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CLINICAL UTILITY OF GENETIC TESTING

Zahra Mehdizadeh Kashi, PhD, HCLD

HOUSEKEEPING

Cell Phones

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Questions

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https://education.lp3network.com/WCC2018

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- Inspector, College of American Pathologists

LEARNING OBJECTIVES

1. Review the history and explore the rapid expansion of genetic testing options.
2. Educate about the 5 main options in genetic testing.
3. Further explain each option, providing the clinician with a greater understanding of what each option is, the science behind this option, and when is the best time to order that option.
4. Explain why pharmacogenetics is important to compounding pharmacists.
5. Provide the clinician a tool that will educate on how to choose the right battery of genetic test based on the presentation of the patient and the selected medicine.
6. Train the provider on additional considerations when ordering pharmacogenetics testing.

OUTLINE

What are genes?

Range of genetic testing

What is pharmacogenetics (PGX)?

Clinical indication of PGX

Case studies of PGX

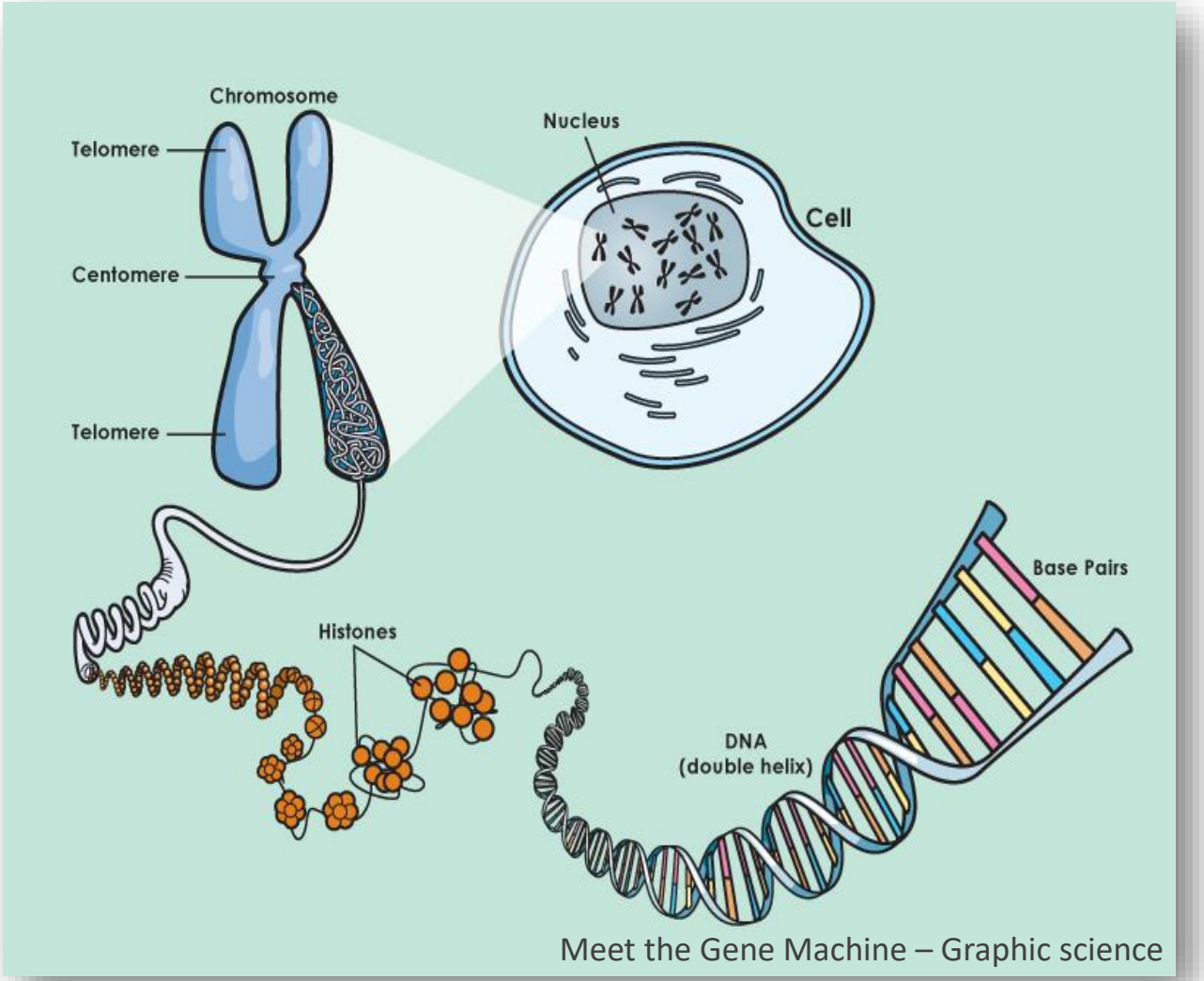
Conclusion

GENES AND GENETIC TESTING

- Genes, the units of heredity present on DNA strands inherited from one's parents are the blueprint for all individual traits
- Code for everything - hair color and height to blood type
- Also code for enzymes including all liver enzymes that are responsible for metabolizing drugs
  - Affected by individual genetic variations
  - Patients with no variants have normal enzyme function, but patients with one or two variants can have enzymes that function more or less than normal enzymes
    - This affects how the body metabolizes drugs, which in turn affects the plasma levels of a drug in the blood
    - Levels that are too high can cause adverse drug reactions (ADRs), and levels that are too low can make treatment ineffective

DNA, CHROMOSOMES AND GENES

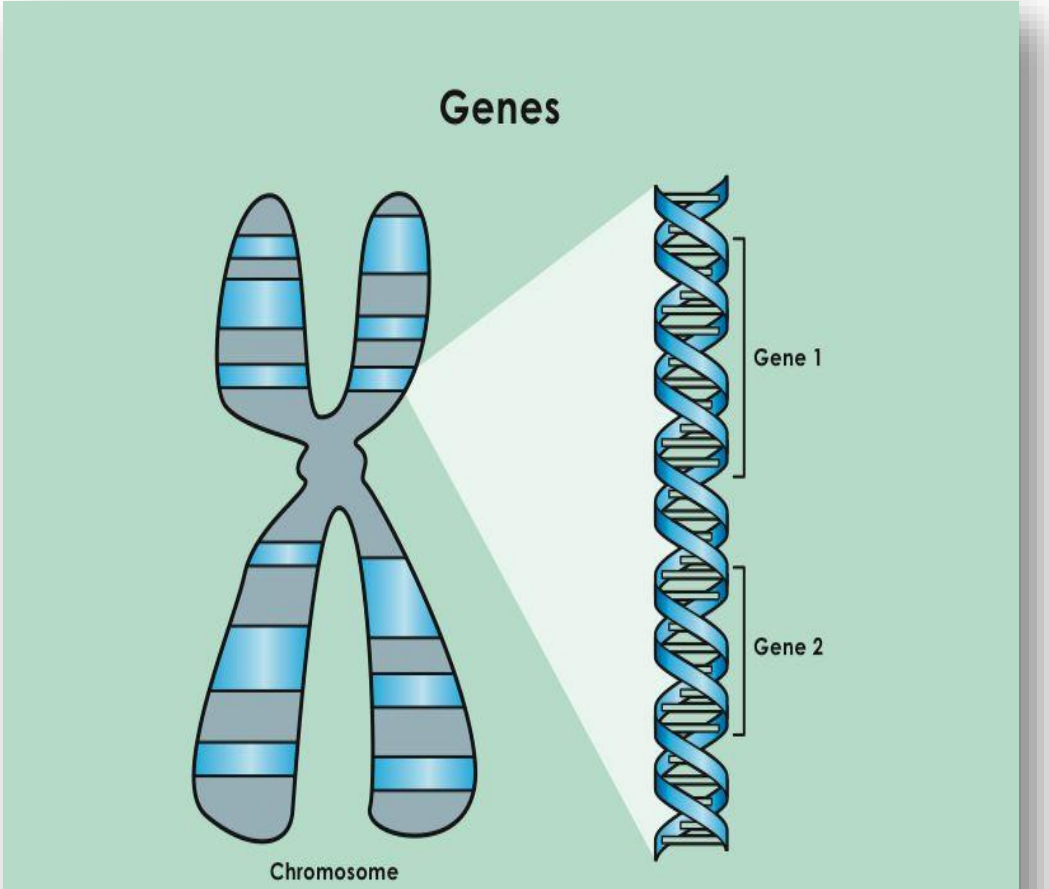
- Deoxyribonucleic acid (DNA)
- Located in the nucleus
- Wrapped up in structures called chromosomes
- 46 Chromosomes – 23 pairs in every cell



Meet the Gene Machine – Graphic science

WHAT IS A GENE?

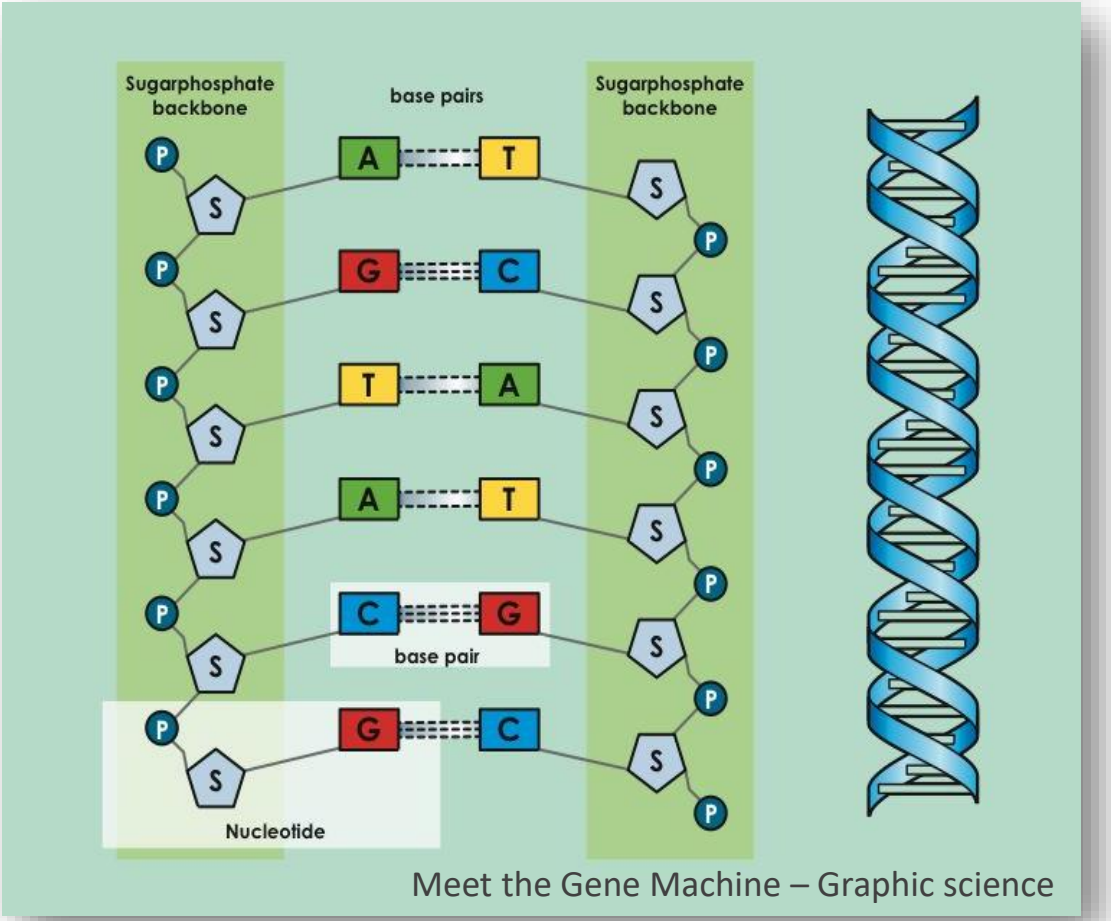
- A part of the DNA that codes for a protein
- Not all the DNA codes for proteins
- 30,000 genes in the human genome



Meet the Gene Machine – Graphic science

NUCLEOTIDES

- The building blocks of DNA are nucleotides
- Each nucleotide has a sugar (S), a phosphate (P) and a nitrogen base (A C G T)
- There are 4 different nitrogen bases in DNA and they can vary from one nucleotide to the next
- The alternating bases provide the CODE
- A permanent alteration of this code (mutation) can affect health
  - A large number of mutations
  - Most have no impact



Meet the Gene Machine – Graphic science

GENETIC TESTS

- DNA testing examines the genetic code in the cells of any human material, from a drop of saliva to a smear of blood or a strand of hair
- Everyone could need DNA testing at some point to check:
  - Genetic disorders
  - Inherited health conditions
  - Health screening
  - Transplantation / Transfusion
  - Paternity or relationship testing
  - Ancestry / genealogy testing (autosomal / mtDNA / Y-Chromosome)
  - Gene therapy
  - Medication assessment / monitoring
  - Forensic testing
  - Even for dating!

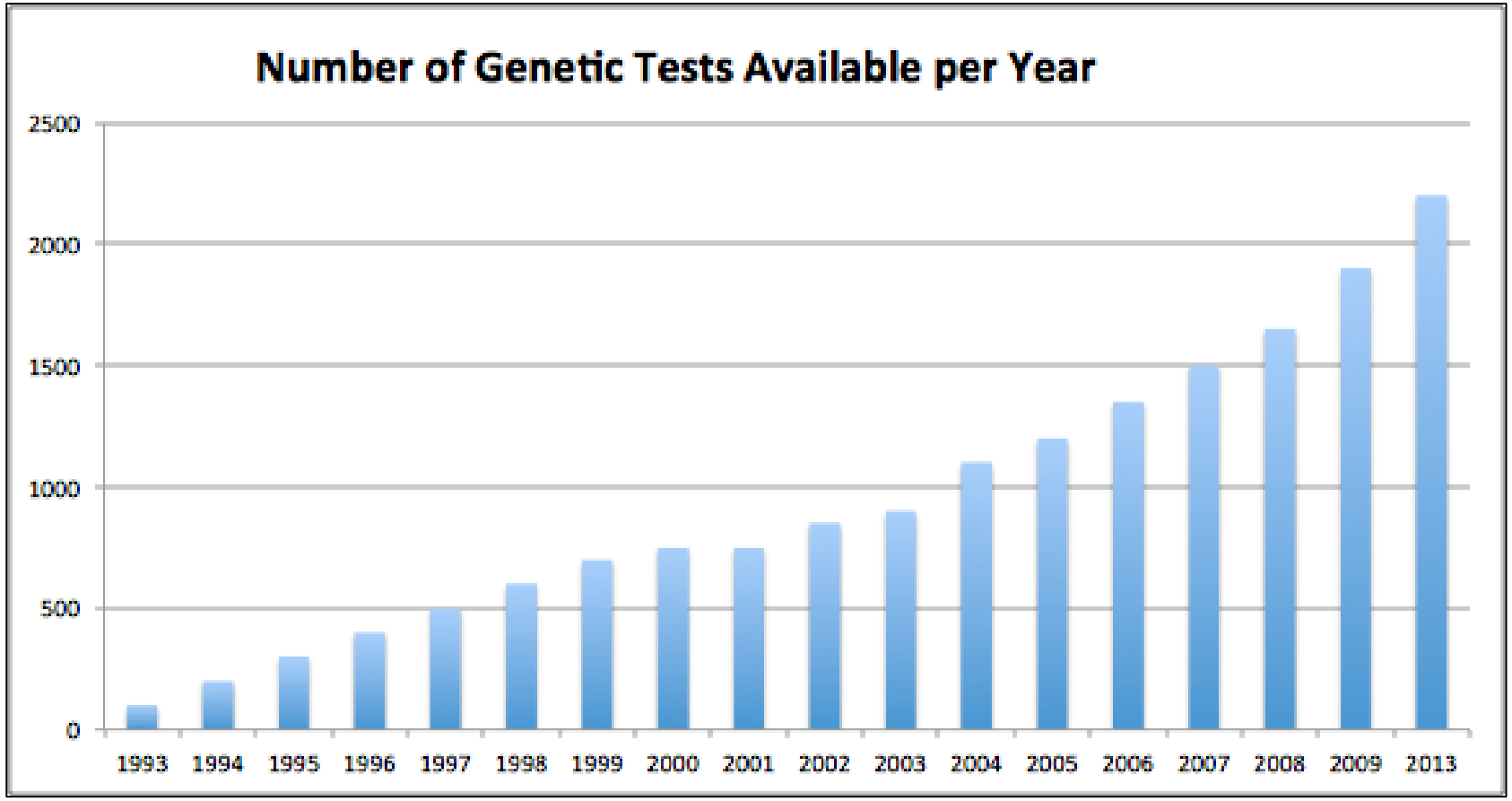
REASON FOR GENETIC TESTING

Diagnostic	Used to confirm a diagnosis based on physical signs
Predictive	Used to detect gene mutations associated with disorders that appear later in life
Carrier Identification	Used by people with a family history of recessive genetic disorders
Prenatal	Used to test a foetus when there is risk of bearing a child with mental or physical disabilities
Newborn Screening	Used as a preventative health measure once the baby is born
Forensic testing	Used to identify an individual for legal purposes
Research testing	Used for finding unknown genes and identifying the function of a gene

CLINICAL GENETIC TESTS

THE NUMBER OF CLINICAL GENETIC TESTS IS BECOMING UNMANAGEABLE.

The CDC estimates that genetic tests for use in the clinical setting have been developed for approximately 2,000 diseases. *Adapted from CDC 2016.*



DIRECT-TO-CONSUMER GENETIC TESTS IN THE MARKET

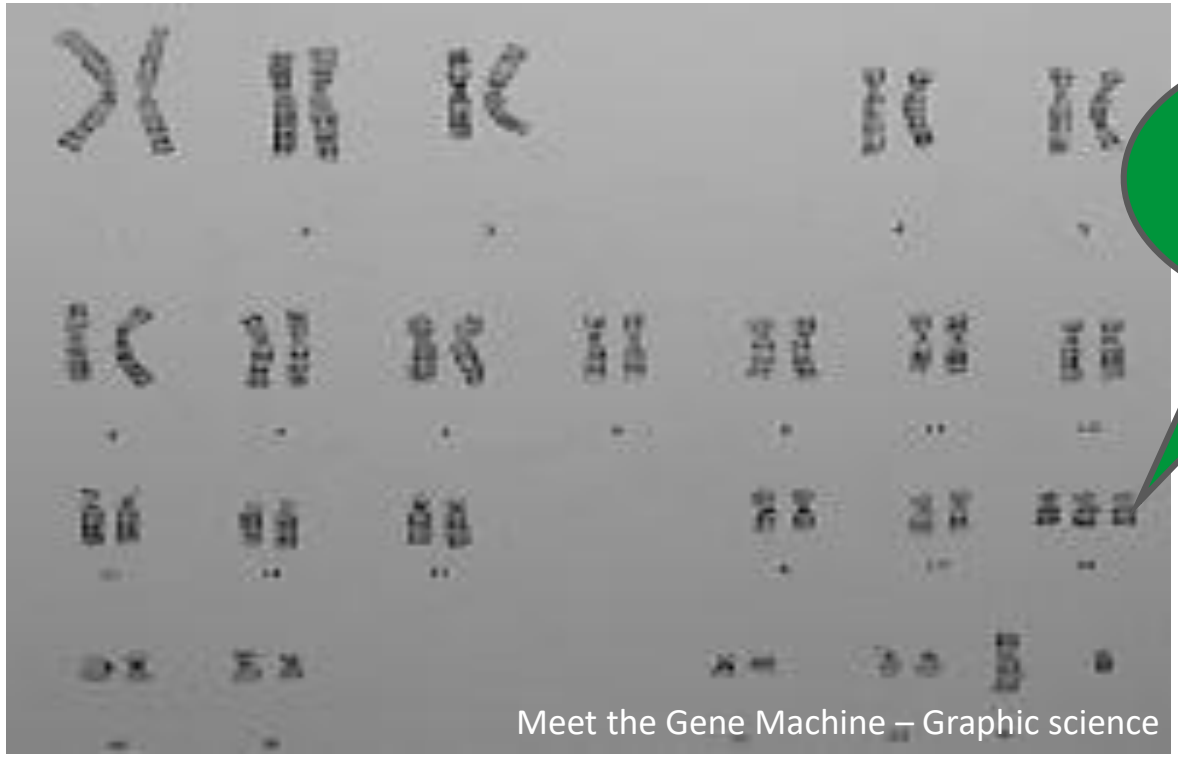
- **Major types of DTC tests include:**
  - **Disease risk and health**
    - Celiac / Parkinson / Alzheimer / carrier status: CF / Sickle cell
  - **Ancestry or genealogy**
    - Where a person's ancestors might have come from, their ethnicity, and family connections
      - Y Chromosome Testing / Mitochondrial DNA Testing / SNP testing
  - **Kinship**
    - Whether tested individuals are biologically related to one another
  - **Lifestyle**
    - Nutrition, fitness, weight loss, skincare, sleep, and even wine preferences
      - Genetic variations related to very specific traits, such as how your body converts the nutrients from food into energy (metabolism), day/night (circadian) rhythm, or the senses of taste and smell
  - **Pharmacogenetics**

TYPES OF GENETIC TESTS

- There is no single genetic test that can detect all genetic conditions
- Types include:
  - (A) Chromosomal Testing
  - (B) Large-scale genomic testing
    - Whole-genome sequencing
    - Exome sequencing
  - (C) Gene Expression Testing
- (D) Single Nucleotide Polymorphism (SNP) testing

(A) ABNORMAL NUMBER OF CHROMOSOMES

- Trisomies – 3 copies rather than 2 copies of a chromosome
- Monosomies – 1 copy rather than 2



(B) WHOLE GENOME SEQUENCING

- Whole genome used in the clinic to aid in the diagnosis of rare congenital disorders and solve diagnostic dilemmas
- Clinical utility include:
    1. Preconception carrier screening
    2. Detection of fetal aneuploidy (cell-free DNA in maternal blood)
    3. Genotyping of neoplasms to individualize cancer treatments
    4. Genetic predisposition screening

Nat Rev Genet. 2013 June ; 14(6): 415–426

(1) PRECONCEPTION CARRIER SCREENING

- Preconception screening for carrier variants associated with rare, recessive disorders
  - Evolved carrier screening used to test for conditions such as Tay-Sachs, Canavan disease in Ashkenazi Jewish descents
- Discovering that reproductive partners are each carriers for a severe recessive condition enables preimplantation genetic diagnosis (PGD)
  - Allows for testing of embryos for a specific genetic variant(s)
  - Embryos lacking the targeted genetic variants are then implanted, preventing transmission of the genetic disease to offspring

(2) NON-INVASIVE PRENATAL TESTING (NIPT)

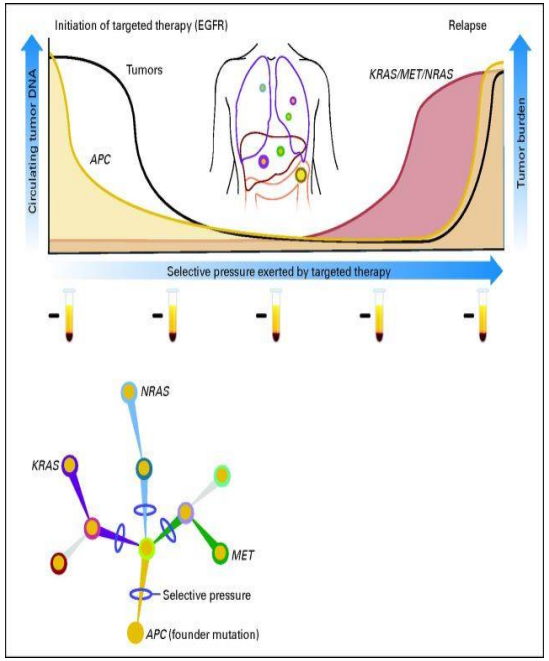
- Detection of aneuploidy by WG testing of cell free DNA
  - Apoptosis of placental cells
  - 10% of total DNA in maternal plasma
  - Detected after 7 weeks gestation
- Cell free DNA is extracted from maternal plasma
- Cell free DNA (~3-20% is fetal in origin) is sequenced
- Millions of sequence reads are generated for each sample per run
- The reads are aligned to determine where they fit in the human genome using bioinformatics

Clinical Chemistry, 2005;51(2), 312-320

(3) GENOTYPING OF NEOPLASMS FOR TREATMENT

TO INVIDUALIZE CANCER TREATMENT

A patient with metastatic colorectal cancer



Detection of tumor-specific DNA mutations in the blood of patients to monitor response and relapse with targeted therapies. Test tubes represent samples of plasma from which circulating free DNA is extracted and used to monitor the presence of cancer-specific aberrations.

Clinical resistance becomes manifest at a later time point

- At the time of presentation, DNA from the primary tumor is used to identify the baseline mutation profile
  - *This case tumor has APC* mutant and *KRAS* wild type (WT)
- At baseline, evaluation of the patient's plasma DNA only identifies *KRAS* WT fragments
- This patient is treated with an anti–epidermal growth factor receptor (EGFR) monoclonal antibody, experiences a clinical response, and has a corresponding decrease in *APC* mutation level, further indicating a decrease in tumor burden
- Continuous monitoring of plasma DNA shows the emergence *KRAS* and *NRAS* mutations and/or *MET* amplification, indicative of the emergence of multiple different resistance clones

(4) GENETIC PREDISPOSITION SCREENING

- Testing a broad range of predispositional Mendelian variants
- The goal is to provide genetically informed predictions of disease risk and medication safety and efficacy enabling disease prevention
  - Monogenic variants for Mendelian syndromes that confer a significant risk for a condition, such as the breast cancer susceptibility gene 1 and 2 (BRCA1/2) variants associated with breast and ovarian cancer, may be revealed in WGS of persons without a personal or family history
- The PeopleSeq (Personal Genome Sequencing Outcomes) Consortium has been formed as the first systematic large-scale longitudinal study of outcomes of predisposition sequencing
  - The MedSeq Project identified a monogenic variant in 21 out of 100 participants (asymptomatic people)

Nat Rev Genet. 2013 June ; 14(6): 415–426

(B) CLINICAL EXOME SEQUENCING

- Evaluates suspected genetic disorders for germline mutations within the coding regions
- Requires samples from the patient and the patient's biological parents
- Useful for Identifying a molecular diagnosis in patients with a known or suspected genetic disorder, which can allow for:
  - Better understanding of the natural history/prognosis
  - Targeted management (anticipatory guidance, management changes, specific therapies)
  - Predictive testing of at-risk family members
  - Testing and exclusion of disease in siblings or other relatives
  - Recurrence risk assessment
  - Reproductive decision-making

Genet Med. 2011 Mar;13(3):255-62. doi: 10.1097/GIM.0b013e3182088158.

Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease.

Worthey EA[1], Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, Serpe JM, Dasu T, Tschannen MR, Veith RL, Basehore MJ, Broeckel U, Tomita-Mitchell A, Arca MJ, Casper JT, Margolis DA, Bick DP, Hessner MJ, Routes JM, Verbsky JW, Jacob HJ, Dimmock DP.

Author information

We report a male child who presented at 15 months with perianal abscesses and proctitis, progressing to transmural pancolitis with colocutaneous fistulae, consistent with a Crohn disease-like illness. The age and severity of the presentation suggested an underlying immune defect; however, despite comprehensive clinical evaluation, we were unable to arrive at a definitive diagnosis, thereby restricting clinical management. After sequencing, we identified 16,124 variants. Subsequent analysis identified a novel, hemizygous missense mutation in the X-linked inhibitor of apoptosis gene, substituting a tyrosine for a highly conserved and functionally important cysteine. X-linked inhibitor of apoptosis was not previously associated with Crohn disease but has a central role in the proinflammatory response and bacterial sensing through the NOD signaling pathway. The mutation was confirmed by Sanger sequencing in a licensed clinical laboratory. Functional assays demonstrated an increased susceptibility to activation-induced cell death and defective responsiveness to NOD2 ligands, consistent with loss of normal X-linked inhibitor of apoptosis protein function in apoptosis and NOD2 signaling. Based on this medical history, genetic and functional data, the child was diagnosed as having an X-linked inhibitor of apoptosis deficiency. Based on this finding, an allogeneic hematopoietic progenitor cell transplant was performed to prevent the development of life-threatening hemophagocytic lymphohistiocytosis, in concordance with the recommended treatment for X-linked inhibitor of apoptosis deficiency. At >42 days posttransplant, the child was able to eat and drink, and there has been no recurrence of gastrointestinal disease, suggesting this mutation also drove the gastrointestinal disease. This report describes the identification of a novel cause of inflammatory bowel disease. Equally importantly, it demonstrates the power of exome sequencing to render a molecular diagnosis in an individual patient in the setting of a novel disease, after all standard diagnoses were exhausted, and illustrates how this technology can be used in a clinical setting.

(C) GENE EXPRESSION

- Although every cell in the body contains the same set of genes, only a fraction of these genes are used in any given cell at any given time based on controlled pattern of “gene expression”
- Gene expression is dynamic
  - The same gene acts differently under different circumstances
  - Example:
    - Siamese cats have colored\"points\" because of a temperature-sensitive pigmentation gene
      - In cooler areas of a cat's body (nose and paws), this gene is expressed to a greater degree

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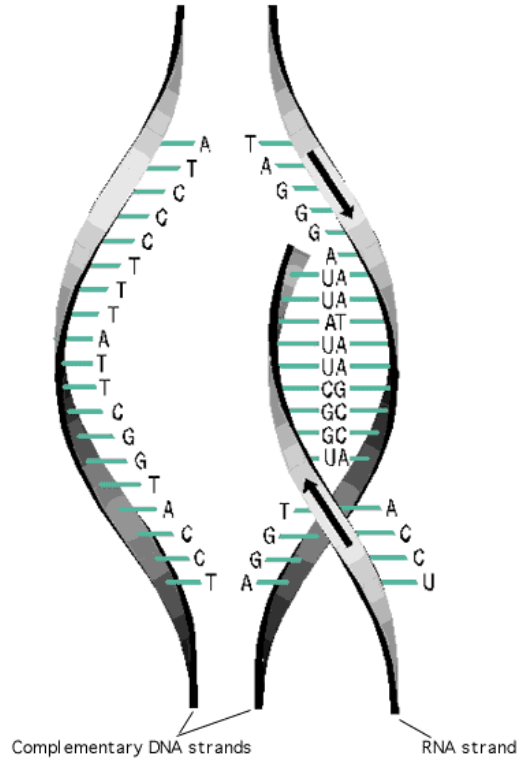
GENE EXPRESSION

Factors controlling gene expression

- Chromosomal activation or deactivation
- Control of initiation of transcription
- Processing of RNA (e.g. splicing)
- Control of RNA transport
- Control of mRNA degradation
- Control of initiation of translation
- Post-translational modifications

Sequences in RNA defines the protein



Adopted from S Teama 2012

ENVIRONMENT INFLUENCE ON GENE EXPRESSION

- Environmental factors can cause epigenetic changes by altering the way molecules bind to DNA or changing the structure of proteins that DNA wraps around
  - Nutrients may behave as transcription factors that bind to DNA and induce gene expression
    - One polymorphism in hepatic lipase gene associated with an increase protective HDL level in response to high fat diet
      - Position 515 CC replacing TT
  - Common genetic variations may alter the expression or functionality of genes
    - Phenylketonuria (PKU) and food containing amino acid phenylalanine
    - Carcinogens modifying gene expression to initiate cancer

CAN WE TEST GENE EXPRESSION?

- To help determine what circumstances trigger expression of various genes, techniques are used to identify which genes are turned on and which are turned off within cells
  - Done by determining which mRNA transcripts are present in a cell
- Possible to study the expression of multiple genes simultaneously by measuring mRNA
  - Microarray analysis
  - Reverse transcription polymerase chain reaction (RT-PCR) levels
  - Measuring protein levels using Western blot

LIMITATION OF GENE EXPRESSION ANALYSIS

- In contrast to genomic data, where a specific variant is present or not, gene expression data will vary inter-individually, temporally, and between different disease states

- In addition, whole blood represents a heterogeneous pool of distinct cells with putatively variable patterns of mRNA expression

- Gene expression might be altered depending on the disease state

(D) SINGLE NUCLEOTIDE POLYMORPHISM

- Within our genes lie small differences in the DNA code

- One common type of difference, called a single nucleotide polymorphism (SNP), occurs when one base in the DNA code is changed
    - Neutral (no effect on the resulting characteristic)
    - Small alterations to dramatic effects on the characteristics

GENOME-WIDE ASSOCATION STUDIES (GWAS)

- Some common genetic variants are associated with risk for complex phenotypes, such as coronary artery disease and type 2 diabetes, in GWAS

PHENOTYPE	GENE	VARIANT
Peptic ulcer	ABO	B
IDDM*	HLA	DR 3,4
Alzheimer dementia	APOE	E4
Deep venous thrombosis	F5	Leiden
Falciparum malaria*	HBB	β^5
AIDS*	CCR5	Δ32
Colorectal cancer	APC	3920A
NIDDM*	PPARγ	12A

GENOME-WIDE ASSOCATION STUDIES (GWAS)

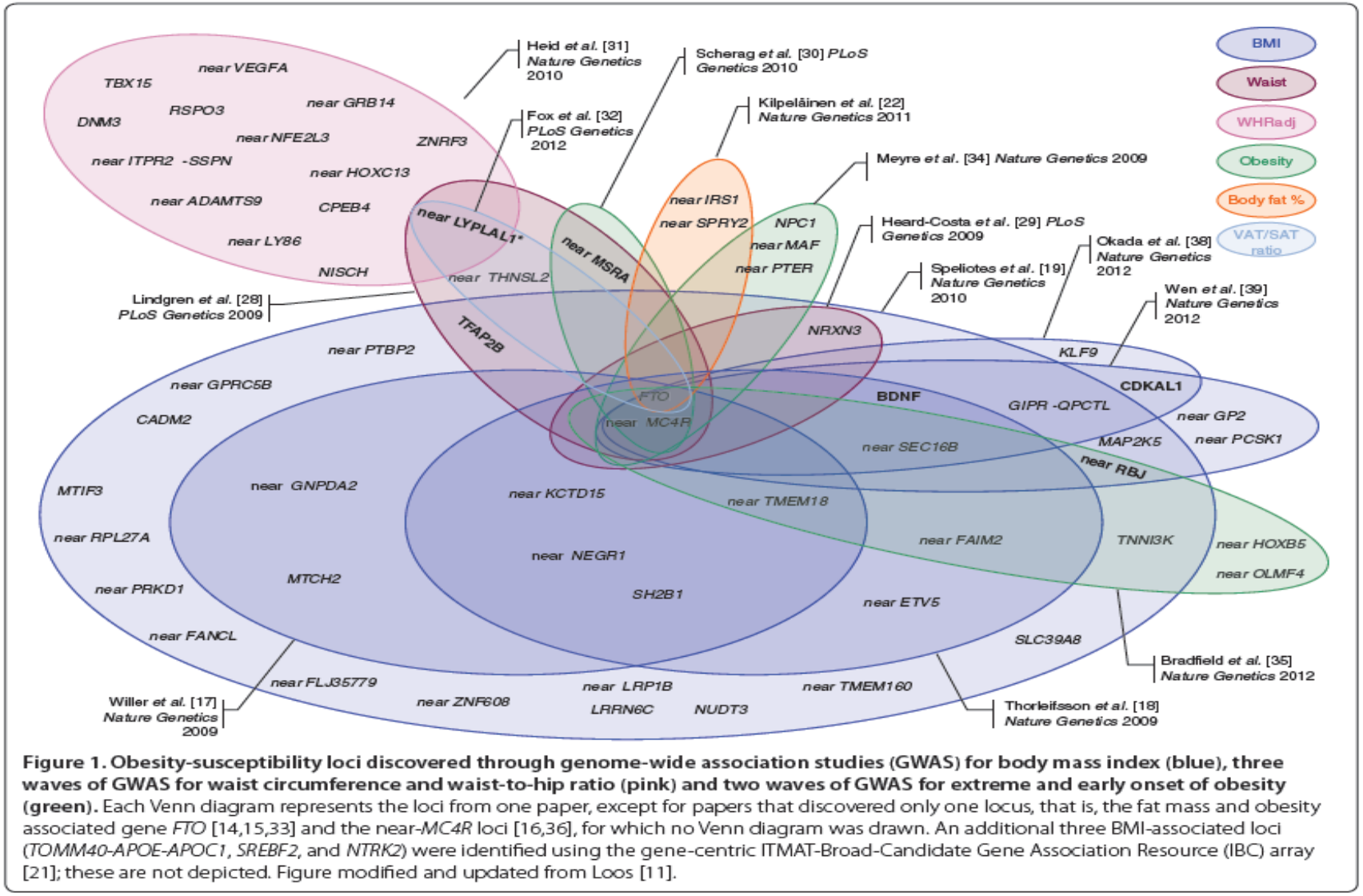


Figure 1. Obesity-susceptibility loci discovered through genome-wide association studies (GWAS) for body mass index (blue), three waves of GWAS for waist circumference and waist-to-hip ratio (pink) and two waves of GWAS for extreme and early onset of obesity (green). Each Venn diagram represents the loci from one paper, except for papers that discovered only one locus, that is, the fat mass and obesity associated gene *FTO* [14,15,33] and the near-*MC4R* loci [16,36], for which no Venn diagram was drawn. An additional three BMI-associated loci (*TOMM40-APOE-APOC1*, *SREBF2*, and *NTRK2*) were identified using the gene-centric ITMAT-Broad-Candidate Gene Association Resource (IBC) array [21]; these are not depicted. Figure modified and updated from Loos [11].

Lu and Loos *Genome Medicine* 2013, 5:55
http://genomemedicine.com/content/5/6/55

WHAT IS SNP?

- In human beings, 99.9 percent bases are same
  - Remaining 0.1 percent makes a person unique
    - Different attributes / characteristics / traits
    - How a person looks
    - Diseases he or she develops
- A Single Nucleotide Polymorphism (SNP) is defined as a single base change in a DNA sequence that occurs in a significant proportion (more than 1 percent) of a large population
  - These variations can be:
    - Harmless (change in phenotype)
    - Harmful (diabetes, cancer, heart disease, Huntington's disease, and hemophilia)
    - Latent (variations found in coding and regulatory regions, are not harmful on their own, and the change in each gene only becomes apparent under certain conditions e.g. susceptibility to lung cancer)

A NORMAL GENE SEQUENCE

aaaccatctaggctatattcggatatcgatctatcggatctatctactagagctactacgatcagggactactacgagcatcgactacgaggcttc
tagaggctatattctaggctactacgatcgatctacgtagctacgagatcgtgtgtgggggggacacagcgatctaatataaatctgatgatcg
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Adopted from University of Rochester “Genetic Testing”

A MUTATED DNA SEQUENCE

aaaccatctaggctatattcggatatcgatctatcggatctatctactagagctactacgatcagggactactacgagcatcgactacgaggcttc
tagaggctatattctaggctactacgatcgatctacgtagctacgagatcgtgtgtgggggggacacagcgatctaatataaatctgatgatcg
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ggctatattcggatgatctatctactagagctgatctatctactagagctgtcgtagtcgtgtgataaaaaaccatctaggctatattcggatatc

Single base pair mutation

Sickle cell anemia

Adopted from University of Rochester “Genetic Testing”

SNPs

- Found in coding and (mostly) noncoding regions
- Occur with very high frequency (1 in 1000 bases to 1 in 100 bases)
- SNPs close to particular gene acts as a marker for that gene
- Significant in:
  - Disease diagnosis
  - Finding predisposition to diseases
  - Drug discovery and development
  - Drug responses
  - Finding human migration patterns

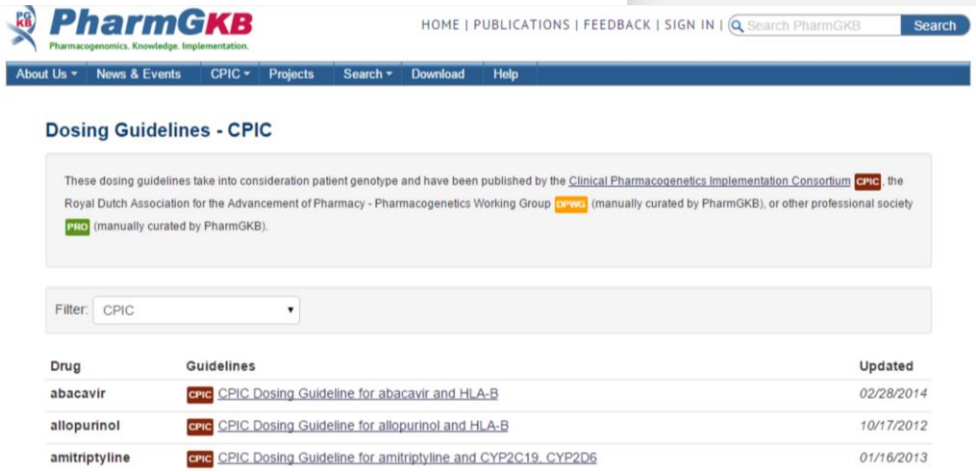
PHARMACOGENETICS

- Study of the genetic factors that influence a person's drug response

The 4 R's:
Right drug, right dose, right patient, right time

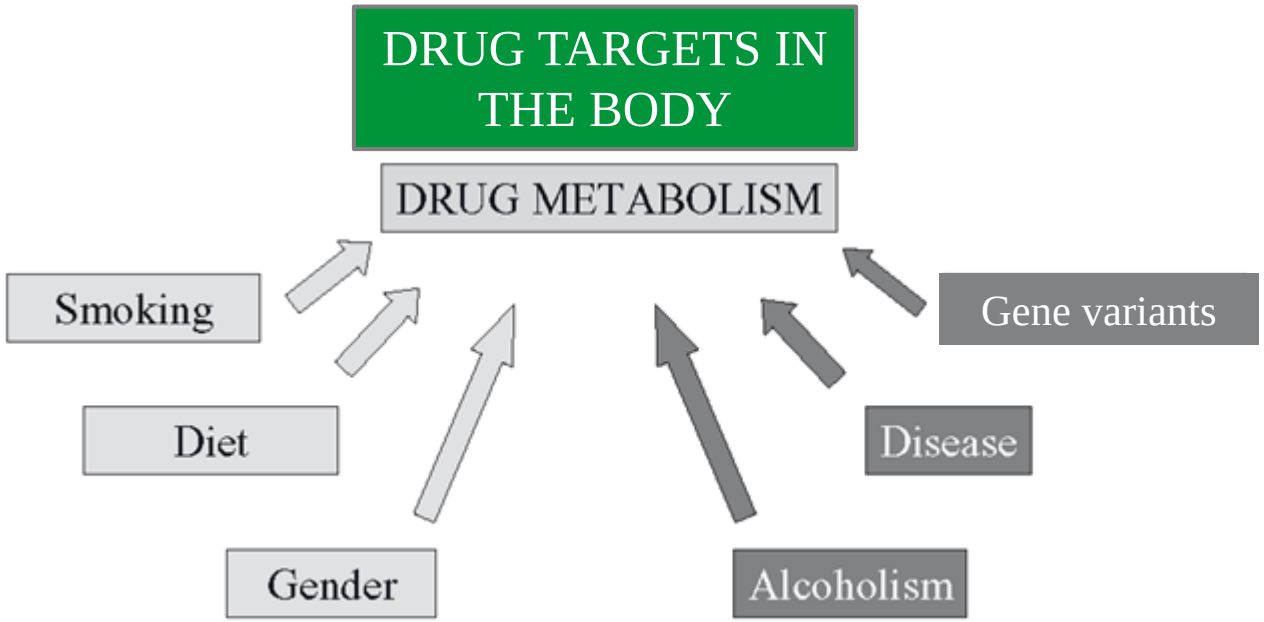
EMBRACING BETTER PATIENT OUTCOMES

- Guidelines based on robust clinical evidence:
  1. FDA PGx Drug Labeling
  2. Clinical Pharmacogenetics Implementation Consortium (CPIC)



WHAT INFLUENCES HOW WE RESPOND TO A DRUG?

http://www.womenshealthmag.com/health/decode-drug-labels



http://what-when-how.com/human-drug-metabolism/effects-of-disease-on-drug-metabolism-factors-affecting-drug-metabolism-human-drug-metabolism/

Combination of environmental factors and genes!

DRUG METABOLISM

WHAT OUR BODY DOES TO A DRUG ONCE WE TAKE IT

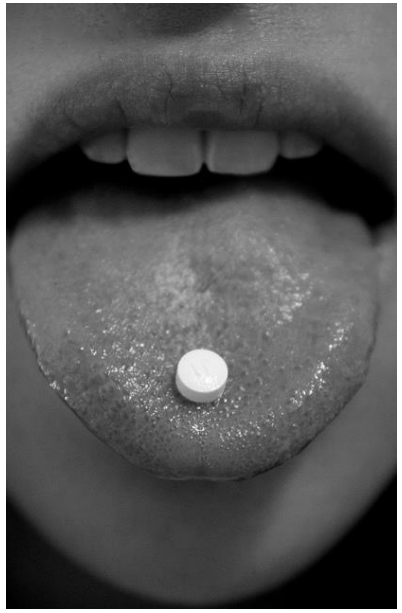


Photo by Flickr user MyLittleOli
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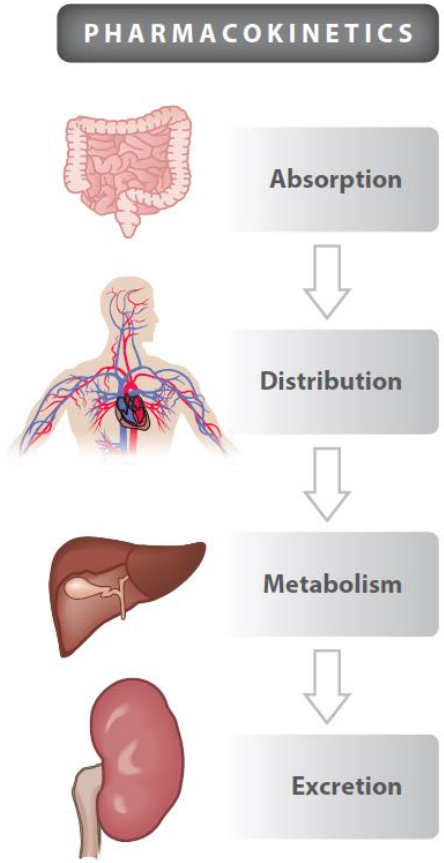


Image source: Pirmohamed (2014) Annu. Rev. Genomics Hum. Genet. 15: 353

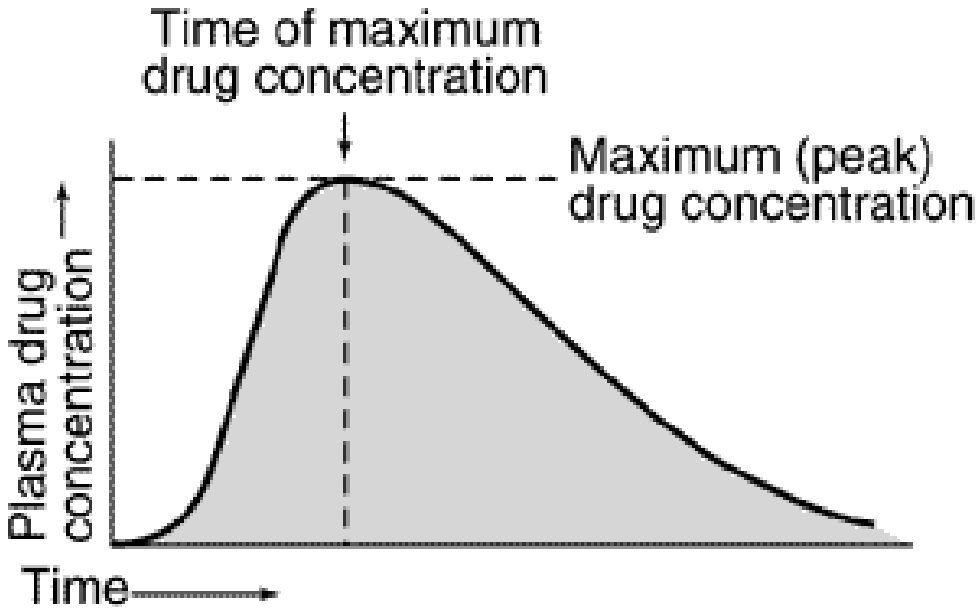
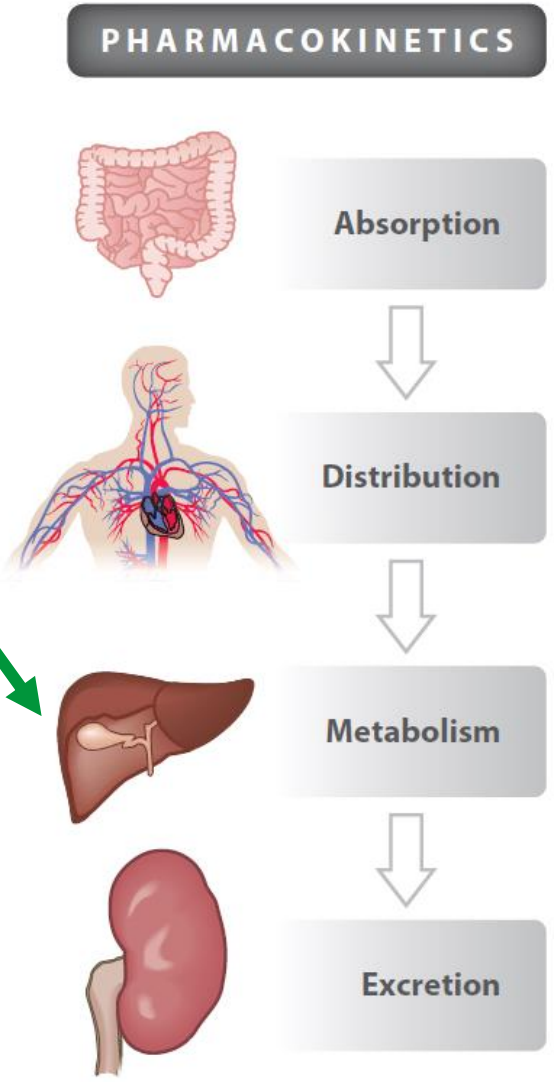


Image source:
http://drtedwilliams.net/kb/index.php?pagename=Plasma%20Concentration%20Time%20Curve

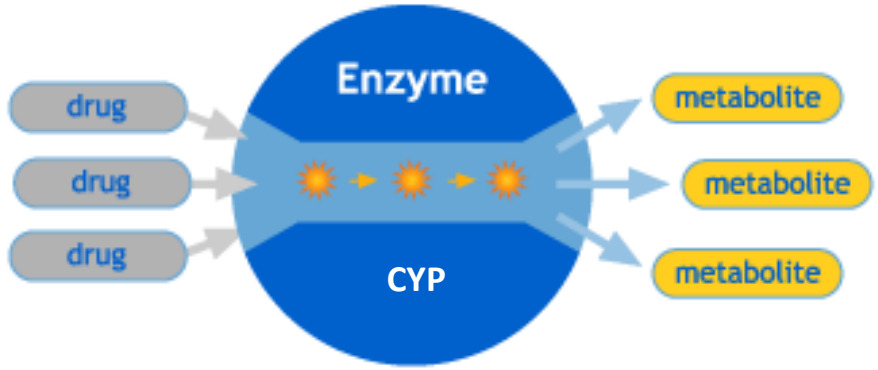
THE MAJOR PHARMACOKINETIC PLAYERS

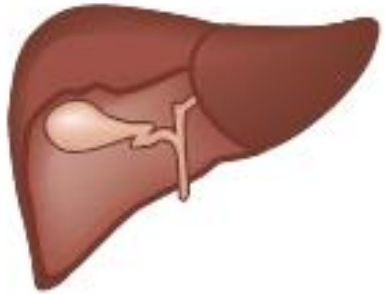
- Enzymes that break-down or activate drugs (Metabolize)
  - *CYP genes* → Cytochrome P450 (CYP) Enzymes
    - E.g. Cyp2D6, Cyp2C9, Cyp2C19, Cyp3A4, Cyp3A5
- Proteins that transport drugs
  - Absorb, transport, excrete drugs into, around, and out of the body
    - SLCO1B1 gene → OATP1B1 protein



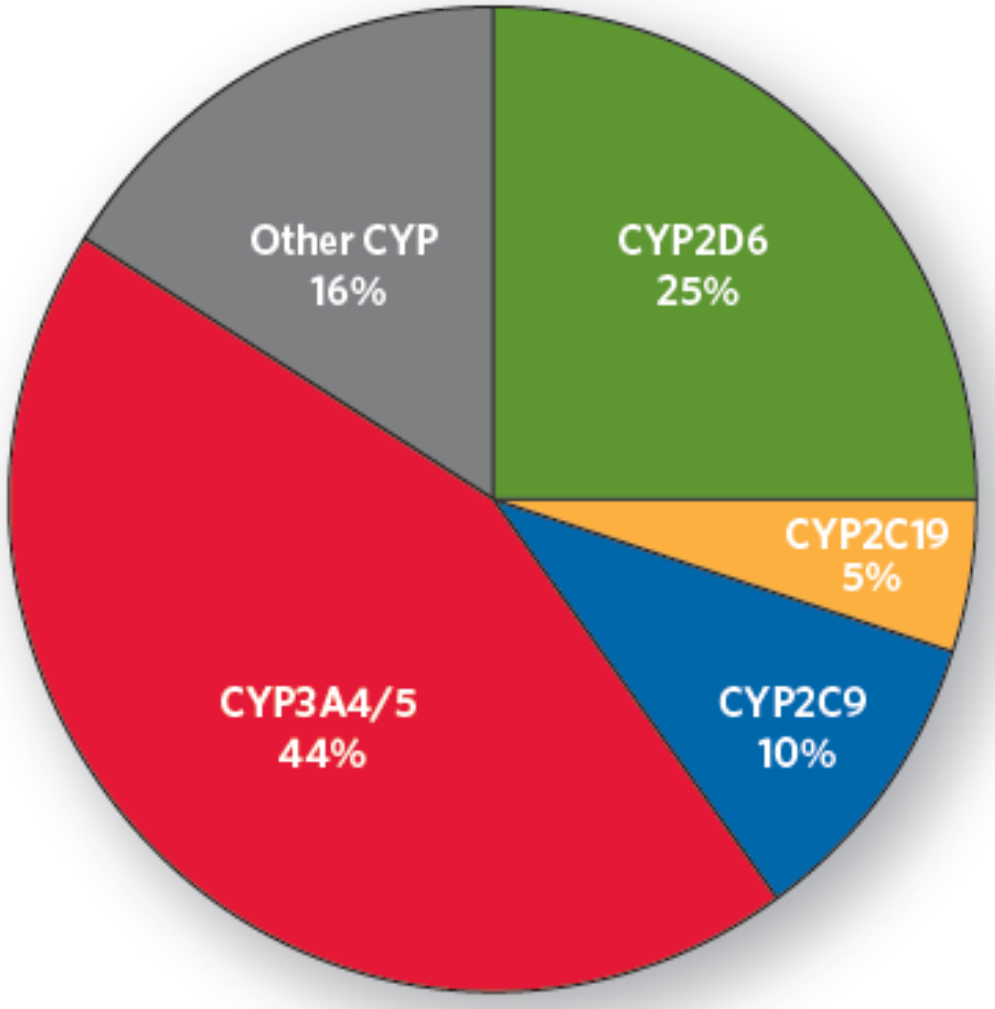
CYTOCHROME P450 (CYP) ENZYMES

- Accounts for ~75% of all drug metabolism!
- Found primarily in the liver
- Large family of related proteins
  - Humans have more than 57 different CYP genes
  - Only a handful do MOST of the work to metabolize drugs
  - A drug is often metabolized by more than one type of CYP enzyme.
  - Each Cyp enzyme has many possible versions
    - CYP2D6 = over 70 different alleles!
    - Different versions lead to different types of "metabolizers"



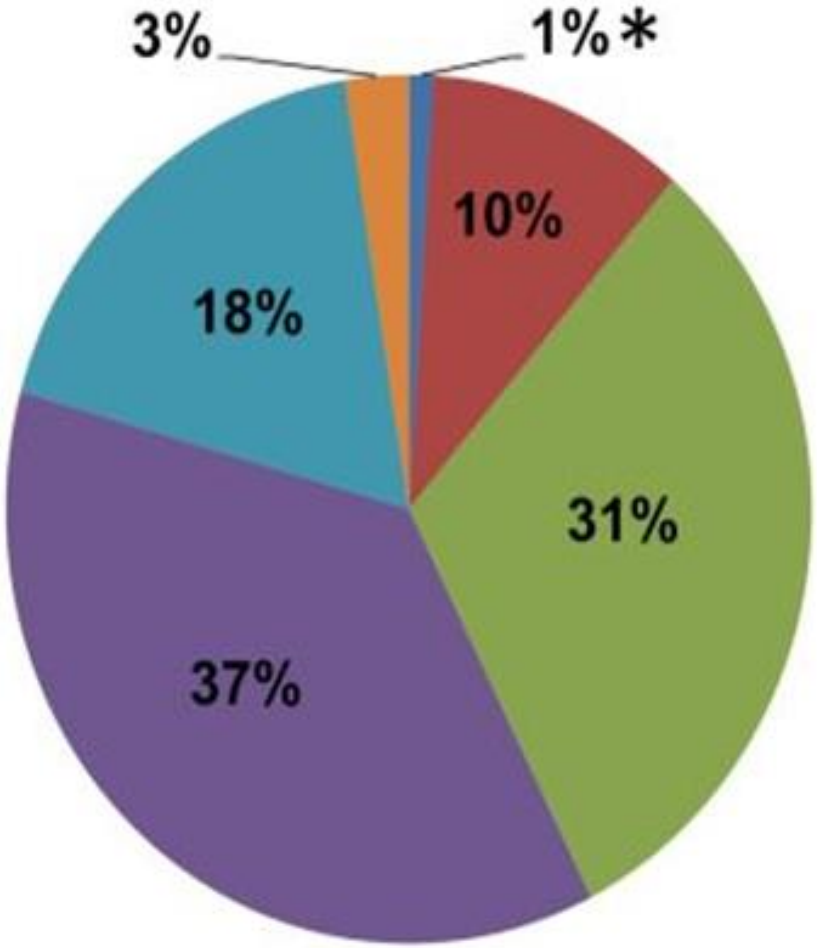


THE MAJOR PHARMACOKINETIC PLAYERS



*Estimated % of drugs metabolized by enzyme type

POPULATION VARIANTS



N=1013

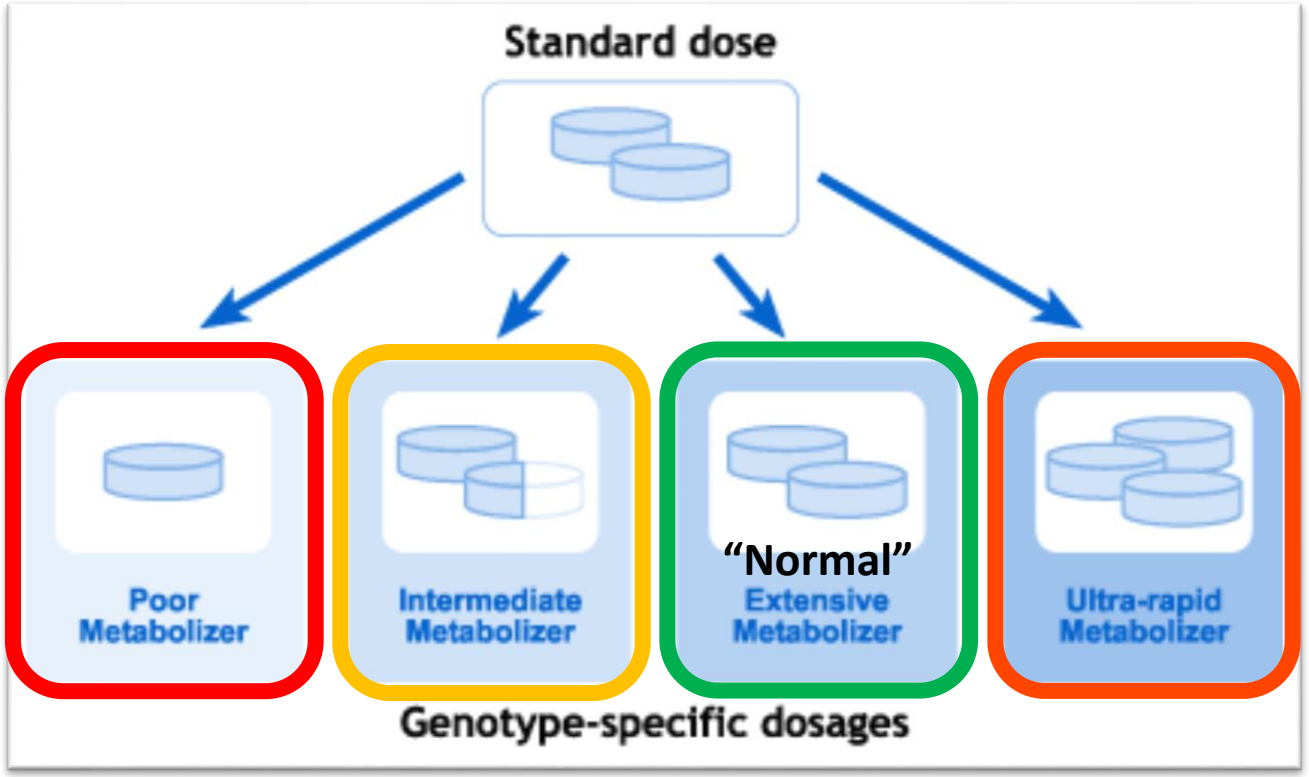
- PGx variant in 0 gene*
- PGx variant in 1 gene
- PGx variants in 2 genes
- PGx variants in 3 genes
- PGx variants in 4 genes
- PGx variant in 5 genes

POTENTIAL CONSEQUENCES OF VARIANTS

- Drug toxicity
- Adverse drug reactions
- Extended pharmacological effects
- Decreased/increased effective dose
- Exacerbation of drug-drug interactions
- Lack of drug efficacy
- Lack of prodrug activation

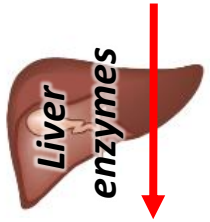
TYPES OF METABOLIZERS AND DRUG DOSES

Gene	Patient Genotype	Patient Phenotype	Indications
CYP2D6	*1/*1dup	Ultra-rapid Metabolizer	A gene duplication was detected, including two functional alleles. This result is consistent with increased enzymatic activity. This phenotype is associated with an elevated risk of toxicity when administering drugs metabolized by CY2D6. Consider alternate medications, or monitor patient closely for adverse drug reactions. Commonly prescribed medications that are metabolized by CYP2D6 include Codeine, Oxycodone, Duloxetine (Cymbalta), Fluoxetine (Prozac), Tramadol and many others.
CYP2C19	*2/*2	Poor Metabolizer	Two non-functional alleles were detected indicating extremely low or non-existent enzymatic activity. Administration of alternative drugs that are not metabolized by CYP2C19, or usage of a reduced dose to prevent toxicity may be warranted. Commonly prescribed medications that are metabolized by CYP2C19 include Sertraline (Zoloft), Citalopram (Celexa), Carisoprodol (Soma), Diazepam (Valium), Omeprazole (Prilosec), and many others.
CYP2C9	*1/*1	Normal Metabolizer	Detection of two normal alleles indicates normal enzymatic activity. Follow standard dosing practices when administering drugs metabolized by CYP2C9. Commonly prescribed medications that are metabolized by CYP2C9 include Ibuprofen, Diclofenac (Voltaren), Celecoxib (Celebrex), Flurbiprofen (Ansaid), Piroxicam (Feldene), warfarin and many others.
VKORC1	GG	Normal Warfarin dose required	The GG genotype for VKORC1 is associated with and average Warfarin dose requirement to achieve efficacy.

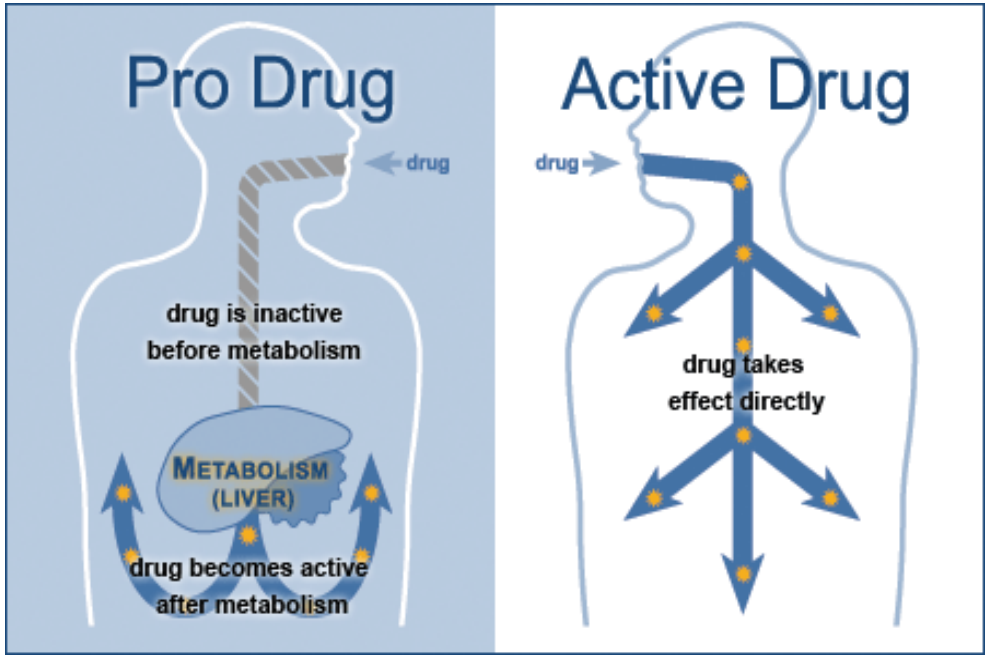


DRUG DOSE ALSO DEPENDS ON DRUG TYPE

Codeine (prodrug)

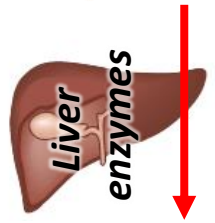


Morphine (active)



Metabolism breaks down Active drug

Morphine (active)



Inactive Metabolites

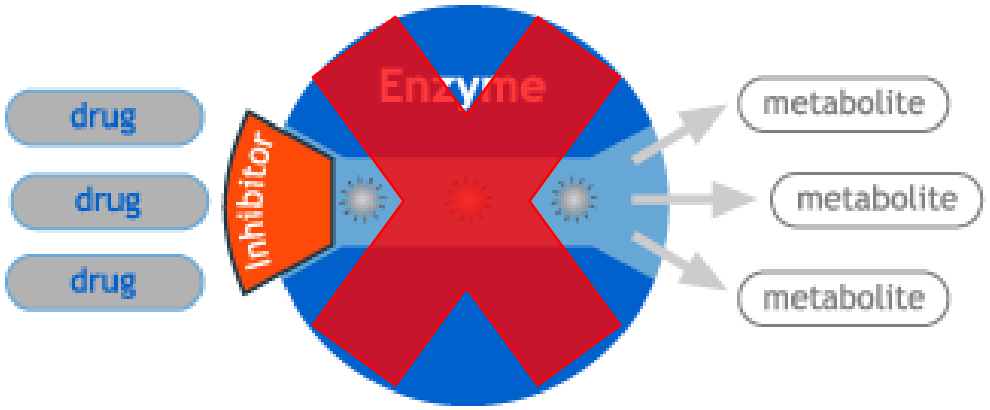
- Codeine is a prodrug that exerts its analgesic effects after metabolism to morphine by CYP2D6
  - What happens if someone is a "poor metabolizer?" **Codeine toxicity**
  - How about an "ultra rapid" metabolizer? **Morphine toxicity**

COMMON PRODRUGS

- Codeine (opioid)
- Amitriptyline (tricyclic antidepressant)
- Clopidogrel (Plavix, platelet inhibitor)
- Simvastatin (Zocor, statin)
- Abacavir (treatment for HIV/AIDS)

DRUG INTERACTIONS: ENZYME INHIBITORS

- **Inhibitors** = block enzymes from working
  - Common source of adverse drug reactions or ineffective doses
- Some drugs ***inhibit*** CYP enzymes
  - Same effect as a "poor metabolizer"



Inhibiting compounds block drug metabolism enzymes. Depending on the drug, inhibition can lead to reduced therapeutic effects or toxic buildup of unmetabolized compounds.

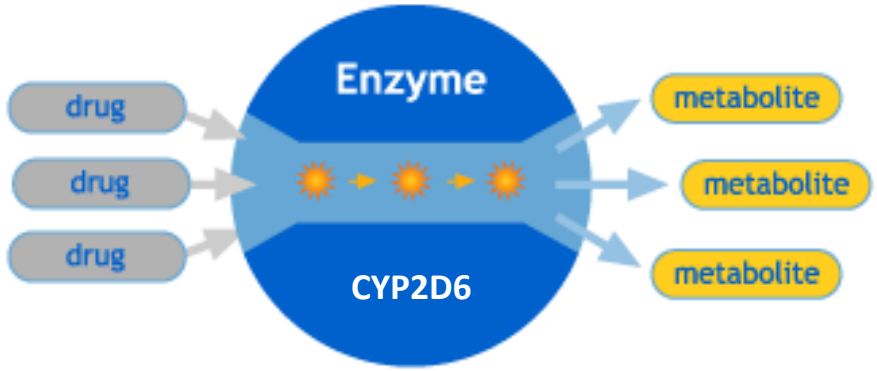
Inhibitor = CYP3A4

Cholesterol-lowering drug
Metab. in-part by CYP3A4

- Too much simvastatin accumulates in body
- Life-threatening myopathies possible
- Muscle aches, spasms, and pain

DRUG INTERACTIONS: INDUCERS AND ACTIVATORS

- **Inducers**: speed up how quickly an enzyme functions
  - Source of adverse drug reactions / ineffective doses
- Some drugs/herbals ***induce*** CYP enzymes
  - Gives same effect as "ultra-rapid metabolizer"



Inducer = CYP3A4 / CYP2C19

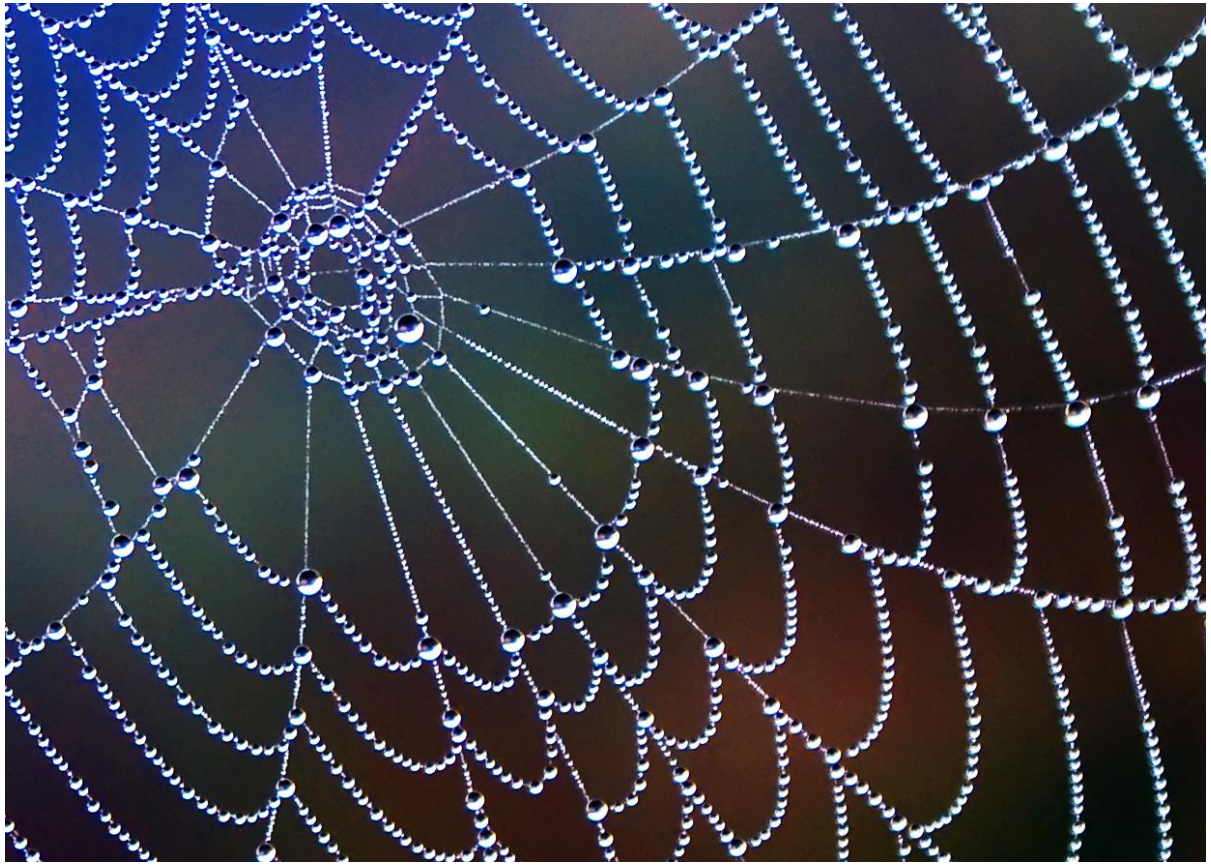
+

Active drug Metabolized by CYP3A4 & CYP2C19

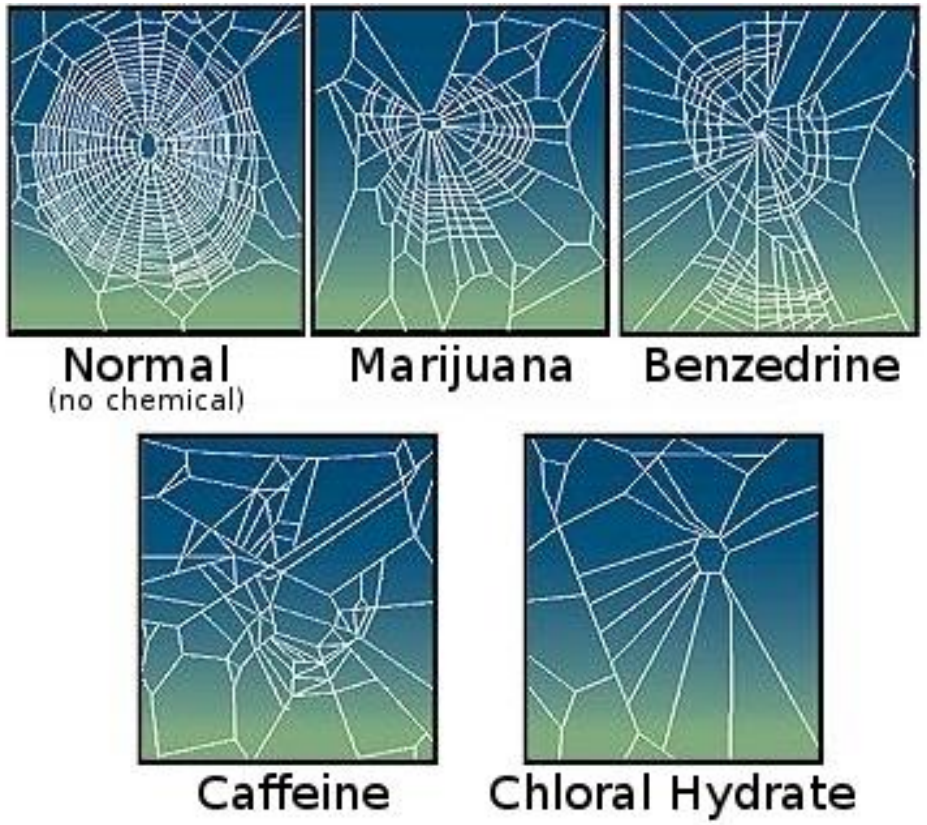
=

- Breaks down too fast
- Typical drug dose ineffective

DRUGS AND ENZYMES



A complex web, indeed!



The webs gets even more complex!

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Link to comprehensive drug interaction table:http://medicine.iupui.edu/clinpharm/ddis/main-table/

VALUE TO THE PRACTICE OF PHARMACY

- Millions of patients are either under- or over-treated, and there are over two million serious ADRs yearly
- Although the *use of compound drugs is a sound alternative for side effects avoidance, they are subject to same above limitations*

PAIN (ADDICTION) MANAGEMENT

GENE TESTS	EFFECT ON PAIN MANAGEMENT
CYP2D6	Key role in the metabolism of opioids including codeine, tramadol, and oxycodone[1,7]
CYP2C19	Impacts dosage requirements for tricyclic antidepressants[8] and methadone
CYP2C9	Crucial to the breakdown of NSAIDS including diclofenac, naproxen, and ibuprofen[6]
CYP3A4/5	Key role in the metabolism of opioids including fentanyl and methadone[5,9]
COMT	Affects morphine dosage requirements and perceptions of pain[10]

CASE STUDY

- 58-yr old female with non-radicular back pain
- Rest, heat, acetaminophen – no relief
- Prescribed tramadol 50 mg every 8 hrs as needed
- She returns after a week - complaining of continued pain
- **Vitals:** pulse:42 / Resp. Rate:16 / BP: 118/85
- **Physical Exam:** alert / in significant pain / pupils normal size
- **Current Meds:** metformin 500 mg daily / simvastatin 40 mg daily / metoprolol 50 mg daily / acetaminophen 1000 mg 3X daily / tramadol 50 mg 3X daily

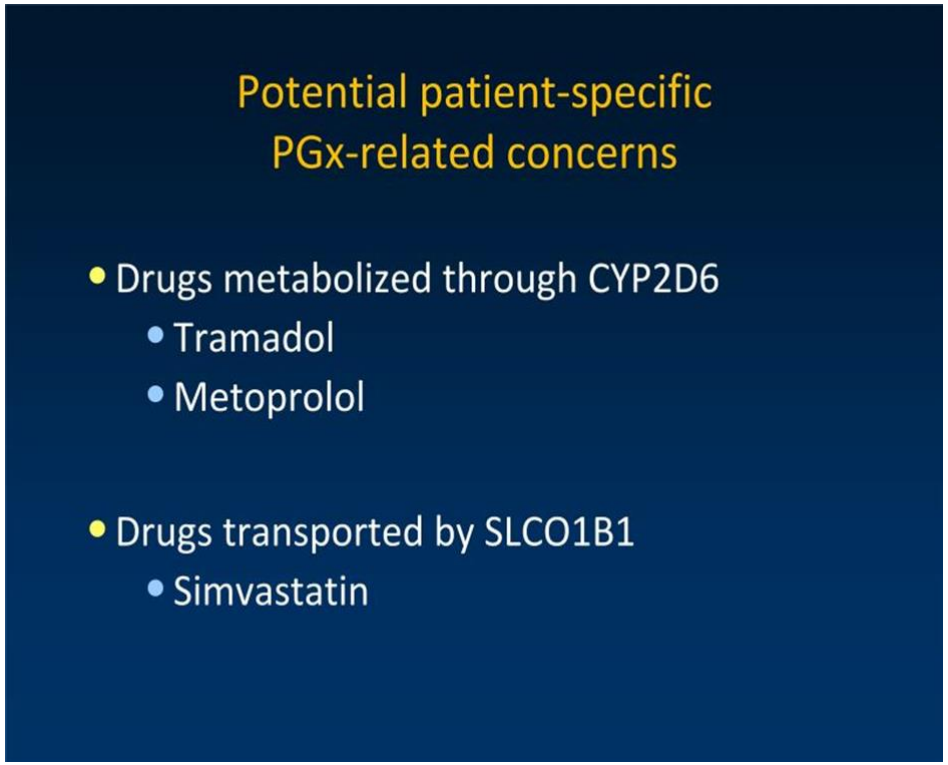
PHARMACOGENOMIC TEST RESULTS

Gene Metabolizing Enzyme	Genotype	Phenotype
SLCO1B1	*1/*5	Increased Risk
CYP2D6	*4/*4	Poor Metabolizer
CYP2C9	*1/*1	Normal
CYP2C19	*1/*2	Normal
CYP3A4	*1/*1	Normal
CYP3A5	*1/*1	Normal

PHARMACOGENOMIC TEST RESULTS

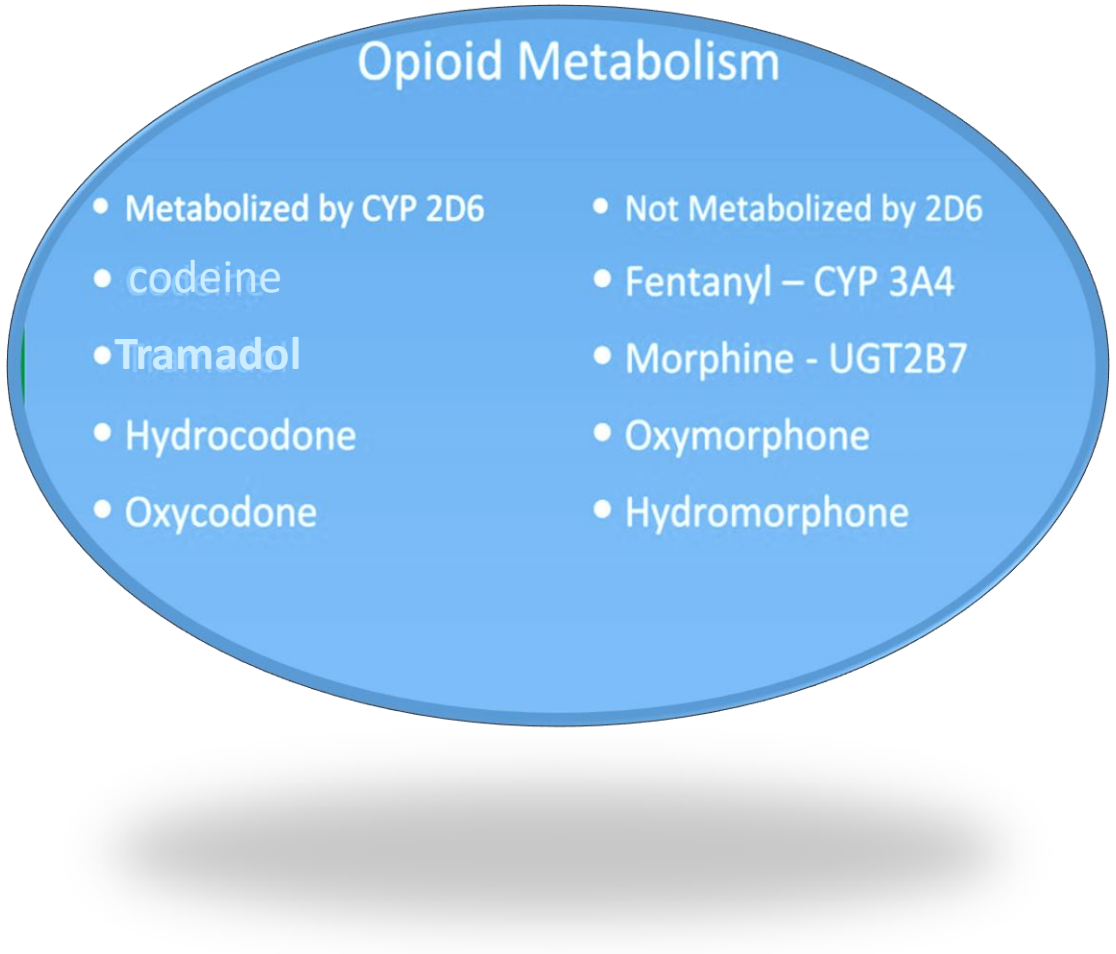
WHICH OF HER MEDS IS OF CONCERN BASED ON HER PGX RESULTS?

- Tramadol
- Acetaminophen
- Metformin
- Simvastatin
- Metroprolol



WHAT DO YOU RECOMMEND?

- Increase dose of tramadol?
- Change to codeine?
- Change to methadone?
- Change to fentanyl?
- Change to hydrocodone?
- Decrease dose of tramadol?



OTHER CONSIDERATIONS FOR THIS CASE

- How about his simvastatin?
  - SLCO1B1 *1/*5 genotype: Increased risk phenotype
- SLCO1B1: transporter regulating hepatic uptake of statins
  - Increased risk of statin-induced myopathy

***CHANGE THE STATIN MEDICATION**

ANOMALOUS URINE TESTING

ANOMALOUS URINE TESTING RESULTS FOR PAIN MANAGEMENT AND ADDICTION TREATMENT EXPLAINED BY PGX TESTING

- Urine testing to confirm use of prescribed medications or illicit drugs plays an important role in clinical management to prevent drug misuse
- Examples where PGx testing was helpful to explain anomalous urine drug testing results were:
    - Diazepam (valium)
    - Methadone
- **Conclusion:** PGx testing helped explain anomalous UA testing results (and ultimately helped physician to craft individualized pain management/addiction treatment plan based on patients PGx profile)

EXAMPLE: DIAZEPAM (VALIUM)

- Diazepam is a benzodiazepine with several clinical uses, including management of anxiety, insomnia, muscle spasms, seizures & alcohol withdrawal
- Diazepam primarily metabolized by CYP2C19 and CYP3A4 to major active metabolite, nordiazepam
- Approximately 3% of Caucasians & 15-20% of Asians have reduced or absent CYP2C19 enzyme activity ("poor metabolizers")
- In these individuals, standard doses of diazepam may lead to a higher diazepam exposure
  - CY2C19 poor metabolizers take longer period of time to emerge from general anesthesia than normal metabolizers

URINE ANALYSIS

- Highly positive valium (diazepam) result with low valium metabolites (nordiazepam, temezepam, oxazepam)
- Normally negligible amounts diazepam excreted unchanged
- Suggestive of urine tampering (pill shaving)….

Confirmatory Test Results

Compound Measured with LC-MS/MS	Cutoff (ng/mL)	Test Outcome	Measured Concentration (ng/mL)	Creatinine Adjusted Value (ng/mg creatinine)
Natural and Semi-Synthetic Opioids				
Codeine	50	Neg		
Morphine	50	**Pos**	> 500	limit
Hydrocodone	50	Neg		
Norhydrocodone	50	Neg		
Hydromorphone	50	Neg		
Oxycodone	50	**Pos**	481.1	1,932.1
Noroxycodone	50	**Pos**	> 500	limit
Oxymorphone	50	**Pos**	> 500	limit
Buprenorphine	5	Neg		
Norbuprenorphine	20	Neg		
Synthetic Opioids				
Fentanyl	5	Neg		
Norfentanyl	8	Neg		
Methadone	100	**Pos**	715.2	2,872.3
EDDP (Methadone metabolite)	100	Neg		
Tramadol	100	Neg		
Tapentadol	20	Neg		
Meperidine	200	Neg		
Normeperidine	200	Neg		
Benzodiazepines				
Alpha-Hydroxyalprazolam	20	Neg		
7-Amino-Clonazepam	50	Neg		
Lorazepam	80	Neg		
Nordiazepam	40	**Pos**	96.0	385.5
Temazepam	50	**Pos**	280.9	1,128.1
Oxazepam	100	**Pos**	413.1	1,659
Diazepam	40	**Pos**	> 400	limit
Stimulants				
Amphetamine	100	Neg		
Methylphenidate*	50	Neg		
Muscle Relaxants				
Carisoprodol	100	Neg		
Meprobamate	100	Neg		
Illicit Drugs				
cTHC (Marijuana metabolite)	25	Neg		
Methamphetamine	250	Neg		
Benzoylecgonine (Cocaine metabolite)	50	Neg		
Phencyclidine (PCP)	25	Neg		
6-MAM (Heroin metabolite)*	10	Neg		
MDMA (Ecstasy)	100	Neg		
Mitragynine (Kratom)	10	Neg		

* Methylphenidate degrades in urine at room temperature after 6 days.

* 6-Monoacetylmorphine (6-MAM) degrades in urine at room temperature after 5 days.

Confirmatory Test Results Continued

Compound Measured with LC-MS/MS	Cutoff (ng/mL)	Test Outcome	Measured Concentration (ng/mL)	Creatinine Adjusted Value (ng/mg creatinine)
Other				
Dextromethorphan	50	Neg		
Dextrorphan	50	Neg		
Gabapentin	1000	**Pos**	> 10,000	limit
Ketamine	50	Neg		
Norketamine	20	Neg		
Naltrexone	50	Neg		
Zolpidem	10	Neg		
Barbiturates				
Butalbital	200	Neg		
Phenobarbital	200	Neg		
Secobarbital	200	Neg		
Alcohol Metabolites				
Ethyl Glucuronide	500	Neg		
Ethyl Sulfate	250	Neg		

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CEO and Laboratory Director

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PGX RESULTS

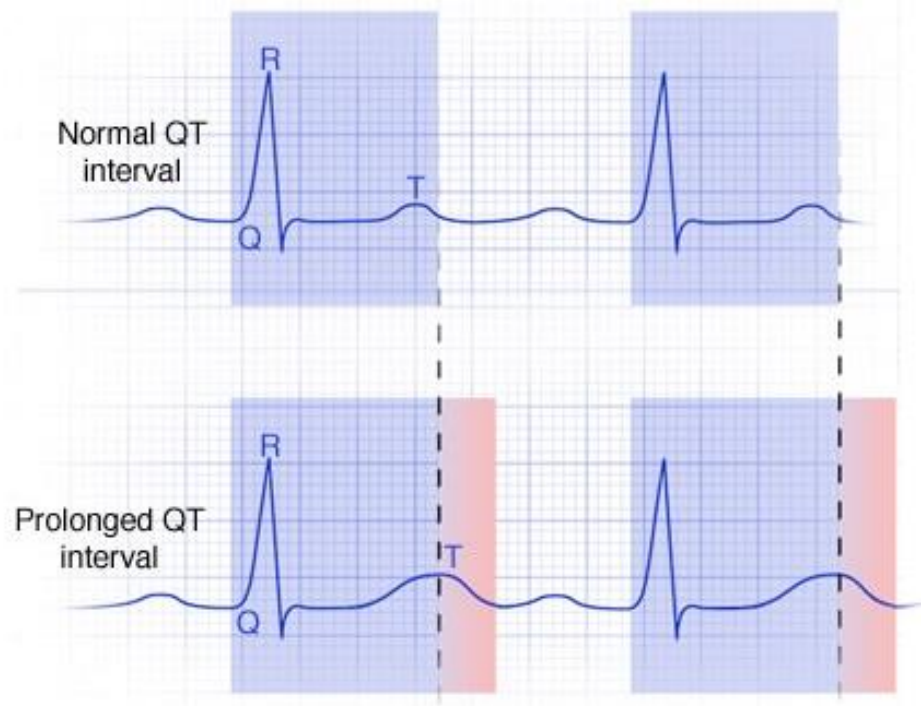
- Absent CYP2C19 enzyme activity (poor metabolizer)
- Explains UA result
- Indicates alternative drug not metabolized by CYP2C19 may be warranted

Gene	Patient Genotype	Patient Phenotype	Indications
CYP2D6	*1/*2A, *1/*35	Extensive (Normal) Metabolizer	The presence of at least one functional allele indicates normal metabolic activity. Follow standard dosing practices when administering drugs metabolized by CYP2D6. Commonly prescribed medications that are metabolized by this enzyme include Codeine, Oxycodone, Duloxetine (Cymbalta), Fluoxetine (Prozac), Tramadol and many others.
CYP2C19	*2/*4	Poor Metabolizer	Two non-functional alleles were detected indicating extremely low or non-existent enzymatic activity. Administration of alternative drugs that are not metabolized by CYP2C19 may be warranted. Commonly prescribed medications that are metabolized by CYP2C19 include Sertraline (Zoloft), Citalopram (Celexa), Carisoprodol (Soma), Diazepam (Valium), Omeprazole (Prilosec), and many others.
CYP2C9	*1/*2	Intermediate Metabolizer	Detection of one normal function allele and one reduced function allele indicates moderately reduced enzymatic activity. There is a possibility of reduced efficacy and an increased risk of toxicity when taking drugs metabolized by CYP2C9. Usage of a reduced dose may be warranted. Commonly prescribed medications that are metabolized by CYP2C9 include Ibuprofen, Diclofenac (Voltaren), Celecoxib (Celebrex), Flurbiprofen (Ansaid), Piroxicam (Feldene), warfarin and many others.
CYP3A4	*1/*1	Normal Expressor (Normal Metabolizer)	CYP3A4 is responsible for the metabolism of approximately 50-60% of clinical drugs used today, including acetaminophen, codeine, cyclosporine A, diazepam, and erythromycin. It is also important for the metabolism of steroid hormones. Although most of the CYP3A4 haplotypes have not been indisputably shown to affect expression or activity in terms of pharmacodynamics or pharmacokinetics, the presence of two normal alleles indicates normal enzyme activity. Co-administration of drugs metabolized by CYP3A4 should not be used in conjunction with CYP3A inhibitors and inducers. Potent inhibitors include Clarithromycin (Biaxin), diltiazem (Cardizem), grapefruit juice, itraconazole (Sporanox), verapamil (Calan), ritonavir, and others. Potent inducers include Carbamazepine, phenobarbital, phenytoin, rifampin and others.
CYP3A5	*3/*3	Non-Expressor (Poor Metabolizer)	In this patient, drugs that are inactivated or activated by CYP3A5 are metabolized at a significantly reduced rate. Lower doses may be required in order to reduce the risk of adverse effects of the drug and to avoid unnecessary dosing.

EXAMPLE: METHADONE

- Methadone (Dolophine) used for the treatment of opioid addiction
- Side effects include methadone-induced QT interval prolongation (can lead to methadone-associated sudden cardiac death)

A prolonged QT interval refers to an abnormality seen on an electrocardiogram. This abnormality reflects a disturbance in how your heart's bottom chambers (ventricles) conduct electricity, leading to abnormal heart rhythms.



METHADONE

- Methadone is primarily excreted in the urine as unchanged drug and metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)
- P450 enzymes important in methadone metabolism include: CYP3A4, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP3A5, CYP3A7, CYP2D6
- Mechanistic link demonstrated between CYP2C19*2 genotype and methadone-induced QT interval prolongation
- CYP2B6*6 genotype deficiently catalyzes methadone metabolism, increases risk for methadone toxicity and drug interactions

URINE ANALYSIS

- Methadone positive results but no methadone metabolite (EDDP) present
- No metabolite suggestive of urine tampering…

Confirmatory Test Results

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Norketamine	20	Neg		
Naltrexone	50	Neg		
Zolpidem	10	Neg		
Barbiturates				
Butalbital	200	Neg		
Phenobarbital	200	Neg		
Secobarbital	200	Neg		
Alcohol Metabolites				
Ethyl Glucuronide	500	Neg		
Ethyl Sulfate	250	Neg		

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CEO and Laboratory Director

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PGX RESULTS

- Absent CYP2C19 enzyme activity (poor metabolizer)
- Absent CYP3A5 enzyme activity (poor metabolizer)
- Explains UA result
- Indicates alternative drug not metabolized by CYP2C19, CYP3A5 may be warranted

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CYP2C9	*1/*2	Intermediate Metabolizer	Detection of one normal function allele and one reduced function allele indicates moderately reduced enzymatic activity. There is a possibility of reduced efficacy and an increased risk of toxicity when taking drugs metabolized by CYP2C9. Usage of a reduced dose may be warranted. Commonly prescribed medications that are metabolized by CYP2C9 include Ibuprofen, Diclofenac (Voltaren), Celecoxib (Celebrex), Flurbiprofen (Ansaid), Piroxicam (Feldene), warfarin and many others.
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CYP3A5	*3/*3	Non-Expressor (Poor Metabolizer)	In this patient, drugs that are inactivated or activated by CYP3A5 are metabolized at a significantly reduced rate. Lower doses may be required in order to reduce the risk of adverse effects of the drug and to avoid unnecessary dosing.

HRT

- One important aspect of compounding pharmacy is hormone replacement therapy (HRT)
- COMT, CYP1A1, and CYP1B1 genes are implicated in ovarian and breast cancer risk via their involvement in estrogen metabolism
  - Certain variants in these three estrogen metabolism genes can produce more DNA damaging (cancer causing) metabolites
- Knowing this information is important for considering HRT
  - Individuals with high risk alleles opt to not take HRT or opt for HRT formulations with lower estrogen profiles

CONCLUSION

- Pharmacogenetics can facilitate improved and more effective pharmacotherapy.
- Individualized treatments based on the presence of gene variants that alter a particular target protein's function but enhance response to a medication is key.
- Physician uptake of clinical pharmacogenetics has been underwhelming, in part due to a perceived lack of clinical utility, inadequate professional guidelines for pharmacogenetics-based management, and limited insurance reimbursement for testing. In the face of these challenges, selected pharmacogenetic examples have managed to achieve acceptance in clinical practice and a number of others are currently being evaluated by randomized controlled trials.

THANK YOU!