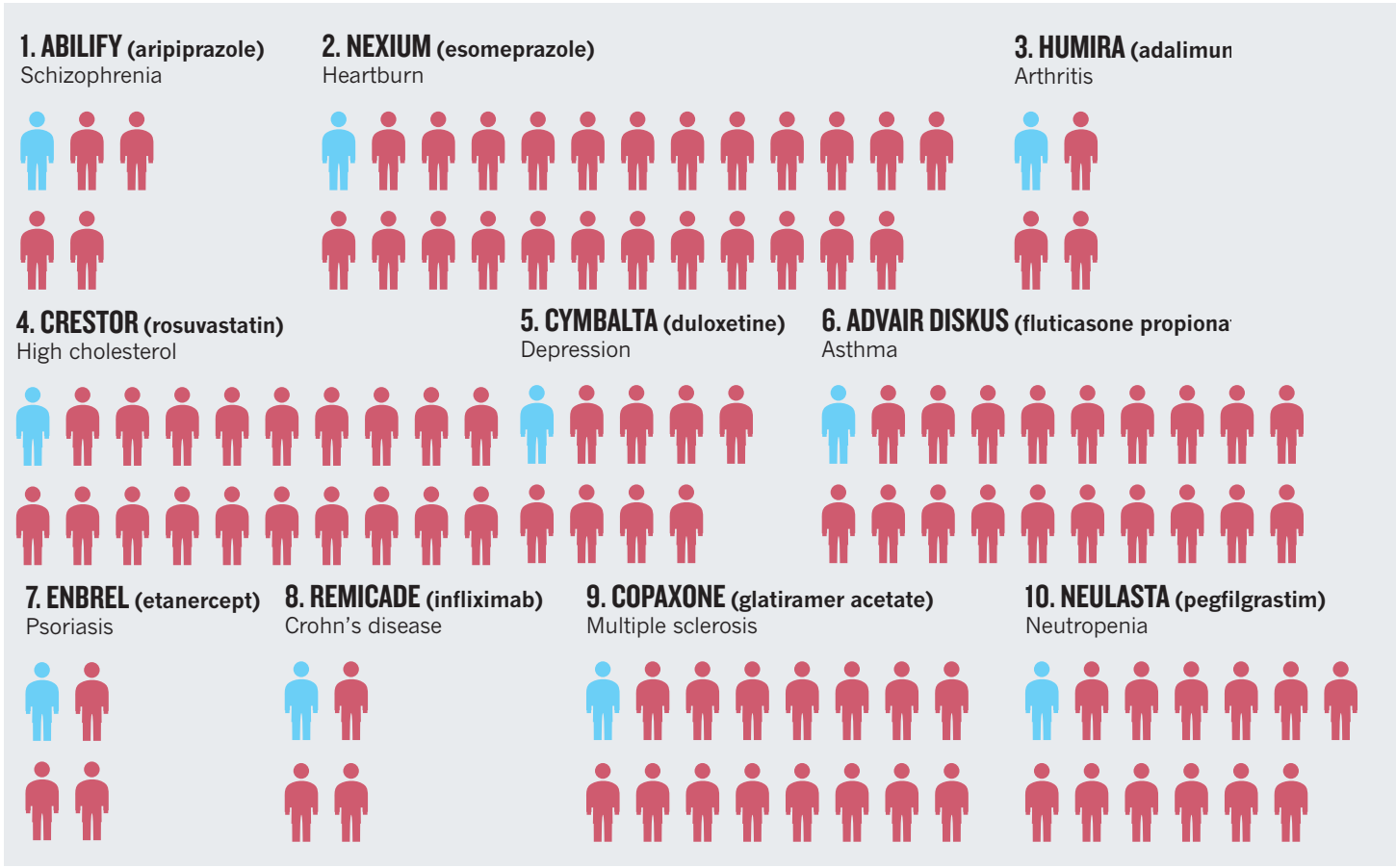


Variation in the Human Genome

- Different versions of a gene are called alleles
  - Example: *CYP2C19* *1/*2

Allele	Function	Frequency of CYP2C19 Variants (%)	
		Caucasian/African American	Asian
1	**Wild-Type**	**-**	**-**
2	**Loss**	**10-15**	**30-35**
3	**Loss**	**<1**	**5-10**
17	**Gain**	**16-22**	**1-3**

Current Model: Imprecision Medicine

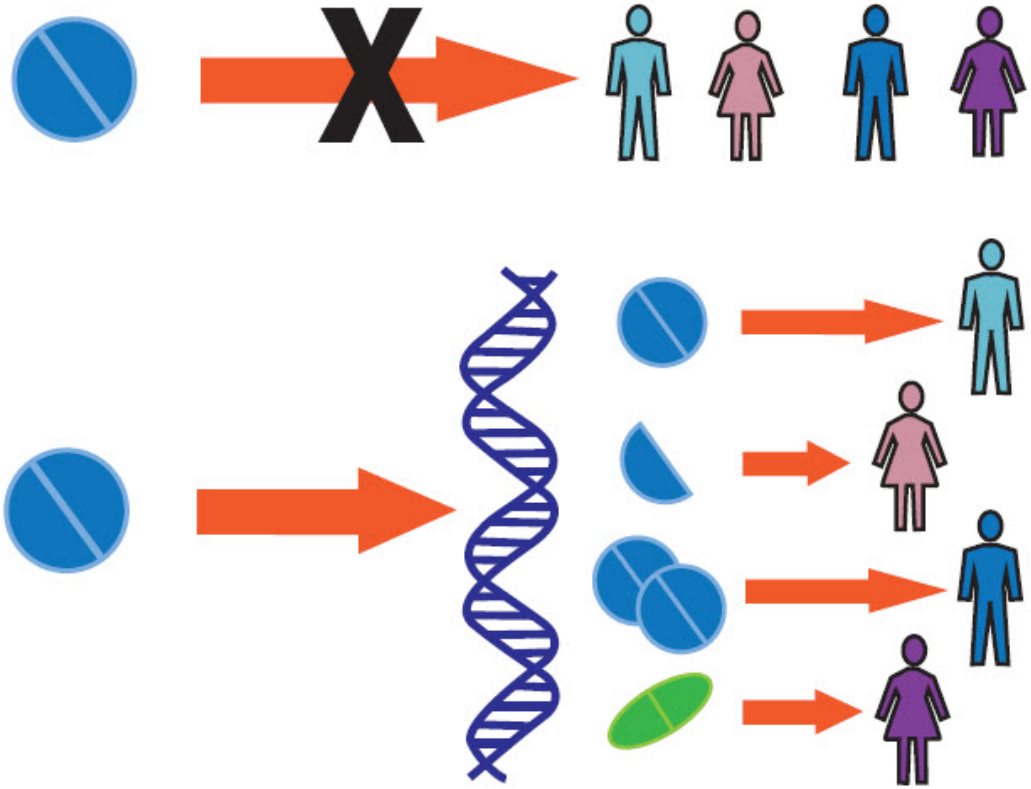


Blue = Treatment Success

Red = Treatment Failure

Schork NJ. *Nature* 2015;520:609-611.

Utilizing Genotype Data



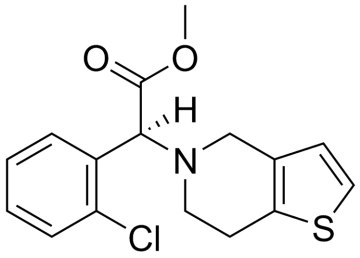
http://medicine.iupui.edu/IIPM

Limitations in Clinical Practice

- Medical record implementation
- Health system logistics
- **Turnaround time**
- **Cost and insurance coverage**
- Privacy and trust
- Clinician education
- **Clear medical recommendations**

Predicting Efficacy

- Approximately 25% of patients on clopidogrel experience a sub-therapeutic response

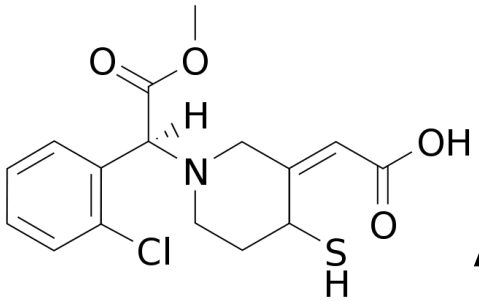


Clopidogrel (Prodrug)

Cytochrome P450
1A2
2B6
X **2C19**

Intermediate Metabolite

Decrease



Cytochrome P450
3A4
3A5
2B6
2C9
X **2C19**

Active Metabolite

Decrease

Therapeutic Failure

Black Box Warning

WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS

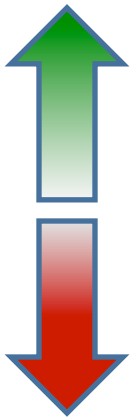
See full prescribing information for complete boxed warning.

- **Effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. (5.1)**
- **Poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) than patients with normal CYP2C19 function. (12.5)**
- **Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy. (12.5)**
- **Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers. (2.3, 5.1)**

Plavix® prescribing information: 3/12/2010

CYP2C19 Polymorphisms

- Genetic variation in *CYP2C19* is common and is influenced by race/ethnicity
  - ***1* = wild-type allele** *(minor allele frequencies)*
  - ***2* = loss-of-function allele** (Caucasians/AA: 10-15% Asians: 30-35%)
  - ***3* = loss-of-function allele** (Caucasians/AA: <1% Asians: 5-10%)
  - ***17* = gain-of-function allele**(Caucasians/AA: 16-22% Asians: 1-3%)

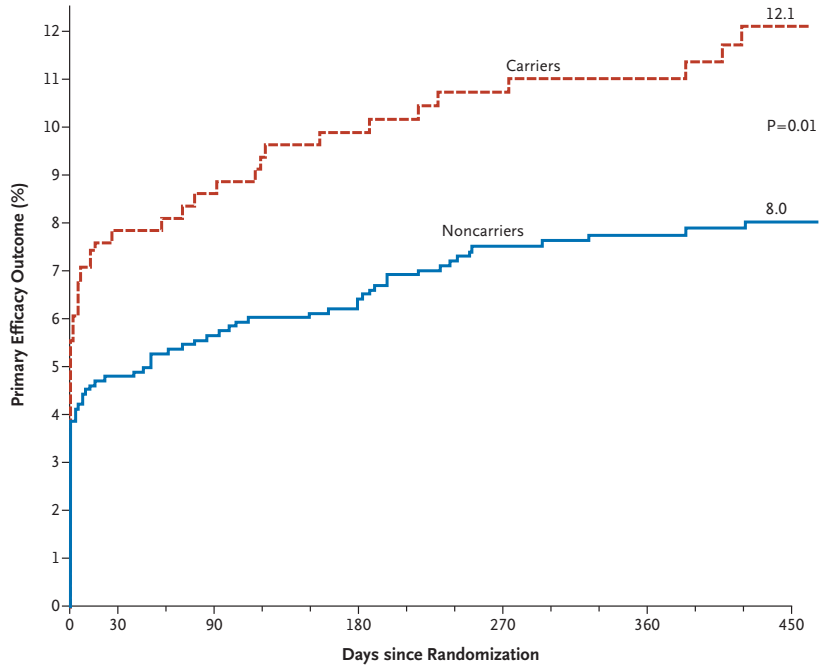


Metabolizer Phenotype	Genotype	U.S. (%)
Ultra-rapid	***17/*17**	1-5%
Rapid	***1/*17**	20-30%
Extensive	***1/*1**	35-50%
Intermediate	***1/*2** or ***1/*3** or ***2/*17** or ***3/*17**	20-30%
Poor	***2/*2** or ***2/*3** or ***3/*3**	1-5%

CYP2C19 Status and Outcomes in Subjects Receiving Clopidogrel

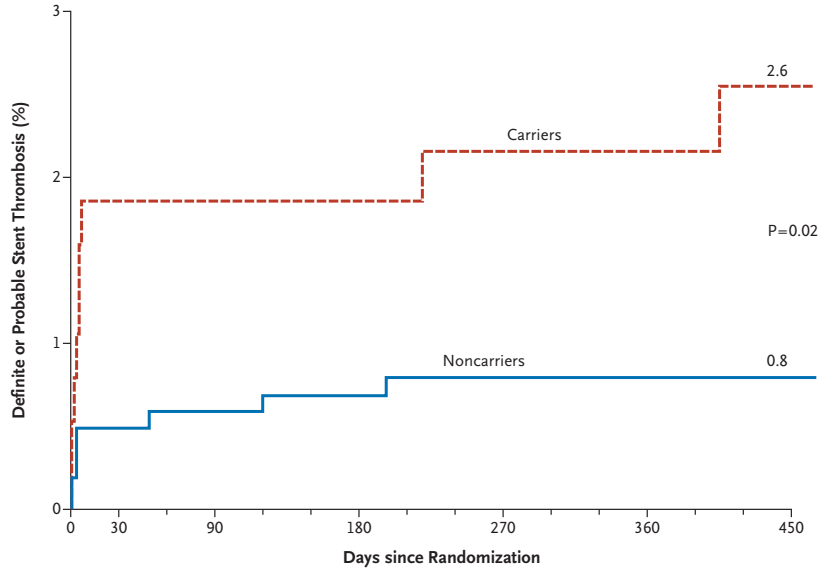
(Composite of Death due to Cardiovascular Causes, Myocardial Infarction, and Stroke)

Primary Efficacy Outcome



No. at Risk							
Carriers	395	364	360	348	306	270	181
Noncarriers	1064	1009	999	980	870	755	542

Stent Thrombosis



No. at Risk							
Carriers	375	368	366	359	316	279	186
Noncarriers	1014	1004	1001	989	885	765	547

Carriers = Reduced Function Allele (*2 or *3)

Mega JL, et al. N Engl J Med 2009;360:354-362.

UNC algorithm

"High risk" patient undergoing PCI

P2Y12 inhibitor initiation
(clinician discretion)

CYP2C19 genotype obtained
(in medical record within 24-48 hours)

Follow-up on *CYP2C19* genotyping result
(continue or switch P2Y12 inhibitor ?)

65-70%	*3-5%*	*25-30%*
EM (**1/*1, *1/*17*)	UEM (**17/*17*)	IM / PM (any **2* or **3* carrier)
Clopidogrel	Clopid, Pras or Ticag *(clinician discretion)*	Prasugrel or Ticagrelor *(clinician discretion)*

PGx Genome

Genes (# of SNPs)	All actionable
CYP2C19 (11)	BCHE (3)
CYP2C9 (2)	RYR1 (3)
CYP2D6 (15)	NAT2 (4)
CYP3A4 (9)	ABCB1 (3)
CYP3A5 (2)	TYMS (3)
DPYD (3)	G6PD (1)
HLA-A (13)	VKORC1 (9)
HLA-B (4)	UGT1A1 (2)
IL28B (3)	TPMT (5)
ITPA (1)	Haplotype for HLA-A, B, C
SLCO1B1 (2)	

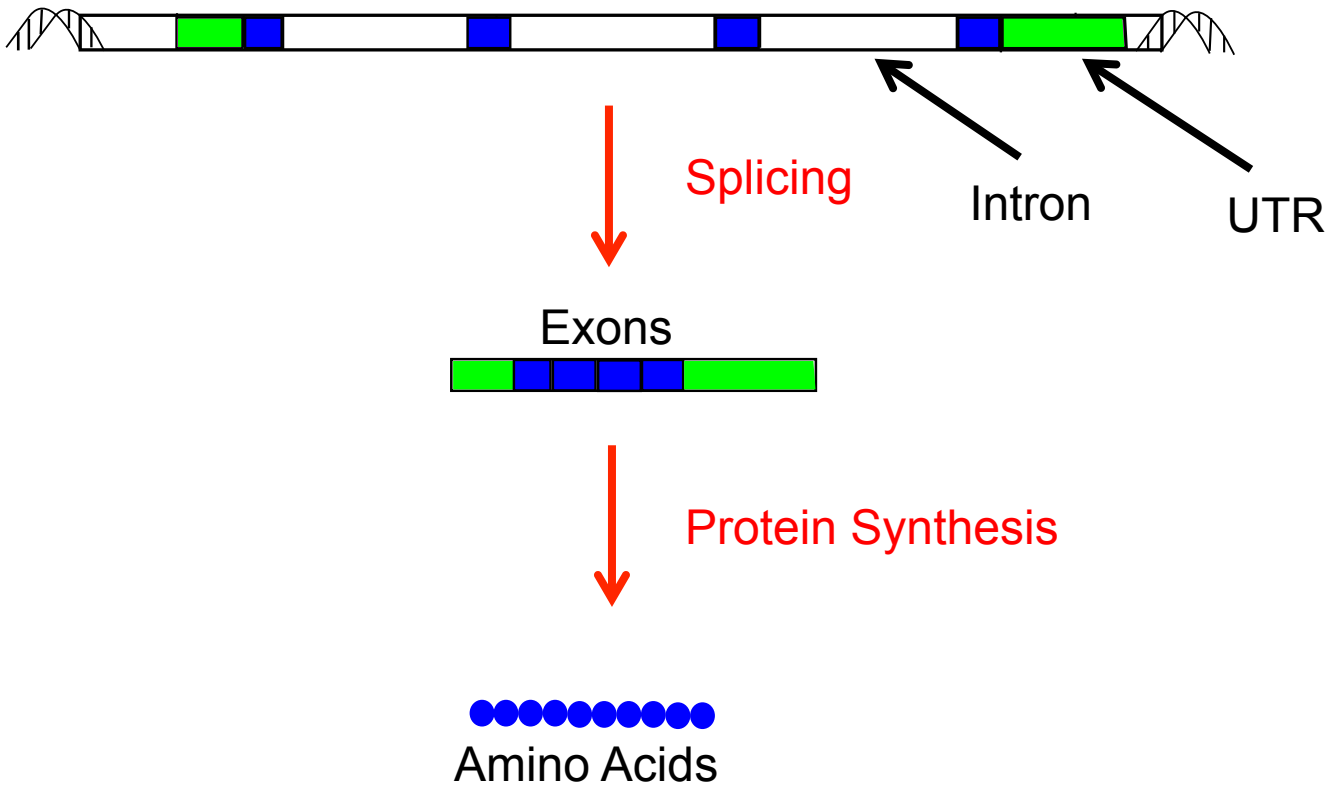
Identify Genes of Interest to Sequence

- UCSC Genome Browser
  - Obtain exon positions and mRNA strands

mRNA/Genomic Alignments

BROWSER	SIZE	IDENTITY	CHROMOSOME	STRAND	START	END	QUERY	START	END	TOTAL
browser	1789	99.9%	10	+	96522438	96612962	NM_000769	1	1789	1799

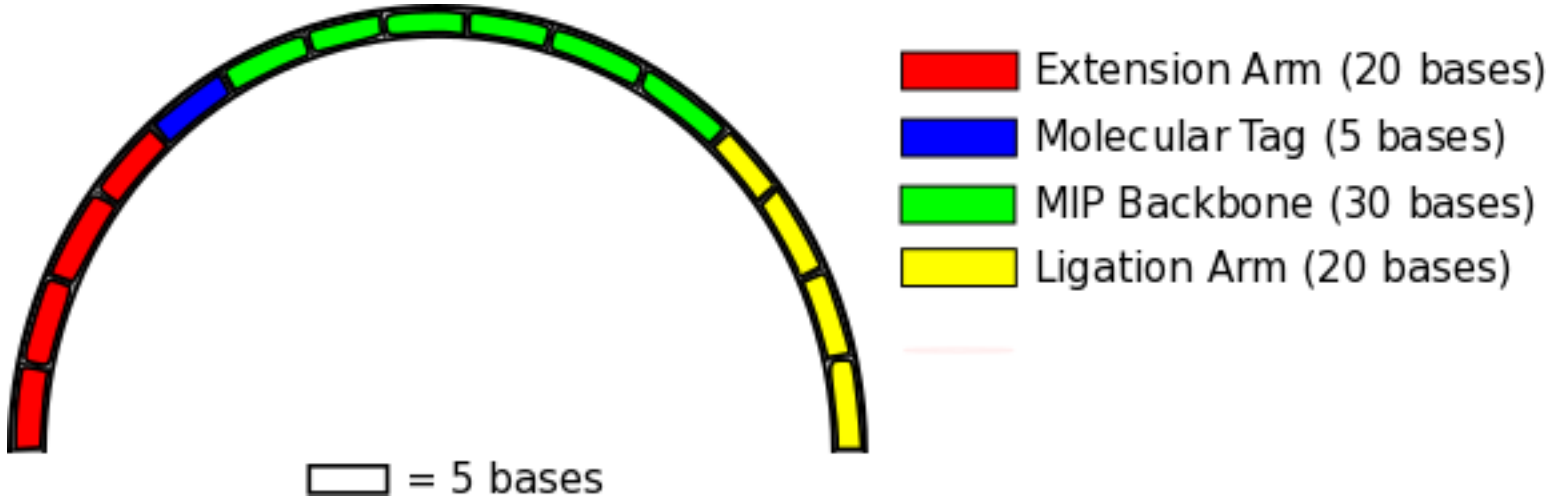
Sequences of Interest



- Sequence all exons for each PGx gene

What is a molecular inversion probe (MIP)?

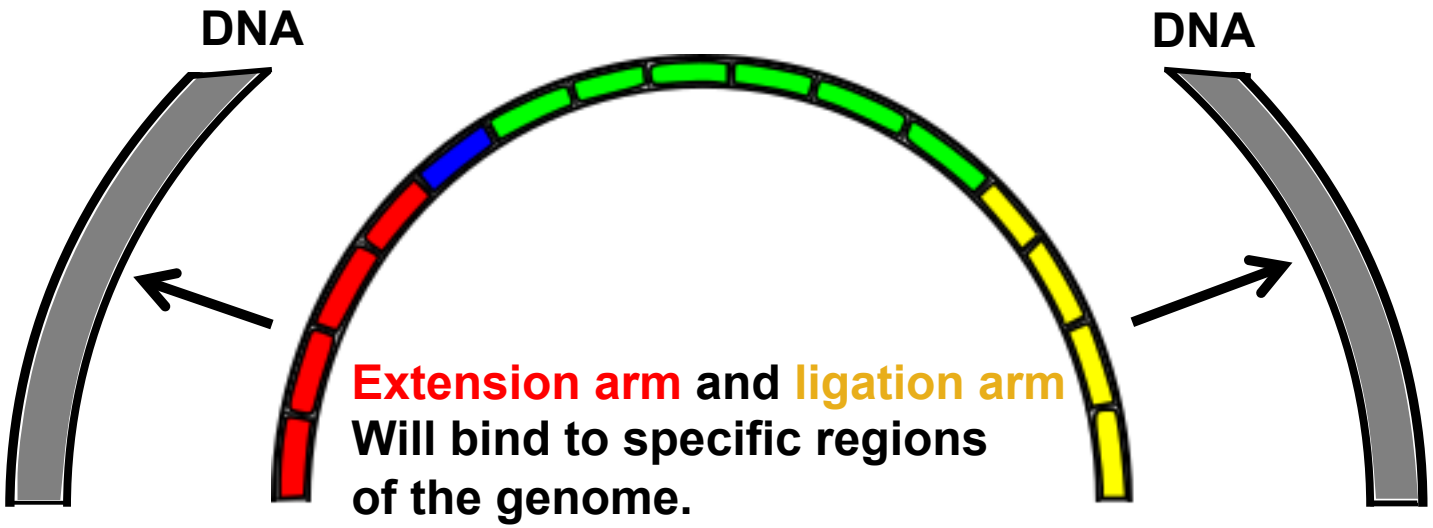
75 oligonucleotide



MIPs capture and enriches specific regions of the genome.

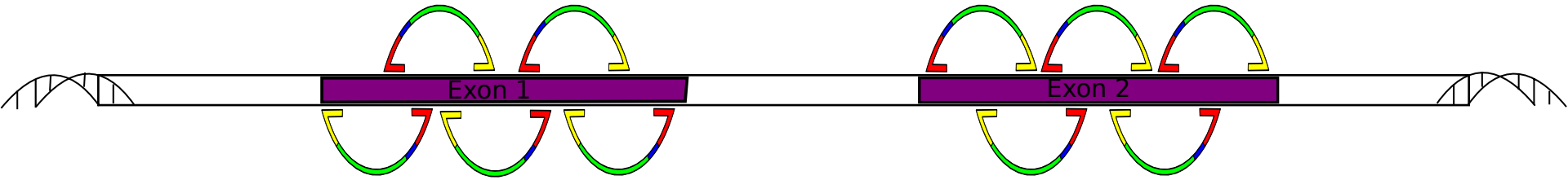
What is a molecular inversion probe (MIP)?

75 oligonucleotide



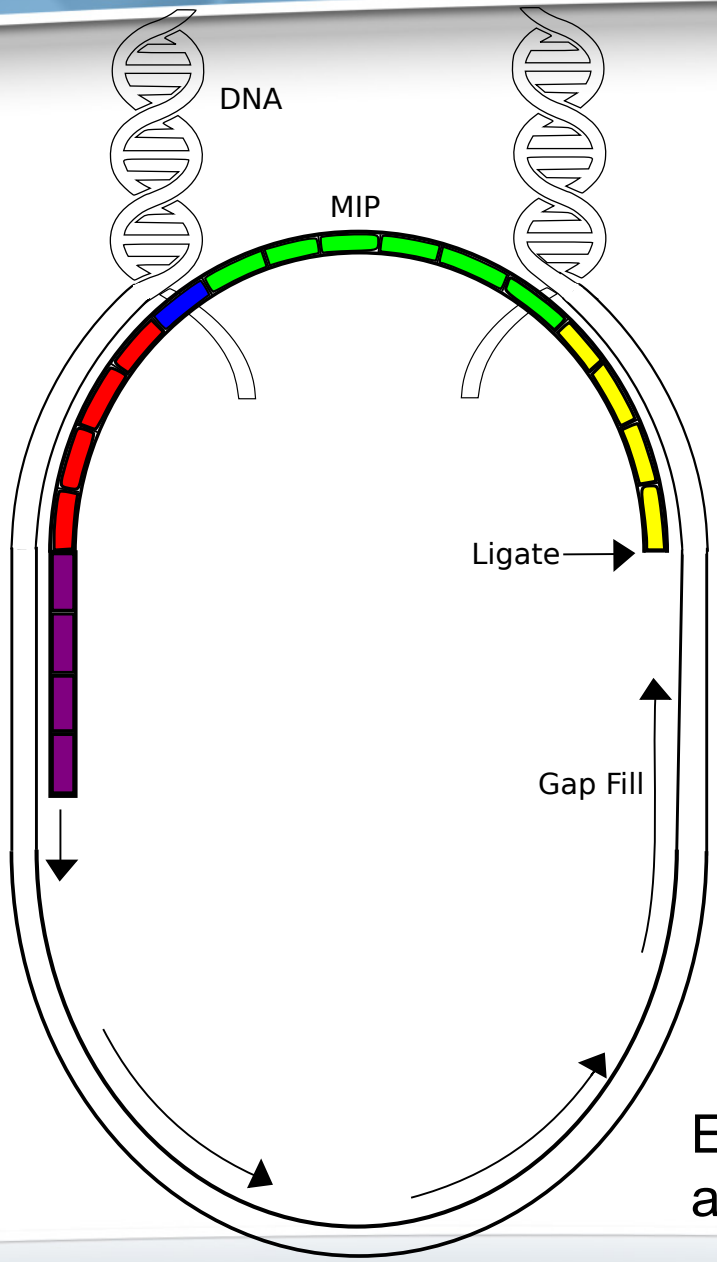
MIPs capture and enriches specific regions of the genome.

MIPs are Tiled to Capture All Sequences of Interest



Each MIP captures 112 bases. Pool together all MIPs to capture all regions of interest.

How are sequences of interest captured?

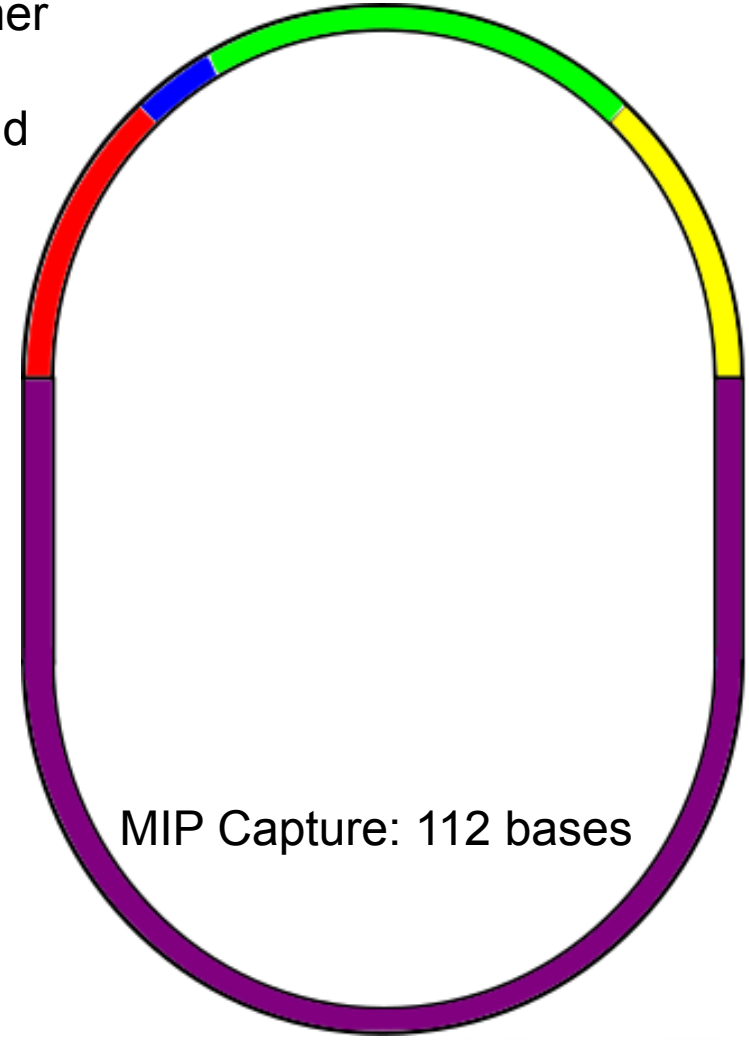


Extension Arm (20 bases)
Molecular Tag (5 bases)
MIP Backbone (30 bases)
Ligation Arm (20 bases)
DNA
MIP Capture (112 bases)

Each MIP captures a unique sequence.

A MIP Capture

75-mer DNA strand

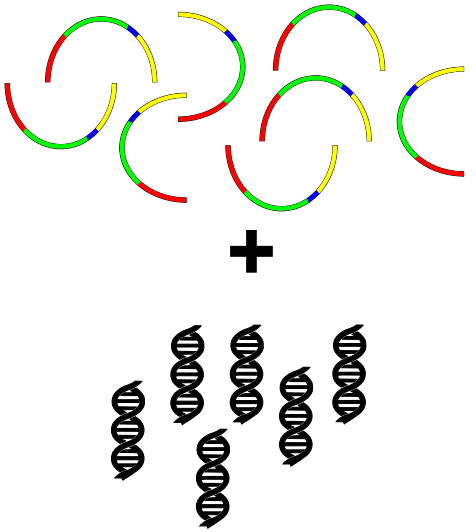


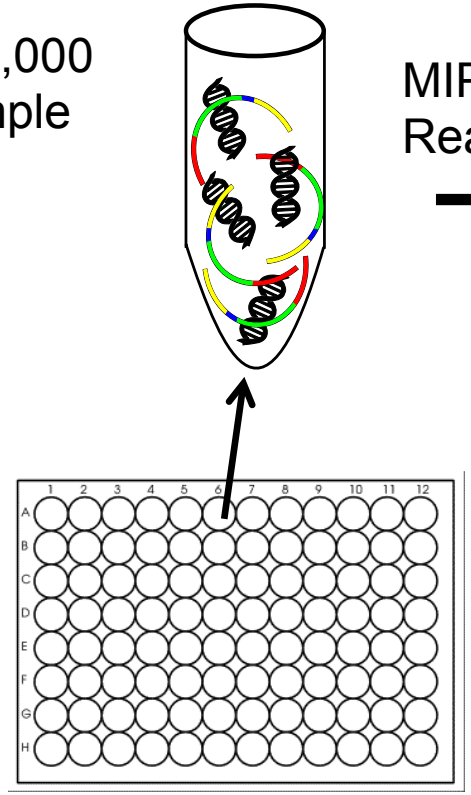
- Extension Arm (20 bases)
- Molecular Tag (5 bases)
- MIP Backbone (30 bases)
- Ligation Arm (20 bases)
- MIP Capture (112 bases)

Each MIP captures a unique sequence.

A MIP Capture Library is Created from Each Sample

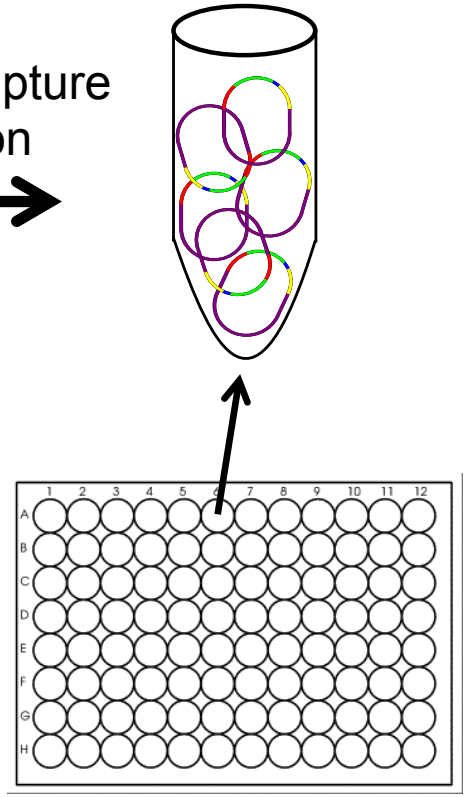
Pool DNA and multiple copies of ~2,000 unique probes together for one sample





Each well has MIPs and DNA for one sample

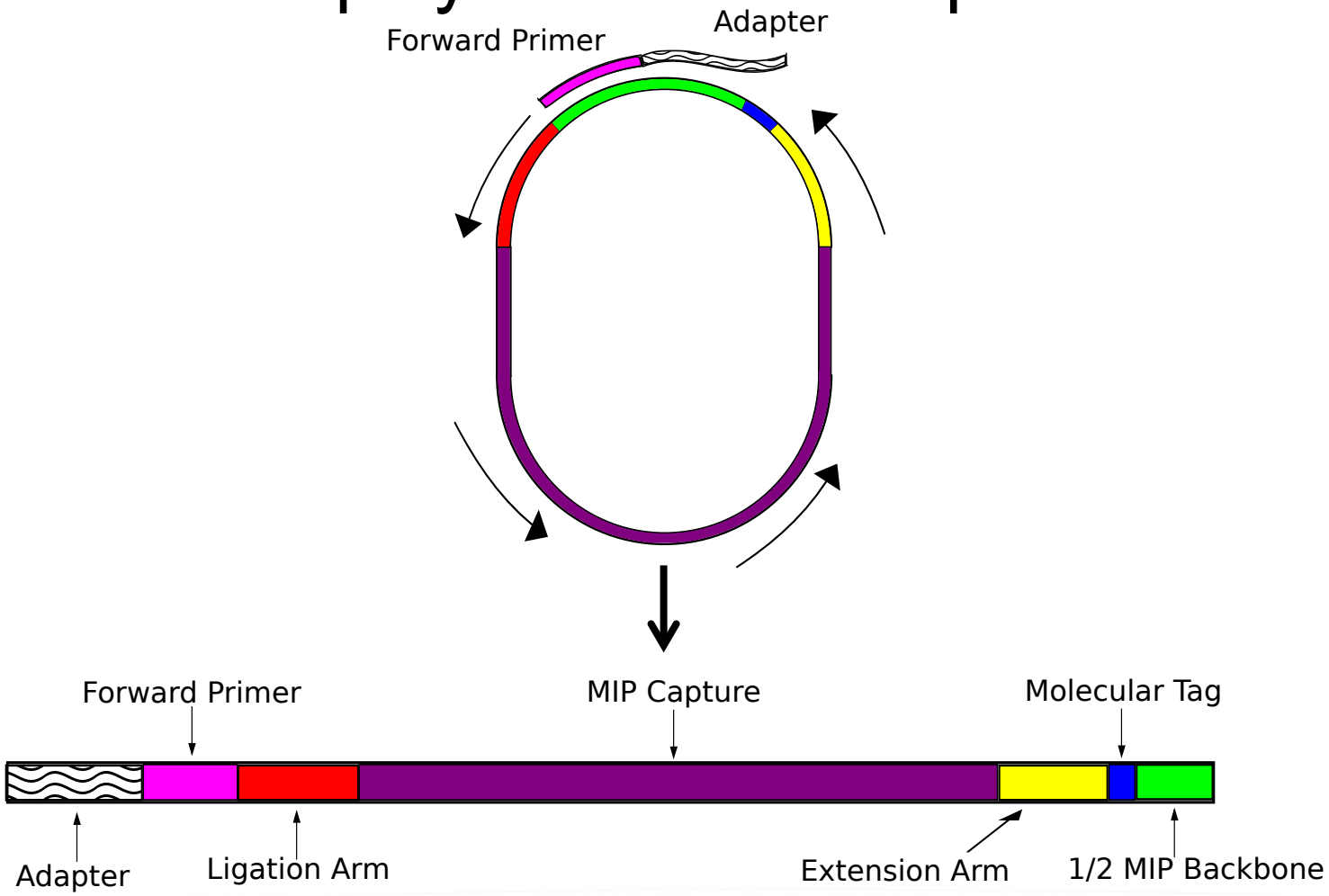
MIP Capture Reaction



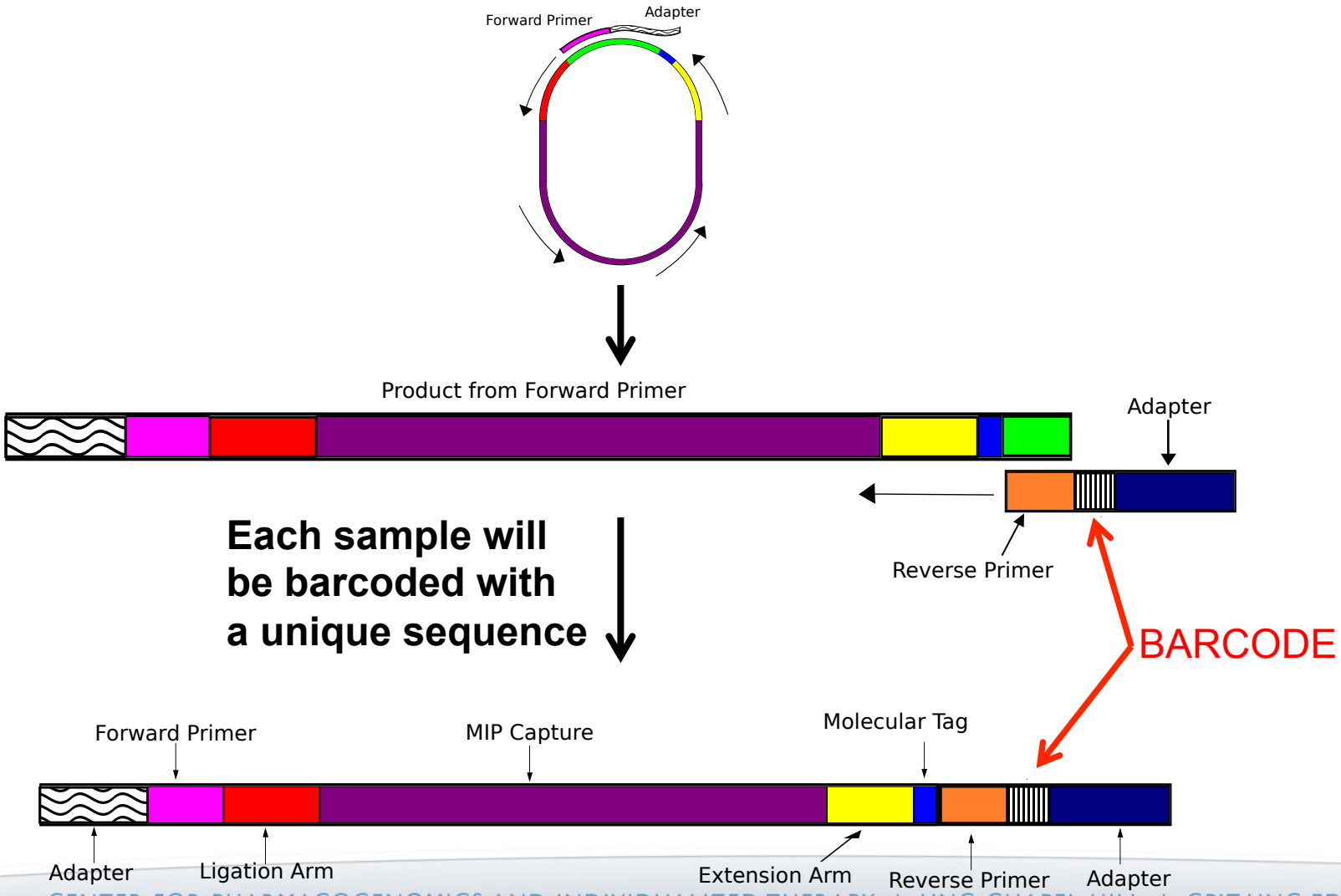
MIP captures for all areas of interest are formed.

Image: http://users.path.ox.ac.uk/~scobbold/tig/plate96.gif

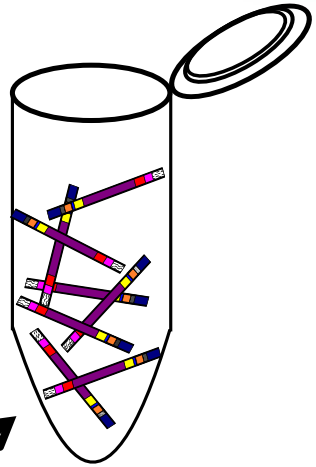
Amplify Each MIP Capture



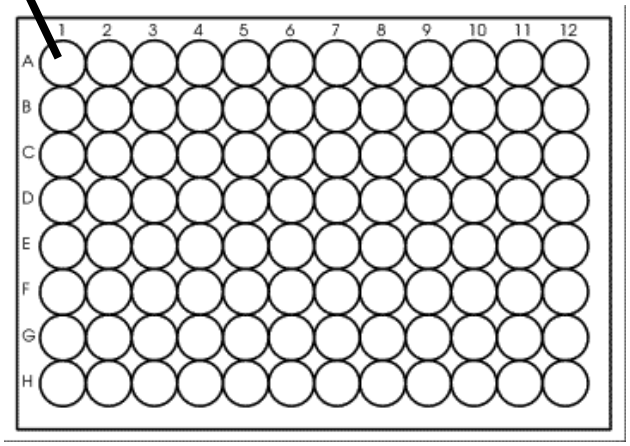
Barcode Each MIP and Prepare Fragment for Sequencing



Pool Together Barcoded Libraries from 96 People



Barcoded library in each well



Pull all wells Together. All barcoded libraries are combined.

Total:
-96 patients
-21 genes,
-75 SNPS (?)

Image: http://users.path.ox.ac.uk/~scobbold/tig/plate96.gif

Align Sequence Fragments to Human Genome

AGTCTGAT ••••• TGTCCGAT •••••
AGTCTGAT ••••• TGTCCGAT •••••
AGTCTGAT ••••• TGTCCGAT •••••

CTACCGAT ••••• AATCTCAC •••••
CTACCGAT ••••• AATCTCAC •••••

File containing the human genome sequence

Scan each fragment across entire genome for alignment

Genotype Output for Patients

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**…**	**…**
CYP2C19	*1/*17 (UM)	*1/*1 (EM)	*2/*3 (PM)	*2/*17 (IM)	…	…
CYP2C9	*1/*1 (EM)	*3/*3 (PM)	*1/*1 (EM)	*1/*2 (IM)	…	…
CYP3A5	*1/*1 (EM)	*3/*3 (PM)	*1/*6 (IM)	*1/*1 (EM)	…	…
TPMT	*1/*1 2 functional	*1/*3A 1 functional, 1 non-functional	*3A/*3A 2 non-functional	*1/*4 1 functional, 1 non-functional	…	…
…	…	…	…	…	…	…
…	…	…	…	…	…	…

Genetic Test Interpretation

Patient Genotypes and Phenotypes		
CYP2D6	Ultrarapid Metabolizer	*2A/*2A
CYP2C19	Intermediate Metabolizer	*1/*1
CYP1A2	Ultrarapid Metabolizer	-163C>A-A/A
CYP2C9	Extensive Metabolizer	*1/*1
SLC6A4	High Activity	L/L
HTR2A	Reduced Activity	G/G

USE AS DIRECTED

bupropion (Wellbutrin®)
desvenlafaxine (Pristiq®)
selegiline (Emsam®)
vilazodone (Vibryd®)

USE WITH CAUTION

amitriptyline (Elavil®)
citalopram (Celexa®)
clomipramine (Anafranil®)
doxepin (Sinequan®)
escitalopram (Lexapro®)
imipramine (Tofranil®)
sertraline (Zoloft®)
trazodone (Desyrel®)

USE WITH INCREASED CAUTION AND WITH MORE FREQUENT MONITORING

desipramine (Norpramine®)
duloxetine (Cymbalta®)
fluoxetine (Prozac®)
fluvoxamine (Luvox®)
mirtazapine (Remeron®)
nortriptyline (Pamelor®)
paroxetine (Paxil®)
venlafaxine (Effexor®)

Winner JG, et al. *Discovery Medicine* 2013;16:219-227.

Pre-emptive Pharmacogenomics at UNC

- No perfect platform for obtaining pharmacogenomics information
- Using multiplex targeted sequencing to determine polymorphisms for actionable genes involved in pharmacotherapy
- High coverage and accuracy when determining polymorphisms
- Relatively inexpensive
- Seeking Clinical Laboratory Improvement Amendments (CLIA) validation
- Low cost!

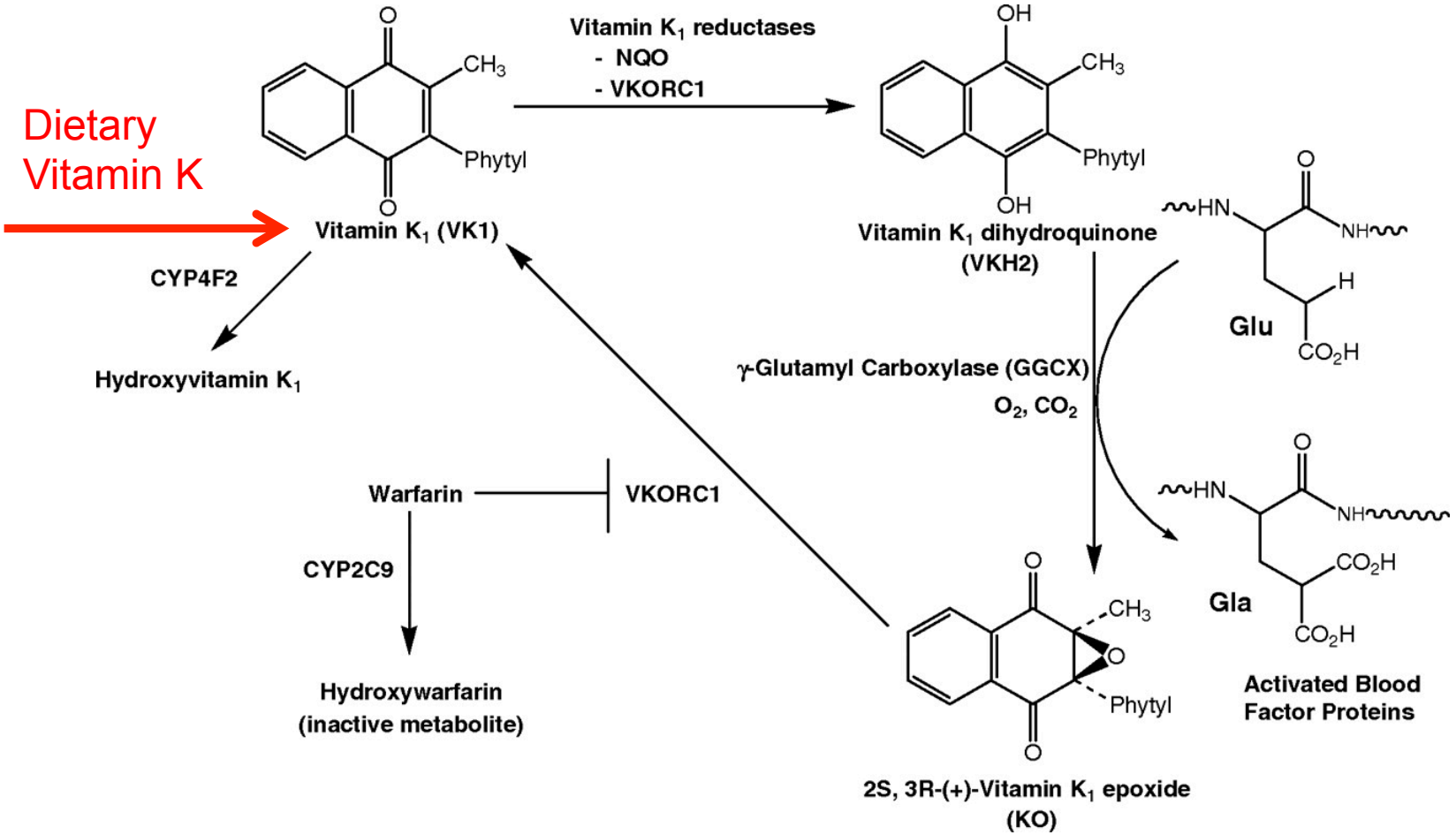
Warfarin: PGx and Dietary Vitamin K

Warfarin

- Anti-thrombotic agent
  - Inhibits vitamin K epoxide reductase (VKOR) complex
  - Synthesis of vitamin-K dependent clotting factors (II, V, VII, IX) is inhibited
  - Indicated in treatment and prophylaxis of venous and arterial thrombotic disorders
  - Substantial inter-individual variability in maintenance dose requirement exists (0.5 to 20 mg/day)
- **Narrow therapeutic index agent**
  - Monitor INR
  - Adverse event: bleeding

Courtesy of C. Lee

Warfarin Metabolism



Consistent dietary vitamin K intake is recommended when taking warfarin.

http://molpharm.aspetjournals.org/content/75/6/1337/F2.large.jpg

VKORC1 Alleles

Allele	Effect	Warfarin Dose Needed
-1639A	**Lower *VKORC1* expression** ***(i.e., less drug target to inhibit)***	**Lower dose**
-1639G	**Higher *VKORC1* expression**	**Higher dose**

Rieder et al. *NEJM* 2005

Population Frequencies of *VKORC1* alleles

-1639A

- Caucasian: 39%
- Black: 11%
- Asian: 91%

Courtesy of C. Lee

CPIC Guidelines for Warfarin

Table 1 Recommended daily warfarin doses (mg/day) to achieve a therapeutic INR based on *CYP2C9* and *VKORC1* genotype using the warfarin product insert approved by the US Food and Drug Administration

VKORC1: −1639G>A	*CYP2C9*1/*1*	*CYP2C9*1/*2*	*CYP2C9*1/*3*	*CYP2C9*2/*2*	*CYP2C9*2/*3*	*CYP2C9*3/*3*
GG	5–7	5–7	3–4	3–4	3–4	0.5–2
GA	5–7	3–4	3–4	3–4	0.5–2	0.5–2
AA	3–4	3–4	0.5–2	0.5–2	0.5–2	0.5–2

Reproduced from updated warfarin (Coumadin) product label.

- Wild-type (CYP2C9*1)
- 2 variant alleles (CYP2C9*2 and *3)

Johnson et al. *Clinical Pharmacology and Therapeutics,* 2011

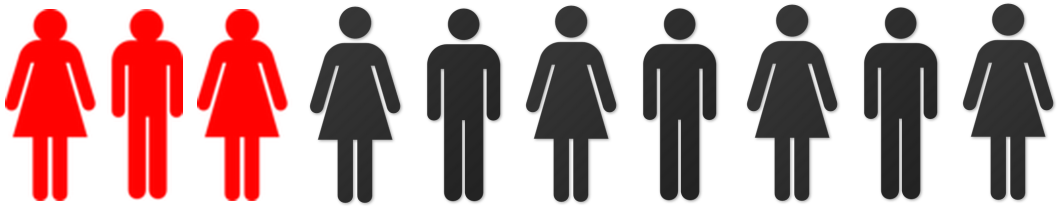
Population Prevalence of *CYP2C9* Genotypes

Group	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
Caucasian	65%	20%	12%	1%	1%	0.5%
African-Am	97%	2%	1%	~0%	~0%	~0%
Japanese	96%	0%	4%	0%	0%	~0%
Korean	98%	0%	2%	0%	0%	~0%

Lee et al. *Pharmacogenetics*, 2002

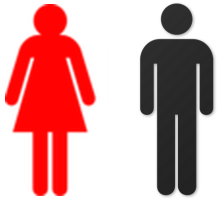
The Genetic Intersection Between Nutrition and Pharmacy: The Potential for Better Disease Management in Hypertension

Hypertension (HTN) is a Major Public Health Concern



3 in 10 adults are pre-hypertensive

3 in 10 adults have HTN

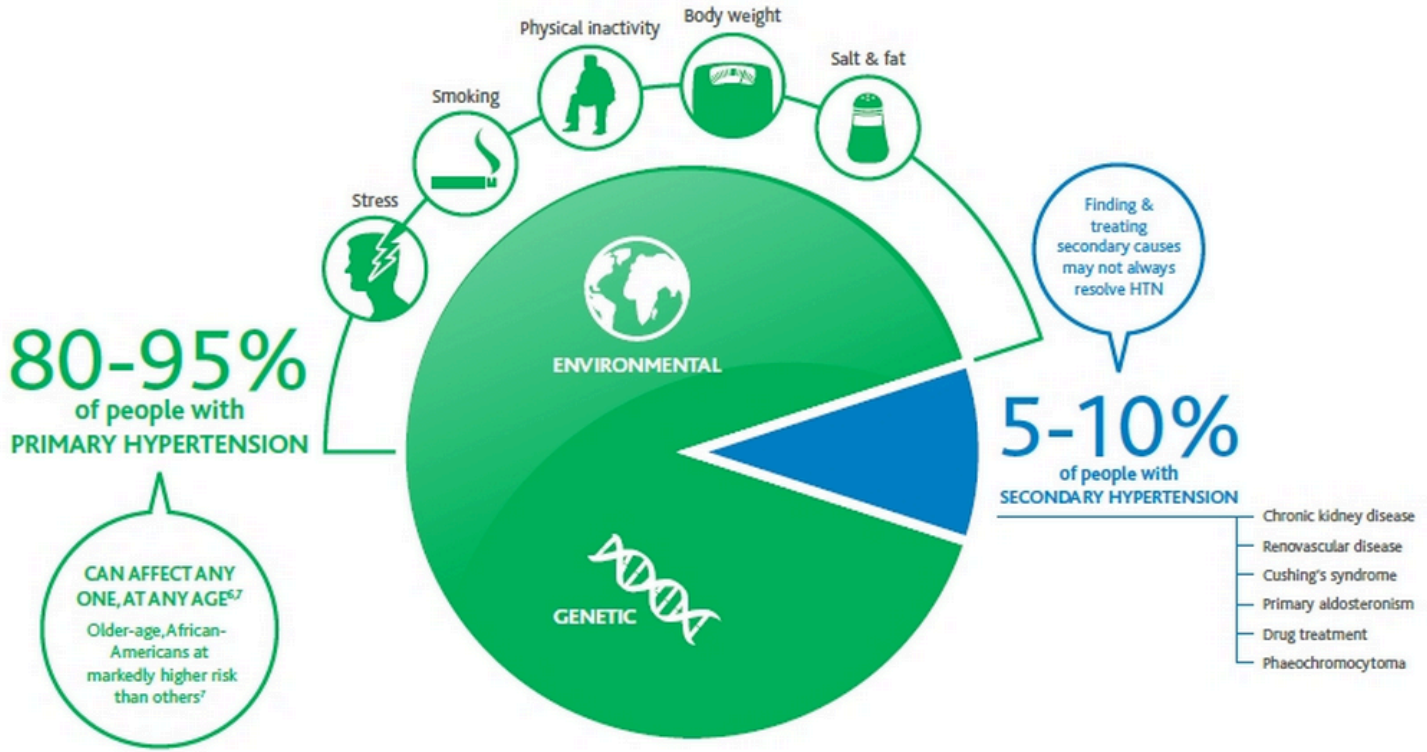


1 in 2 adults with HTN DO NOT have their BP under control

- Increase risk of cardiovascular mortality with increasing BP
- HTN accounts for 54% of strokes and 47% of IHD
- Estimated lifetime risk of developing HTN is ~90%

(2015 Scientific Report of the 2015 Dietary Guidelines Advisory Committee, 2015 CDC Stroke Fact Sheet, Lawes et al. *Lancet*, 2008)

Causes and Risk Factors for HTN: Focusing on Salt Intake and Genetics

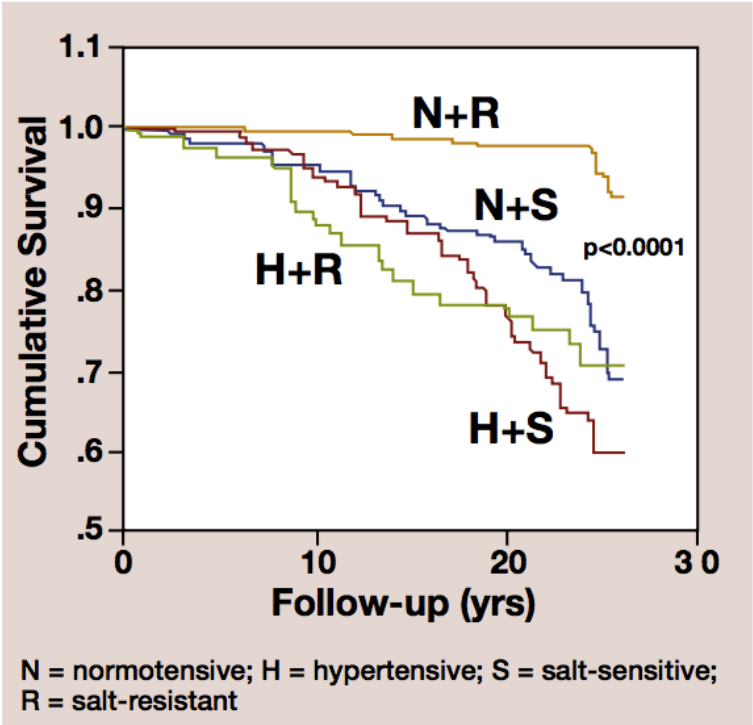


References: 1. Dosh SA. *J Fam Pract.* 2001;50:707–12. 2. Taler SJ. *Prim Care Clin Office Pract* 2008; 35: 489–500. 3. Calhoun DA, Jones D, Textor S, *et al. Hypertension* 2008; 51: 1403–19. 4. Pisoni R, Ahmed MI, Calhoun DA. *Curr Cardiol Rep.* 2009; 11: 407–13. 5. Rossi GP. *Curr Hypertens Rep.* 2010; 12: 342–348. 6. Liebson PR. *Prev Cardiol.* 2009; 12:189-97. 7. Levine DA, Lewis CE, Williams OD, *et al. Hypertension* 2011; 57:39-47.

What are the benefits of identifying the salt sensitive phenotype?

- Observational study
- Cohort of 708 subjects:
  - 278 with HTN
  - 338 salt sensitive
  - Followed for up to 27 years
- Salt sensitivity was found to be a significant risk factor for mortality

FIGURE 1. SALT SENSITIVITY REDUCES SURVIVAL IN NORMAL AND HYPERTENSIVE PARTICIPANTS



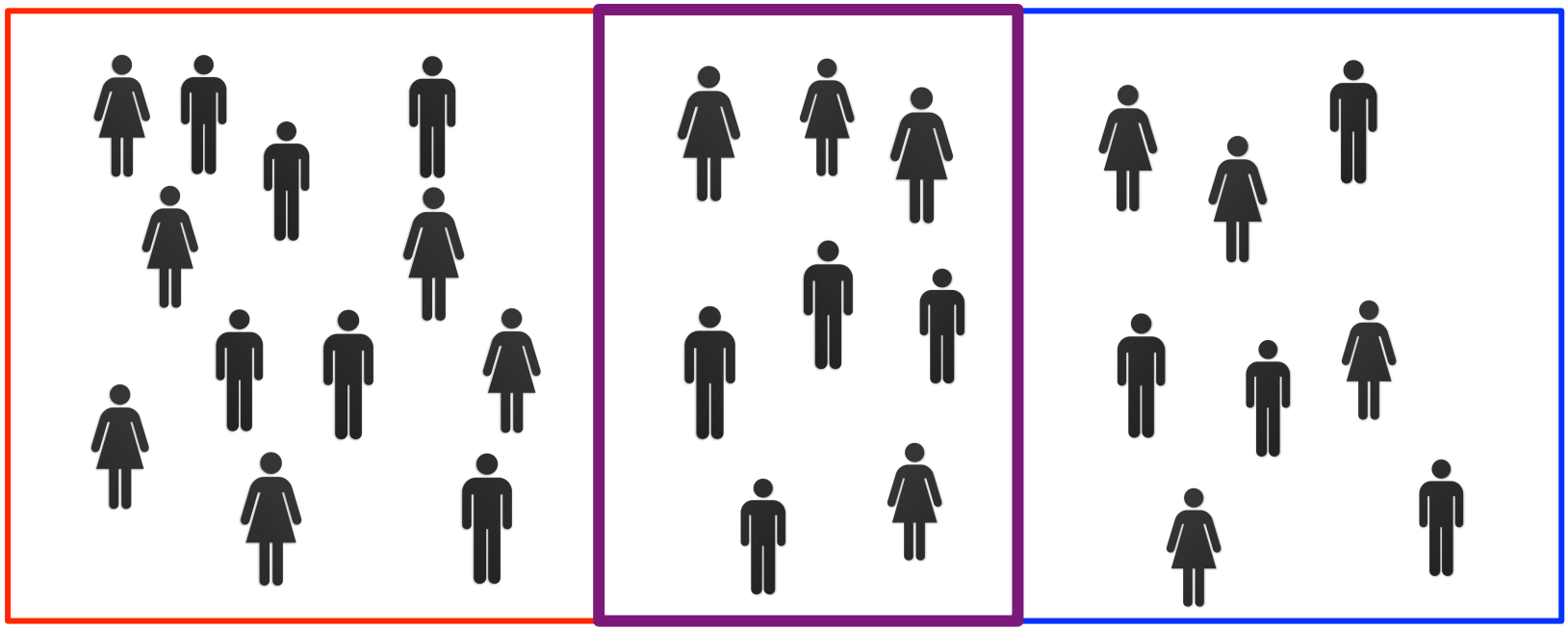
N = normotensive; H = hypertensive; S = salt-sensitive; R = salt-resistant

Weinberger et al., *Hypertension*, 2001

The Intersection Between Salt Sensitivity and Hypertension

Hypertensive (30.4%), 67 Million People

Salt Sensitive (26.4%), 58 Million People



Hypertensive & Salt Resistant (18%), 40 Million

Hypertensive & Salt Sensitive (12%) 26 Million

Normotensive & Salt Sensitive (14%), 31 Million

Felder et al., *Curr Opin Nephrol Hypertens*, 2013

Using MIPs to Help Elucidate Genetics Associated with Salt Sensitivity Hypertension

Goals:

- Focus on genetic regions that are associated with both hypertension and salt-sensitivity

- Sequence genes where haplotype structures will help clarify variants and significant SNPs that have been shown to be significantly associated with salt-sensitivity

Samples from Previously Published Cohort

- 55 HTN, 130 normotensive white subjects from UVA (discovery cohort)
- 211 white HTN subjects for the replication cohort
- Study looked at 17 candidate genes, 35 SNPs. 2 variants were associated with SS (2 in SLC4A5)
- Replication was confirmed in 2nd cohort
- SLC4A5 was significantly associated with SS in 2 separate white populations

Example MIP Targets

Gene	Function
ADD1 exons	Gene responsible for the modulation activity of sodium transport systems. It increases renal tubular Na^+/K^+ ATPase activity. Variants in this gene result in greater blood pressure response when dietary sodium is varied. (Meneton et al. *Physiological Reviews,* 2005)
GRK4 exons	Polymorphisms cause hyperphosphorylation, desensitization, and internalization of the dopamine 1 receptor and increasing the expression of angiotensin type 1 receptor. Renal dopamine receptors are responsible for 50% of sodium excretion during moderate sodium excess. Polymorphisms in these receptors are linked to hypertension and salt sensitivity. (Robin et al. *Current Opinion in Nephrology and Hypertension,* 2013)
SLC4A5 exons	Gene codes for a protein that transports sodium and bicarbonate across the cell membrane in the distal nephron. Knocking out this transporter results in sodium retention and hypertension. (Felder et al. *Current Opinion in Nephrology and Hypertension,* 2013)

Genes	Function
4. UMOD (2 SNPs)	Codes for uromodulin, the most abundant urinary protein and is secreted by epithelial cells lining the thick ascending limb of the loop of Henle in the kidney. Multiple GWAS studies have identified 2 promoter SNPs as independent susceptibility to CKD and hypertension. (Matteo et al. *Nature Medicine*, 2013)
5. AGT (6 SNPs)	Angiotensinogen is the precursor for the angiotensin peptides (e.g. angiotensin I, II, III). Angiotensin II is a potent constrictor of all blood vessels. This gene helps control blood pressure and variants have been shown to increase salt sensitivity. (Hunt et al. *American Journal of Hypertension*, 1999)
6. CYP11B2 (1 SNP)	Codes for an enzyme in the adrenal cortex responsible for the synthesis of mineralocorticoid aldosterone. Aldosterone is stimulated when angiotensin II or high potassium levels are present. This results in sodium retention and potassium excretion. Greater risk of salt sensitivity has been observed with variants in this gene. (Pamies-Andreu et al. *Journal of Human Hypertension*, 2003)
7. SGK1 (2 SNPs)	Gene plays a central role in regulating the epithelial sodium channels in the distal nephron. Variants in this gene are associated with salt sensitivity and hypertension. (Rao et al. *Journal of Human Hypertension*, 2012)
8. NEDD4L (2 SNPs)	Gene regulates epithelial sodium channels in the distal nephron. Variants in this gene is associated with increased blood pressure and salt sensitivity. (Dahlberg et al. *PLoS ONE*, 2007)

Key Points

- Response to salt varies.
- Salt sensitivity is an independent risk factor of mortality.
- Using genetics to differentiate the salt-sensitive phenotype has the potential to identify patients who would benefit from dietary interventions and pharmaceutical treatments.

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