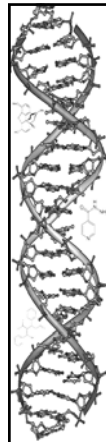


Pharmacogenetics:

Everyone's just a little different!

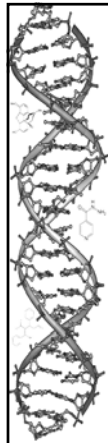
Alan P. Agins, Ph.D.

President, PRN Associates
Continuing Medical Education, Tucson, AZ



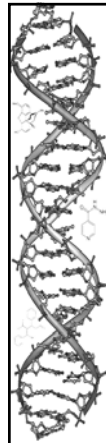
"If it were not for the great variability among individuals, medicine might as well be a science and not an art."

Sir William Osler, 1892



Pharmacogenetics:

- Relatively new field of study within the realm of pharmacology
- Patients can respond differently to a given therapeutic agent even if they have the same illness.
- The same dose of a given drug in some patients causes very different plasma levels and different therapeutic response that cannot be explained by weight, age or gender

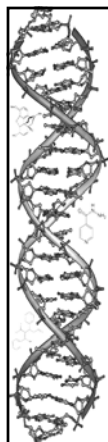


Pharmacogenetics

The study of genetically determined interindividual differences in therapeutic response to drugs and susceptibility to adverse effects

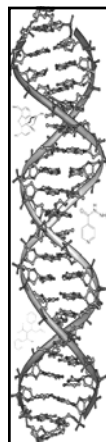
Pharmacogenomics:

Use of genome based techniques in drug development

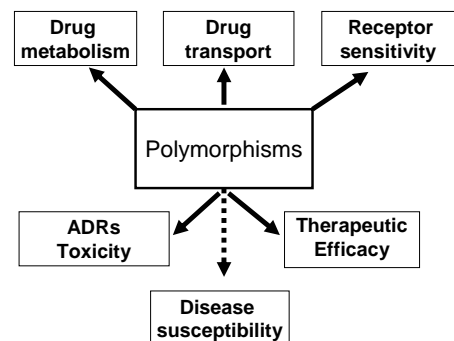


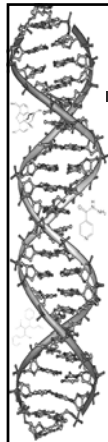
Pharmacogenetics

- The differences in the response to a given drug can be due to two major pharmacological factors that can vary with genetic influence:
 - Pharmacokinetic:
 - Genetically based differences in the processes influencing bioavailability
 - Absorption, distribution, metabolism, elimination
 - Pharmacodynamic:
 - Genetically based differences in the targets at which the drug acts
 - Receptors, enzymes, ion channels, etc



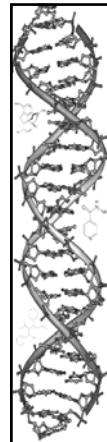
Consequences of Polymorphisms





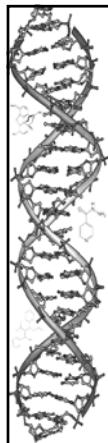
Polymorphism

- Genetic variation occurring with a frequency of 1% or more in the population
- 1. **SNP** (single nucleotide polymorphism):
- 2. **Insertion/deletion polymorphism**: insertion or deletion of a few nucleotides
- 3. **Variable number tandem repeats**: variation in the number of times a sequence of several hundred base pairs is repeated
- 4. **Simple tandem repeats**: 2-4 nucleotides repeated a variable number of times



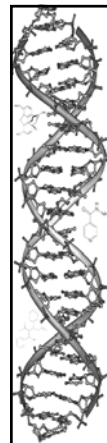
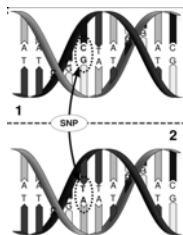
Polymorphism vs. Mutation

- **Polymorphism is defined as a variation in more than 1% of the population.**
- **Mutations are rare differences which occur in less than 1% of the population (usually much less than 1%).**
- **It is estimated that about 10 million SNPs exist in human populations**
- **Most of these SNPs are neutral**



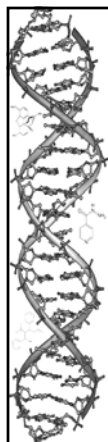
Single Nucleotide Polymorphism

- Most frequent type
- Difference in a single base of the genomic sequence
- Usually 1/1000 bases
- Most do not influence the structure or function of proteins



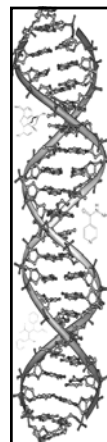
SNPs can occur

- **In Exons**
 - may alter the structure of proteins and may lead to functional consequences
- **In Introns**
 - may influence splicing
- **In Regulatory regions**
 - may influence expression of the gene



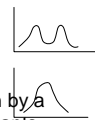
Pharmacogenetics

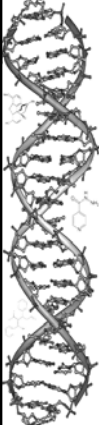
- **Genotype**: gene structure encoding for the given characteristics
- **Phenotype**: the manifestation of the genotype, which can be observed and can be influenced by other factors:
 - Other gene products
 - Environment
 - Acquired characteristics
- Frequency and functional relevance of genetic polymorphisms differs greatly among ethnic groups



Pharmacogenetics

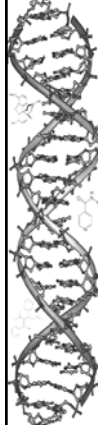
- **Determination of genotype**:
 - PCR, gene sequencing
- **Determination of phenotype**:
 - Generally “after-the-fact”
 - After administration of a drug
 - Measure pharmacokinetic parameters (half-life, clearance, plasma levels)
 - Or, after unusual response or toxicity
- **Distribution of phenotypes in the population**:
 - Multimodal (usually bi- or trimodal) distribution - indicates determination by a single gene having polymorphic variants





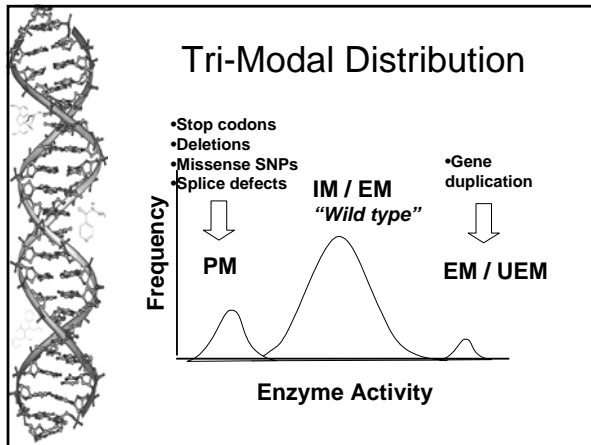
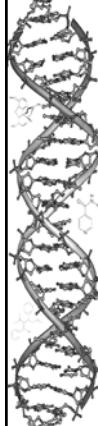
Pharmacogenetics

- SNP Polymorphisms in metabolic pathways are usually bi- or trimodal = two or three phenotypes
 - Enhanced (extensive) metabolizer
 - High rate of metabolism – often resulting in low plasma concentration of the drug
 - usually heterozygote or homozygote dominant
 - Intermediate metabolizer
 - Poor metabolizer or non-metabolizer:
 - Slow or no metabolism of the drug resulting in high plasma concentration for an extended time
 - Usually homozygote recessive



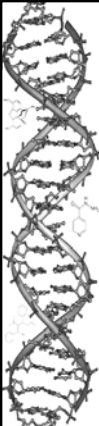
Pharmacogenetics and Predicted vs Unpredicted ADRs

	Augmented: related to the therapeutic effect	Idiosyncratic: related to pharmacogenetics
Predictability	Yes	No
Dose dependence	+++	+ / -
Frequency	Common	Rare
Morbidity	+/-	+++

Pharmacogenetics of Drug Metabolism

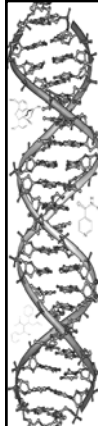
- Drug metabolism is crucial in determining therapeutic and adverse effects
- Genetic factors play an important role in individual differences of drug metabolism
 - Phase I
 - Oxidation, reduction, hydroxylation, dealkylation, etc.
 - Cytochrome P450 enzymes in gut and liver
 - Phase II
 - Conjugation with glucuronic acid, glutathione, sulfate, acetate, etc
 - Aim: to increase water solubility
 - Usually in the cytosol



Pharmacogenetics and Cytochrome P450

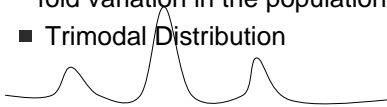
Major P450 Isoforms

- CYP3A4 -
- CYP2D6 - Polymorphism
- CYP2C19 - Polymorphism
- CYP2C9 - Polymorphism
- CYP1A2
- CYP2E1

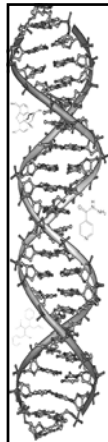


Cytochrome P450 CYP2D6 Polymorphism

- Discovered in the 1970s
- One of the most widely studied polymorphisms in drug metabolism
- 2% of total liver CYP content
- More than 50 alleles, up to a 1000-fold variation in the population
- Trimodal Distribution

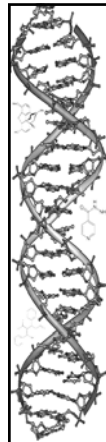


The graph shows a trimodal distribution with three distinct peaks of varying heights, representing the different metabolic phenotypes of CYP2D6.



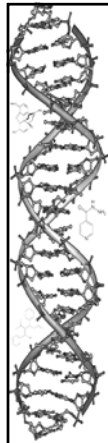
CYP2D6 Poor Metabolizers (PM)

- Inheritance of two mutant *CYP2D6* alleles, due to nucleotide substitutions, deletions, insertions or gene conversions
- No enzyme protein or very poor enzyme activity; impaired metabolism of CYP2D6 substrates
 - Caucasians 8 – 10%
 - American Blacks 1 – 3%
 - Japanese / Chinese < 1%
- Clinical considerations: higher plasma drug level due to decreased drug clearance; exaggerated clinical outcome and increased risk of dose-dependent side effects; may have to lower drug dose



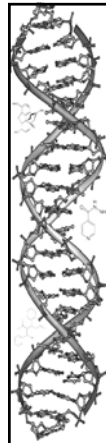
CYP2D6 Ultraextensive Metabolizers (UEM)

- Inheritance of alleles with duplication or amplification (up to 13 copies) of functional *CYP2D6* genes
- Excessive amount of enzyme expressed, high metabolic capacity
- Frequency: from 2% in Swedish population to 30% in Ethiopian population
- Clinical considerations: Possibly higher than normal drug dose required for efficacy; side effects if metabolites are toxic



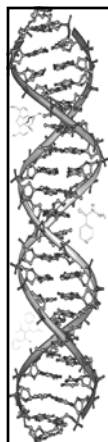
CYP2D6 Extensive Metabolizers (EM)

- Individuals who are either homozygous for the normal-functioning alleles or functional mutant alleles, or heterozygous with one active and one mutant allele
- Largest, but most diverse population, can have wide range of metabolic capacity
- Clinical considerations: high or low end of the group may need drug dose adjustment for acceptable efficacy and safety



Drugs Metabolized by CYP2D6

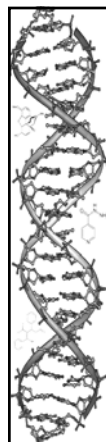
- | | |
|-----------------------------|---------------------------|
| ■ Tricyclic Antidepressants | ■ Dextromethorphan |
| ■ Venlafaxine | ■ Beta Blockers |
| ■ Fluoxetine | - Metoprolol |
| ■ Paroxetine | - Propranolol |
| ■ Antipsychotics | - Timolol |
| ■ Haloperidol | ■ Opioids |
| ■ Perphenazine | - Codeine |
| ■ Risperidone | - Hydrocodone |
| ■ Atomoxetine | - Oxycodone |
| | - Tramadol |



Tailored Dosing

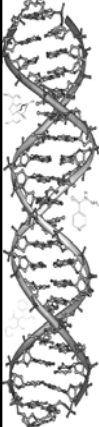
- **Recommend dosage adjustment to Atomoxetine in CYP2D6 PM and those taking strong 2D6 inhibitors**
 - Individual > 70 kg: start at 40 mg/day
 - Individual ≤ 70 kg: start at 0.5 mg/kg/day.
 - *Increase to the usual target dose of 80 mg/day and 1.2 mg/kg/day, respectively, only if treatment fails to improve symptoms after 4 weeks and the initial doses are well tolerated.

Strattera (atomoxetine) package insert: <http://pi.lilly.com/us/strattera-pi.pdf>



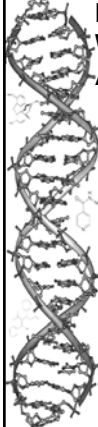
Interesting tidbit on CYP2D6

- CYP 2D6 also present in brain
- Functionally associated with dopamine transporter
- Might have a role in dopaminergic transmission
- Some studies have suggested differences in personality traits between PMs and Ems
 - Type A vs Type B personality
 - Higher levels of anxiety / impulsivity (PMs)



Cytochrome P450 CYP2C19 Polymorphism

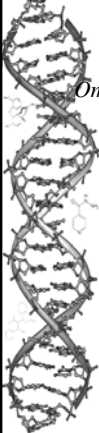
- Poor Metabolizers
 - 3–5% of Caucasian
 - 15–25% of Asians
 - (Chinese, Japanese, Koreans, Indians, etc)
- May affect clearance of:
 - amitriptyline, **diazepam**, clomipramine, **phenytoin**, progesterone, propranolol, **PPIs** (lansoprazole, omeprazole, pantoprazole, rabeprazole, etc), warfarin



Examples: Why diazepam metabolism is slower in Asians compared to Caucasians?

Genotype	Allele	Diazepam $t_{1/2}$
EM	CYP2C19 *1/*1	20 hours
PM	CYP2C19 *2/*2	84 hours

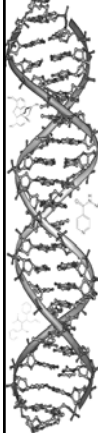
↑
About 15 – 25% of Asians have high frequency of mutant alleles CYP2C19



CYP2C19 Polymorphism and Treatment of H.Pylori

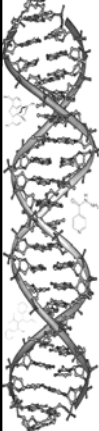
Genotype	Allele	Cure rate
Wild type	CYP2C19 *1/*1	29 %
Htz	CYP2C19 *1/*2	60 %
Hmz variant	CYP2C19 *2/*2	100 %

Omeprazole
Due to higher concentration and longer duration of omeprazole



Cytochrome P450 CYP2C9 Polymorphism

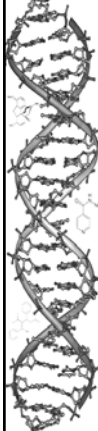
- More than 50 SNPs have been described in the regulatory and coding regions of the CYP2C9 gene
- Some of them are associated with reduced enzyme activity
- 10–35% of Caucasians are poor metabolizers
- May affect clearance of:
 - **Phenytoin, S-warfarin**
 - losartan, valsartan, glipizide, glyburide, rosiglitazone NSAIDs, celecoxib, rosuvastatin



Frequency of CYP2C9 Phenotype in Various Populations

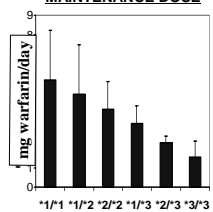
Racial group	Decreased Activity				
	*1/*1 (%)	*1/*2 (%)	*1/*3 (%)	*2/*2 (%)	*3/*3 (%)
Caucasians	65	20	12	1	0.4
African American	97	2	1	0	0
Chinese	97	0	4	0	0
Japanese	96	0	4	0	0
Korean	98	0	2	0	0
Turkish	62	18	17	1	1
Spanish	50	16	24	2	0

C. R. Lee, J. A. Gildstein and J. A. Pieper, Pharmacogenetics 12:251-263, 2002



Effect of CYP2C9 Genotype on Warfarin-Related Outcomes

MAINTENANCE DOSE **TIME TO STABLE ANTICOAGULATION**

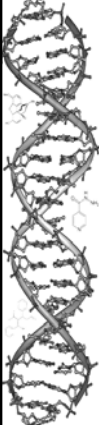


CYP2C9-WT ~ 90 days
CYP2C9-Variant ~180 days


*2 or *3 carriers take longer to reach stable anticoagulation

- Variant alleles have significant clinical impact
- Still large variability in warfarin dose (15-fold) in *1/*1 "controls"?

(Higashi et al., JAMA 2002)



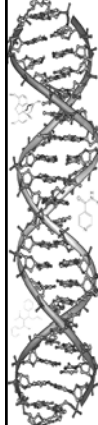
CYP2C9 Polymorphism and Phenytoin Toxicity



Ataxia, nystagmus, drowsiness, gingival hyperplasia grade II

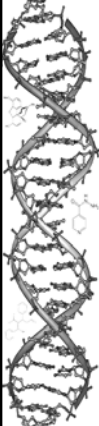
Phenytoin dose = 300 mg/day
Plasma phenytoin level = 33.2 µg/ml

Genotype : CYP2C9*3/*3



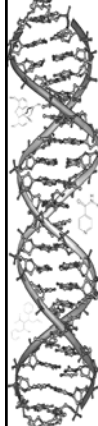
Other non-polymorphism factors that may affect Cytochrome P450

- Alcohol
- Other drugs
- Caffeine
- Constituents of tobacco
- Char-broiled foods
- Cruciferous vegetables
- Grapefruit juice
- Air or water pollutants
- Induction or Inhibition
- More likely to affect
 - CYP3A4
 - CYP1A2
 - CYP2E1



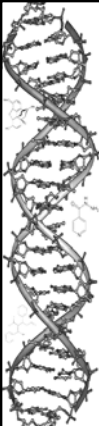
Polymorphism of phase II metabolism: Acetylation

- N-acetyltransferase (NAT)
 - Speed of acetylation is genetically determined: bimodal distribution, slow and fast acetylators
 - Autosomal recessive inheritance
 - The rate of slow acetylators increases with age
 - Rate of slow acetylators is higher:
 - Gilbert syndrome, rheumatoid arthritis, ischaemic heart disease



Drugs that are substrates for N-acetyltransferase

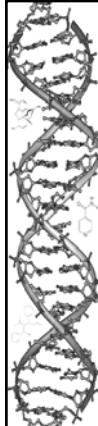
- isoniazid
- sulfamethazine
- sulfapyridine
- sulfasalazine
- clonazepam
- hydralazine



Ethnic Differences In The Distribution Of Acetylator Phenotype

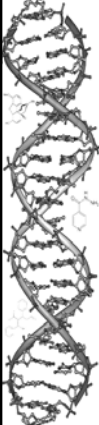
Population	% Slow	% Fast (Hetero)	% Fast (Homo)
Caucasians	58.6	35.9	5.5
Blacks	54.6	38.6	6.8
Japanese	12	45.3	42.7
Chinese	22	49.8	28.2

From: Kalo W. Clin Pharmacokinet 7:373-4000, 1982.



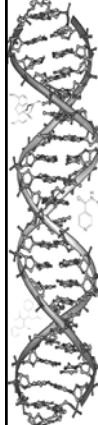
Clinical Concerns

- Slow acetylators more likely to develop toxicity from various NAT substrates
- Isoniazid – neurotoxicity, elevated transaminases
- Sulfasalazine – cyanosis, hemolysis, transient reticulocytosis
- Greater risk of sulfonamide hypersensitivity in HIV pts ????



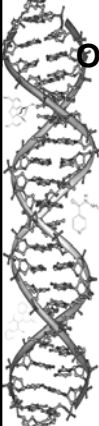
Other Enzyme Polymorphisms

- Glucose-6-phosphate dehydrogenase
 - Most frequent pharmacogenetic enzymopathy
 - ~ 130 enzyme variants, only some are abnormal
 - Antimalaria drugs (primaquine), antibiotics (sulfonamides, chloramphenicol, nitrofurantoin), other medicines
 - Can cause fatal hemolysis in some patients
 - Favism: hemolysis after consumption of legumes, gooseberry, blackcurrant



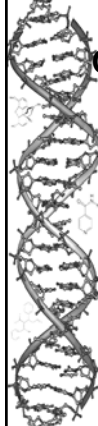
Incidence Of G6PD Deficiency In Different Ethnic Populations

<u>Ethnic Group</u>	<u>Incidence(%)</u>
Kurds	53
Iraqi	24
Indians-Parsees	16
Javanese	13
Filipinos	13
Iranians	8
North Africa	<4
Chinese	2
Greeks	0.7-3
Micronesians	<1



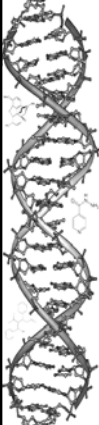
Other Enzyme Polymorphisms

- Alcohol dehydrogenase (ADH)
 - Speed of ethanol \Rightarrow acetaldehyde reaction is increased
 - Acetaldehyde dehydrogenase activity is unaffected, so acetaldehyde is not metabolised at a sufficient rate
 - Acetaldehyde is accumulated causing flushing and tachycardia
 - Frequency: 5-20% in caucasians, 90% among Chinese



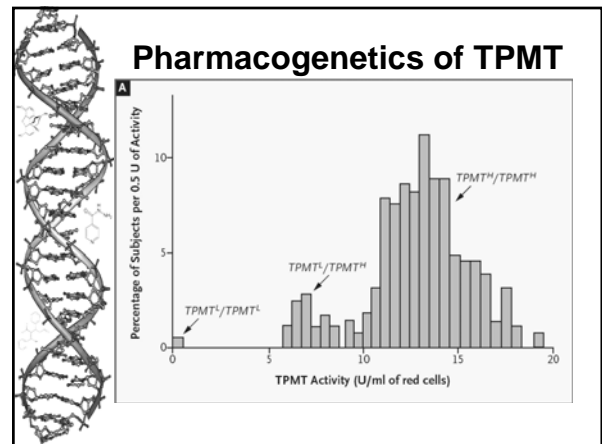
Other Enzyme Polymorphisms

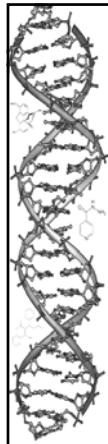
- Serum cholinesterase
 - Activity of serum cholinesterase (pseudocholinesterase) is reduced in some people (1/25000)
 - Administration of succinylcholine causes paralysis of breathing muscles



Other Enzyme Polymorphisms

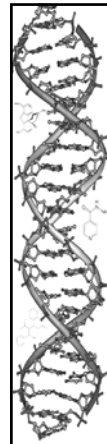
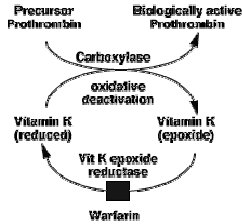
- Thiopurine S-methyltransferase (TPMT)
- Drugs:
 - 6-mercaptopurine
 - azathiopurine
- Diseases:
 - Acute lymphoblastic leukemia
 - Inflammatory bowel disease
- Toxicity:
 - Fatal myelosuppression
 - Hematopoietic toxicity





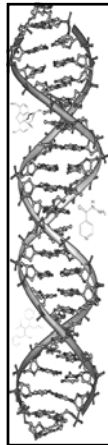
Other Enzyme Polymorphisms Vitamin K epoxide reductase

- Polymorphisms in the *vitamin K epoxide reductase complex (VKORC1)* are the major contributor to warfarin dose variability (30%)
- Some mutations make VKORC1 less susceptible to suppression by warfarin



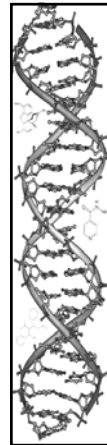
Other Enzyme Polymorphisms Vitamin K epoxide reductase

- Two main haplotypes: low-dose haplotype group (A) and a high-dose haplotype group B
- Group A *VKORC1* polymorphisms lead to a more rapid achievement of a therapeutic INR, but also a shorter time to reach an INR over 4, which is associated with bleeding
- African-Americans less sensitive
- Asians more sensitive



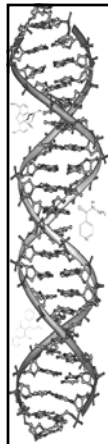
Drug Receptor Polymorphisms

- B-adrenergic receptors**
- $\beta 1$ receptor gene
 - Some SNPs result in reduced sensitivity to beta-blockers, others have increased sensitivity to beta-blockers
- $\beta 2$ receptor gene
 - Some SNPs lead to strong resistance to beta 2 agonists
 - One SNP appears to be associated with response to antidepressants



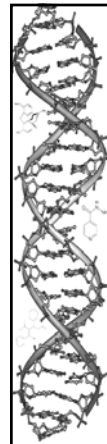
Polymorphism in Serotonin Transporter

- Allelic variation in 5HT Transporter function (5HTTLPR polymorphism)
- 3 genotypes: *ss*, *sl*, *ll*
- Functional polymorphism ?
- May lead to
 - Increased susceptibility to anxious and depressive features
 - Less favourable antidepressant response in patients affected by mood disorders



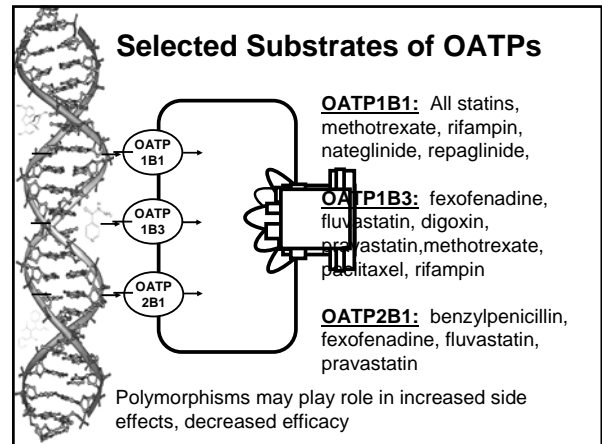
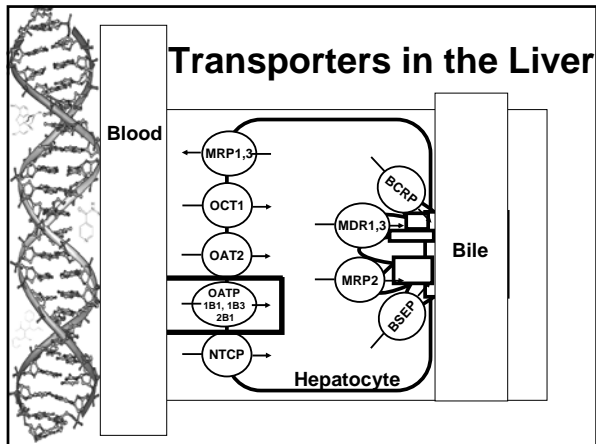
Polymorphism in Serotonin Transporter

- Polymorphism:
- Might be related to affective disorders
 - May affect treatment response to antidepressants, lithium
 - Efficacy
 - Tolerability
 - Time to benefit
 - May explain differences in antidepressant-induced mania in some pts



Polymorphism in Drug Transporters

- PGP: P-glycoprotein, ATP dependent efflux transporter**
 - MDR: Gene family that codes for PGPs, multidrug resistance genes (MDR1 only)
 - ABC transporter (ATP binding cassette)
- OATP: Organic Anion Transport Protein**
- OCTP: Organic Cation Transport Protein**
- OATP and OCTP are influx transporters

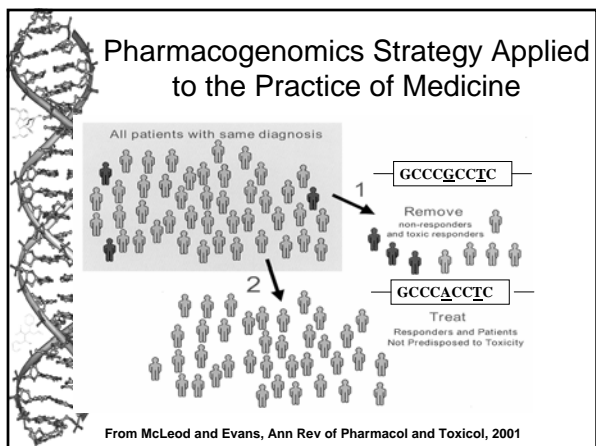


Psychopharmacogenetics

- Various dopamine receptor / transporter polymorphisms and response to antipsychotics, potential for TD, etc
- Polymorphisms in Serotonin receptors / transporters and depression / anxiety risk, treatment outcomes, etc
- Polymorphisms in nicotinic (ACh) receptors and nicotine dependence, etc

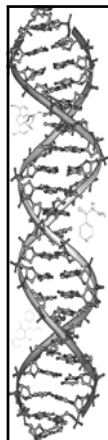
What is the Goal of Pharmacogenetic Studies

- Identify and categorize the genetic factors that underlie the differences and apply this in clinical practice
- Rational, individual therapy
- Screening for those patients who carry the genes which place them at risk in case of certain therapies
- Discovering which drugs are potentially dangerous for carriers of a given polymorphism
- Establishing the frequency of pharmacogenetic phenotypes



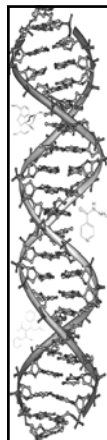
What are the barriers to the implementation of pharmacogenomic testing?

- Cost of the testing
- Lack of Third Party payment
- Interpretation of results
- Privacy / Anonymity



What is the cost of the test?

- Wide range of prices and “packages”
- Current range for 2D6 testing is about \$300 to \$600 dollars with some labs charging more.
- A key consideration is that this is a one time cost that will inform the use of 70 drugs



Is interpretation difficult?

- It is fairly straightforward to learn how to interpret a 2D6 result in order to guide clinical decision making
- Howeverit will be very challenging to integrate the output of genotyping from multiple genes particularly in patients taking multiple drugs