Implementing Pharmacogenetic Testing Into Clinical Laboratories

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Key learning objectives

- Recognize decisions in implementing pharmacogenetic testing
- Learn about strengths and limitations of CYP genotyping
- Appreciate interpretive challenges associated with pharmacogenomic results

Overview

- How to Decide
  - Tests to offer
  - Platforms
- How to interpret
  - Complex genotypes
  - Complex phenotypes
- What to tell the health care provider
  - Report contents

Decisions: Tests to offer

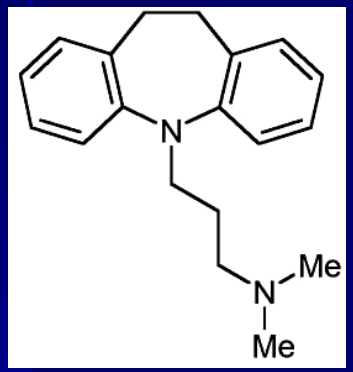
- Clinical validity (mainly rely on published studies)
- Clinical utility (Is it useful?)

Case presentation

- 7 yr old boy
- Treated successfully for severe ADD with **imipramine** (Tofranil), 150 mg given at night
- Complained of thirst and then collapsed suddenly at school
- Resuscitation unsuccessful
- Autopsy negative
- Toxicology…

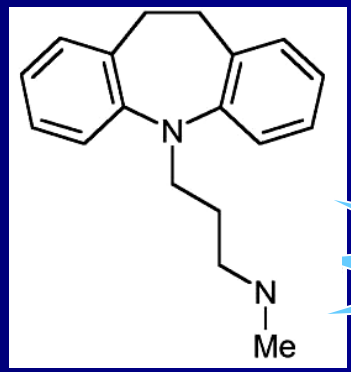
Metabolism of imipramine

imipramine



CYP3A4

desipramine



CYP2D6

Inactive Metabolites

CYP2D6

Inactive Metabolites

Imipramine/desipramine toxicity

- Early S/S are mild and anticholinergic
- Serious S/S include hypotension, coma, arrhythmias, cardiorespiratory arrest
- Progression from mild to life-threatening S/S may be rapid
- Poor metabolizers (CYP2D6) are at risk for toxicity with standard doses

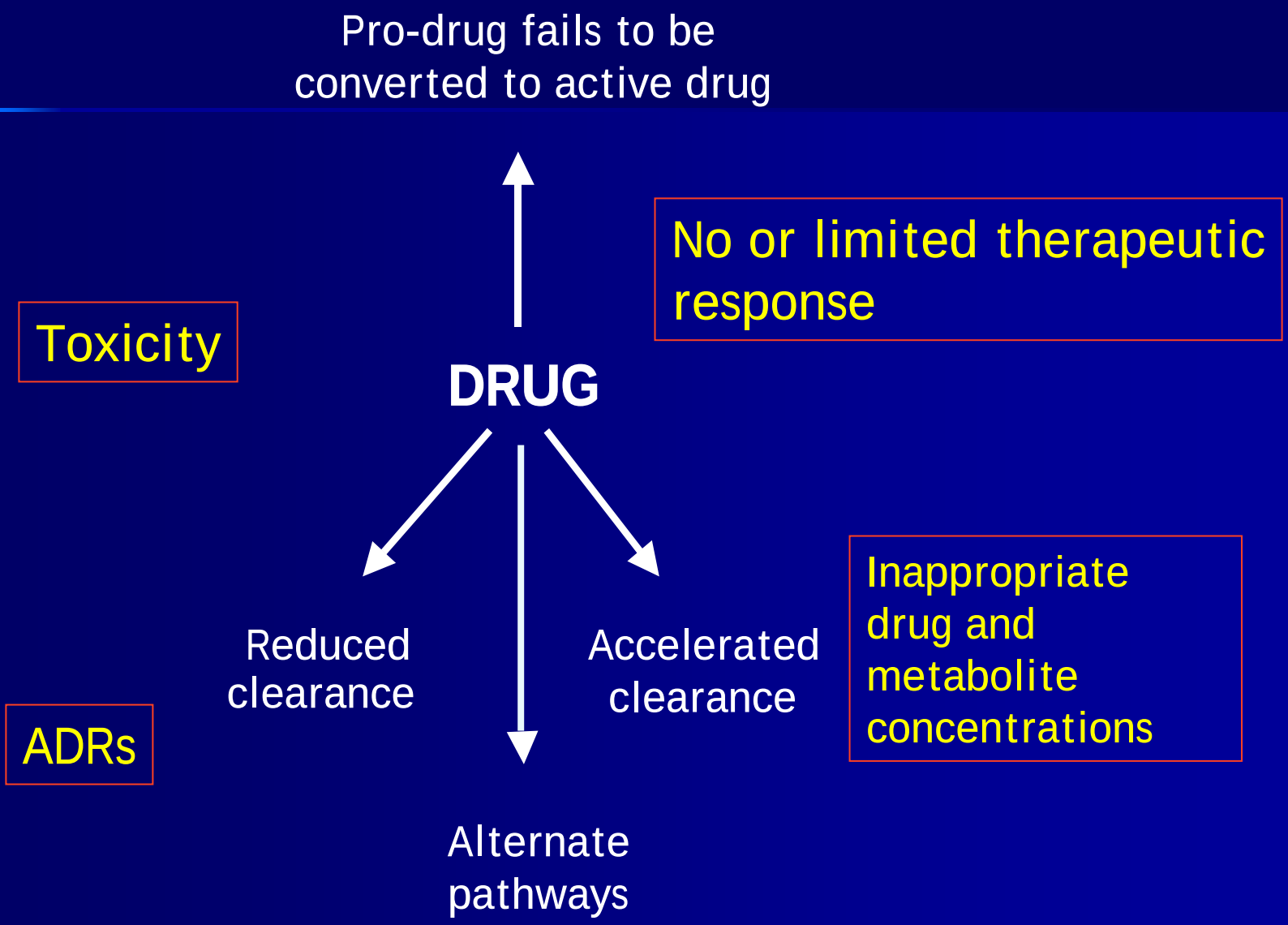
Case presentation (cont)

	Imipramine	Desipramine	Total	Desipramine / Imipramine Ratio
Therapeutic			**150-300 ng/mL**	**1-2**
Serum (ng/mL) (15 min after collapse)	69	942	1,011	13.7
Cardiac blood (ng/mL)	1,100	13,000	14,100	11.8
Femoral blood (ng/mL)	470	6,000	6,470	12.8
Liver (mg/g)	35,000	449,000	484,000	12.8

Utility of pharmacogenomics

- Select the best drug, at the best dose
  - narrow therapeutic index
  - narrow opportunity for efficacy
  - known population of non-responders
  - long period of time required to assess efficacy
  - drugs with well understood phenotype-genotype relationships
- Investigate adverse drug reactions (ADRs)

Consequences of variant metabolism



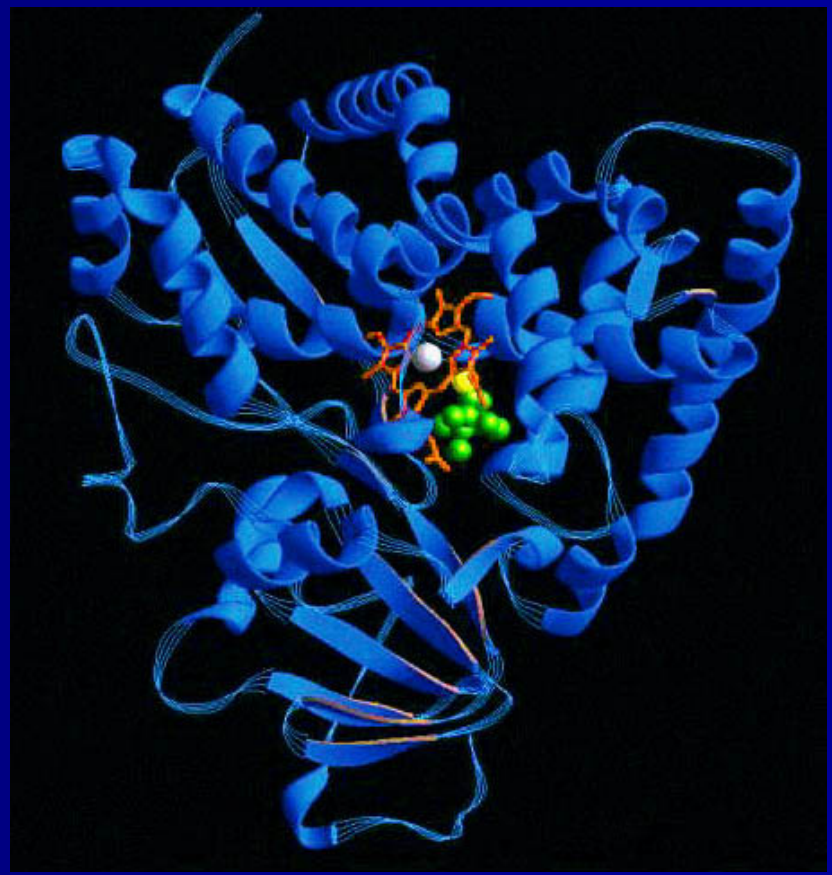
Pharmacogenetics “usefulness”

- Avoid drugs not likely to work
- Predict patients’ responses (or lack of) to medication and propensity to develop side effects
- Optimize drug dosage to avoid excessive or suboptimal concentration
- Identify genetic variants contributing to variability in drug response.

Cytochrome P450s (CYPs)

Genetic variants are associated with altered drug levels, but not with disease

- CYP2D6: estimated to metabolize 25% of all prescribed drugs
- CYP2C9: 5%
- CYP2C19: 15%
- CYP3A4/5: 50%



Examples of drugs metabolized by

CYP2D6

- Antiarrhythmics
- Antipsychotics
- Antidepressants
- Opiates
- Selective serotonin reuptake inhibitor (SSRI)

CYP2C9

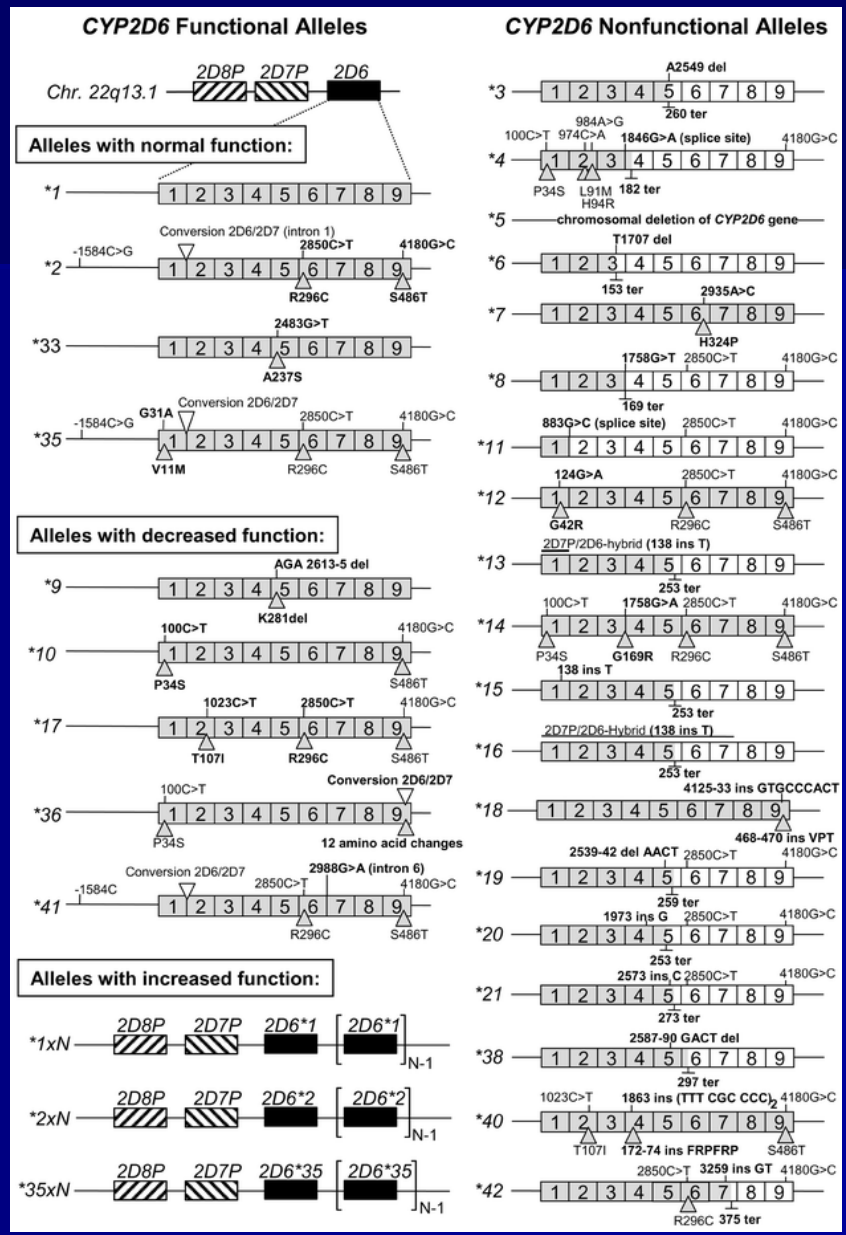
- Anticonvulsants
- Antidepressants
- Hypoglycemics
- Anticoagulants
- Antibacterial
- Cancer Chemotherapy
- Nonsteroidal anti-inflammatory drugs

CYP2C19

- Anticonvulsants
- Antidepressants
- Anti-ulcer
- Antimalaria
- Cancer Chemotherapy
- Proton pump inhibitors

Alleles of *CYP2D6*

- More than 80 known
  - SNPs
  - INDELs
  - Complete gene deletion or duplications
- Clinical significance of most unclear



Zanger UM,et al. Naunyn Schmiedebergs Arch Pharmacol 2004

CYP2C9- CYP2C19 most common clinically relevant variants

	CYP2C9	CYP2C19
Functional	*1	*1
Decreased Function	*2 *3*4*5	*8 *9
Non Functional	*6	*2 *3 *4 *5 *6

Decisions: Platforms

- Test Complexity
  - Number of sequence variants
  - Repeats (UGT1A1)
  - Single base changes/deletions/insertions
  - Full gene deletions/duplications
  - Full gene analysis
  - Haplotypes vs. genotypes
    - Molecular haplotyping?

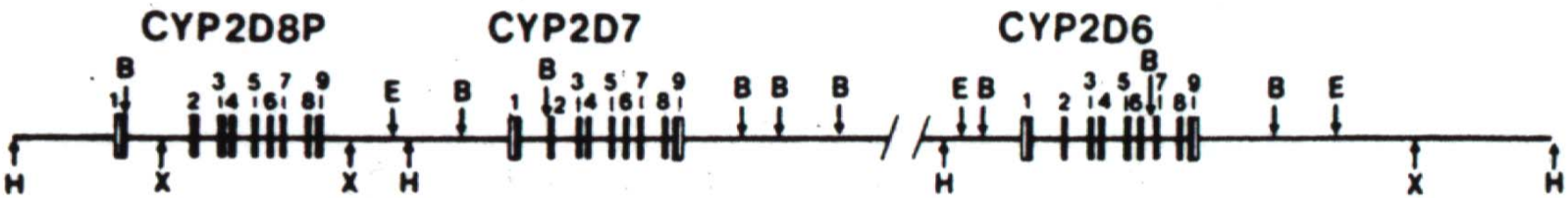
IVD, ASR or laboratory developed

- FDA cleared IVDs
  - Cytochrome p450 2D6
  - Cytochrome p450 2C19
  - UGT1A1
- Other tests with clinical interest
  - Cytochrome p450 2C9
  - VKORC1 for warfarin sensitivity
  - MTHFR for methotrexate sensitivity
  - TPMT for azathioprine sensitivity
  - NAT2
  - Many others

Gene patents

- Patent
  - the mutation (cyp2D6 *4)
  - the use of the mutation (UGT1A1, TPMT)
  - the method
- License from
  - Negotiating with the license holder
  - Using ASRs/IVDs that manufacturer has a licensing agreement

CYPs: Analytical challenges



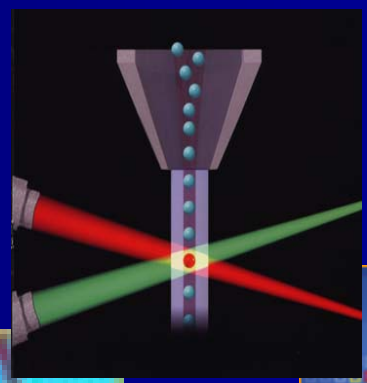
Lancet 1990; 336: 529-32

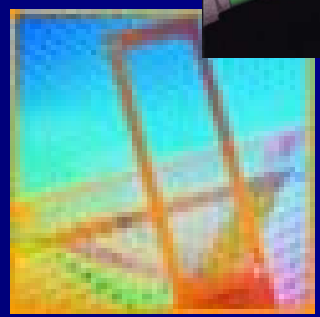
- Superfamily/Pseudogenes:
  - 57 genes, 33 pseudogenes
- Gene dose important
- Many SNPs are common among alleles
- Many rare variants
- Importance of haplotypes not known

Commercial CYP genotyping product examples

- Roche Light Cycler Assays
- Tm Bioscience: 3 genes, 26 variants
- Roche AmpliChip: 2 genes, 29 variants
- GE CodeLink: 9 genes, 113 variants

- Third Wave Invader: 1 gene
- Gentris: 2 genes

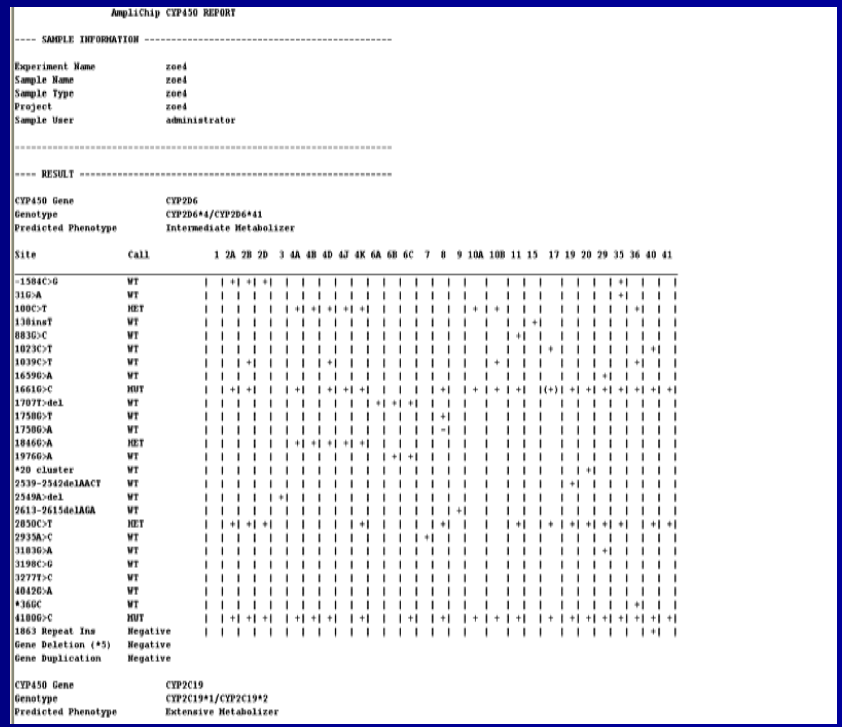




- AutoGenomics
- Biotage
- Genelex
- Genaissance
- Nanogen
- Nanosphere Verigene

AmpliChip

- Solid support microarray
- FDA approved for 2D6,2C9
- 2 genes
  - 2D6, 27
  - 2C19, 2 alleles
- Deletion
- Duplication
  - Allele with duplication



AmpliChip CYP450 REPORT

---- SAMPLE INFORMATION ----

Experiment Name	zoe4
Sample Name	zoe4
Sample Type	zoe4
Project	zoe4
Sample User	administrator

---- RESULT ----

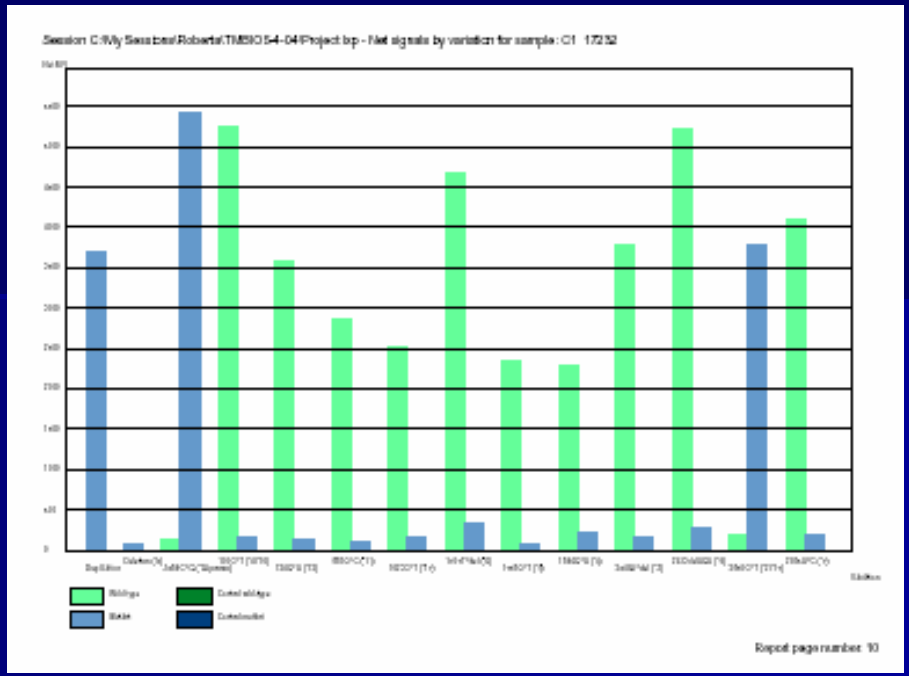
CYP450 Gene	CYP2D6
Genotype	CYP2D6*4/CYP2D6*41
Predicted Phenotype	Intermediate Metabolizer

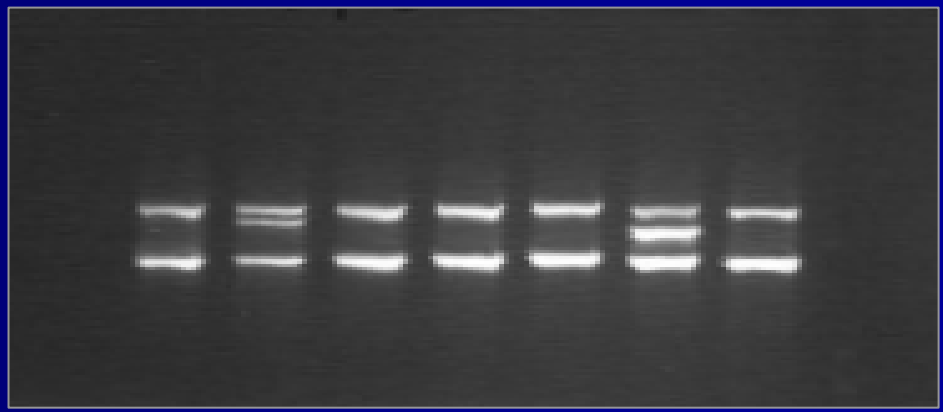
Site	Call	1	2A	2B	2D	3	4A	4B	4D	4J	4K	6A	6B	6C	7	8	9	10A	10B	11	15	17	19	20	29	35	36	40	41
-1584C>G	WT	|	+|	+|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|
31G>A	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|
100C>T	HET	|	|	|	|	|	+|	+|	+|	+|	+|	|	|	|	|	|	|	+	+	|	|	|	|	|	|	+|	|	|
138insT	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|	|
883G>C	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|	|
1023C>T	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+	|	|	|	|	|	|	+|	|
1039C>T	WT	|	|	+|	|	|	|	+|	|	|	|	|	|	|	|	|	|	+	|	|	|	|	|	|	+|	|	|
1659G>A	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
1661G>C	MUT	|	+|	+|	|	+|	|	+|	+|	+|	|	|	|	+|	|	+	+	|	+|	|	(+)|	+|	+|	+|	+|	+|	+|
1707T>del	WT	|	|	|	|	|	|	|	|	|	+|	+|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
1758G>T	WT	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|
1758G>A	WT	|	|	|	|	|	|	|	|	|	|	|	|	-|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
1846G>A	HET	|	|	|	|	|	+|	+|	+|	+|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
1976G>A	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	+|	|	|	|	|	|	|	|	|	|	|	|	|
*20 cluster	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|
2539-2542delAACT	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|
2549A>del	WT	|	|	|	|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
2613-2615delAGA	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|
2850C>T	HET	|	+|	+|	+|	|	|	|	|	+|	|	|	|	|	+|	|	|	+|	|	+|	|	+|	+|	|	+|	+|
2935A>C	WT	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
31836A	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|	|	|	|
3198C>G	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
3277T>C	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
4042G>A	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
*36GC	WT	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|
41800>C	MUT	|	+|	+|	+|	|	+|	+|	+|	|	+|	|	+|	|	+	|	+|	|	+	|	+|	+|	+|	+|	+|	+|	+|
1863 Repeat Ins	Negative	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	+|	|	|
Gene Deletion (*5)	Negative																														
Gene Duplication	Negative																														

CYP450 Gene	CYP2C19
Genotype	CYP2C19*1/CYP2C19*2
Predicted Phenotype	Extensive Metabolizer

Luminex Tag-It

- Three genes
  - 26 variants
- 2D6
  - 12 alleles
    - Deletion
  - Duplication (determine allele?)
- 2C9
  - 5 alleles
- 2C19
  - 7 alleles





Analytical validation

- Limited validation for FDA approved tests
- Validation for laboratory developed tests
  - Accuracy - compare to independent analysis laboratory and/or method
  - Reproducibility – within run/between run
  - Compare to published allele frequencies

Frequency of variant CYPs

Gene	Allele	Enzyme activity	Caucasians		Asians		SouthEast Asian	African American		Middle East	
CYP2C9	*2	deficient	8-13%	17%			0.50%	1-4%			5%
	*3	deficient	6-10%	7%	1.7-5%	7.5%	10%	0.5-1.5%	5%		5%
CYP2C19	*2	deficient	13%	16%	32%	32.5%	20%	17%	15%		20%
	*3	deficient			6-10%	10%	5%				
CYP2D6	*3	deficient	2%	0.5%				2%			
	*4	deficient	12-21%	19.5%				1-9%	5%	3.5%	20%
	*5	deficient	4-6%	2%	5-13%	5%	10%	4-6%	5%	1%	0%
	*10	decreased	3%	1.5%	35-50%	48%	50%	6-10%	5%	3%	15%
	*17	decreased						34%	35%	3%	5%
	Dup	increased	1-4%	1.5%	1-2%	1.6%		0.13	5%		5%

Interpretation: CYP phenotypes

Poor Metabolizer (PM): gene absent or nonfunctional

Intermediate Metabolizer (IM)

Extensive (normal) Metabolizer (EM)

Ultra-rapid Metabolizer (UM):
multiple copies of functional genes

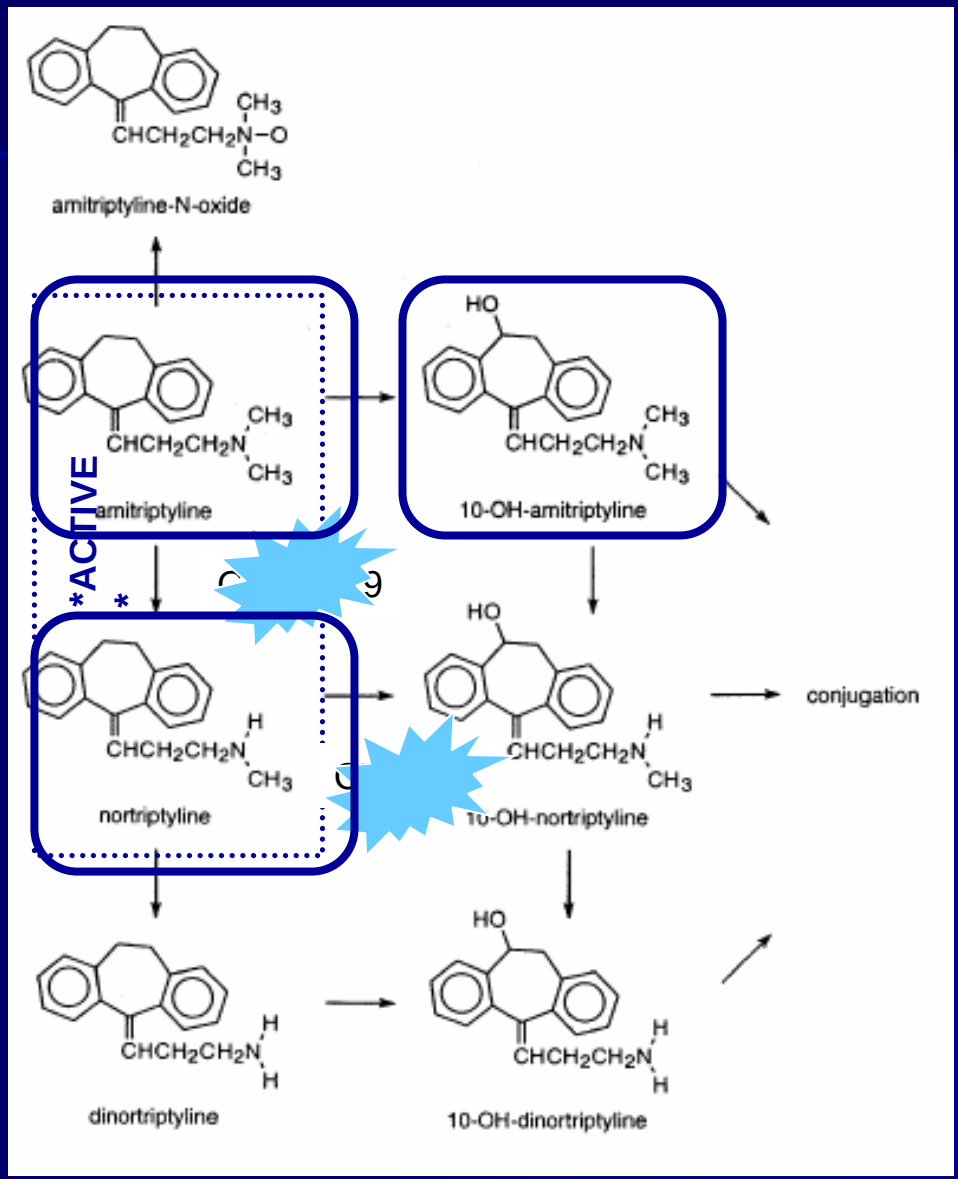
Predicting phenotypes from genotypes

Alleles	Predicted phenotype
F/F	EM
F/DF	EM
F/NF	EM
DF/DF	IM
DF/NF	PM
NF/NF	PM
DUP-F	UM

Example: Antidepressants and CYPs

Brand Name	*Generic Name*	*Mechanism*	*CYP Metabolism*
Effexor	Venlafaxine	*mixed*	*CYP3A4, CYP2D6, CYP2C9, CYP2C19*
Wellbutrin	Bupropion HCL	*mixed*	*CYP2B6, CYP2D6, CYP1A2, CYP3A4 +*
Desyrel	Trazodone	*mixed*	*CYP3A4, CYP2D6*
Zoloft	Sertraline	*SSRI*	*CYP3A4, CYP2D6, CYP2C9, CYP2C19*
Prozac	Fluoxetine	*SSRI*	*CYP2B6, CYP2C9, CYP2C19, CYP3A4+*
Celexa	Citalopram	*SSRI*	*CYP3A4, CYP2C19, CYP2D6*
Lexapro	Escitalopram	*SSRI*	*CYP3A4, CYP2C19*
Elavil	Amitriptyline	*TCA*	*CYP3A4, CYP2D6, CYP2C19, CYP2B6+*
Norpramin	Desipramine	*TCA*	*CYP2D6, CYP1A2*
Sinequan	Doxepine	*TCA*	*CYP2D6, CYP1A2, CYP3A4*
Pamelor	Nortriptyline	*TCA*	*CYP1A2, CYP2D6, CYP2C19, CYP3A4*
Anafranil	Clomipramine	*TCA*	*CYP2D6, CYP1A2, CYP2C19, CYP3A4*

Example 1: Amitriptyline

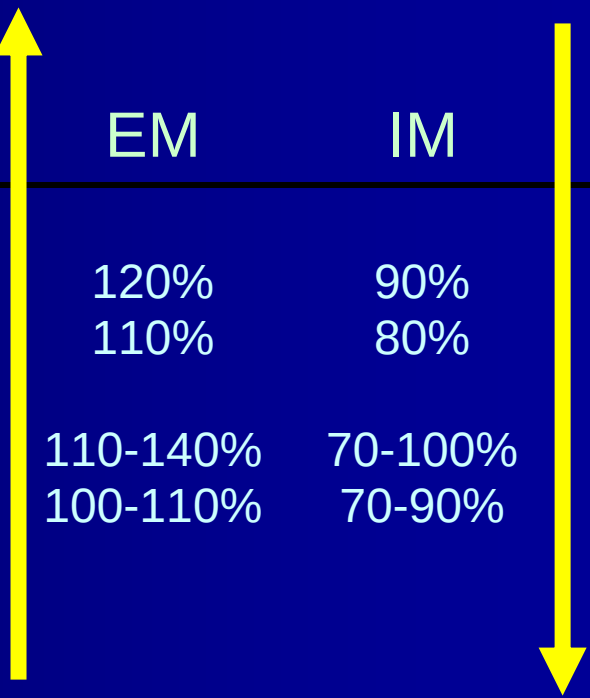


Baselt, 2004

Genotype-based dosing

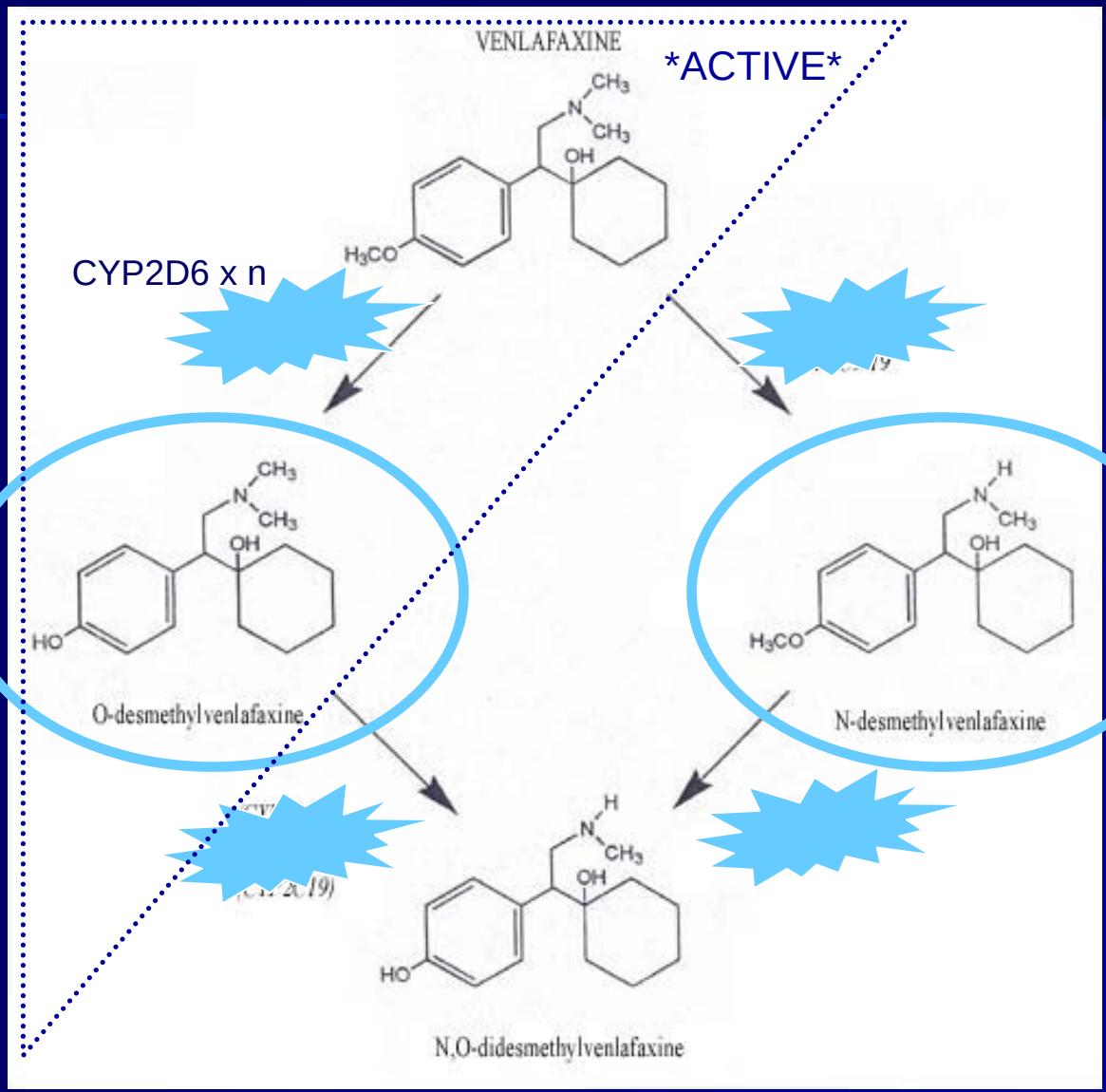
for drugs that are *inactivated* by the affected enzyme

	Usual Dose		UM	EM	IM	PM
Amitriptyline Nortriptyline	150 mg	CYP2D6 CYP2C19	230% N/A	120% 110%	90% 80%	50% 60%
Others		CYP2D6 CYP2C19	230-300% N/A	110-140% 100-110%	70-100% 70-90%	20-90% 40-70%



Kirchheiner J et al. Acta Psychiatr Scand, 104, 2001

Example 2: Venlafaxine

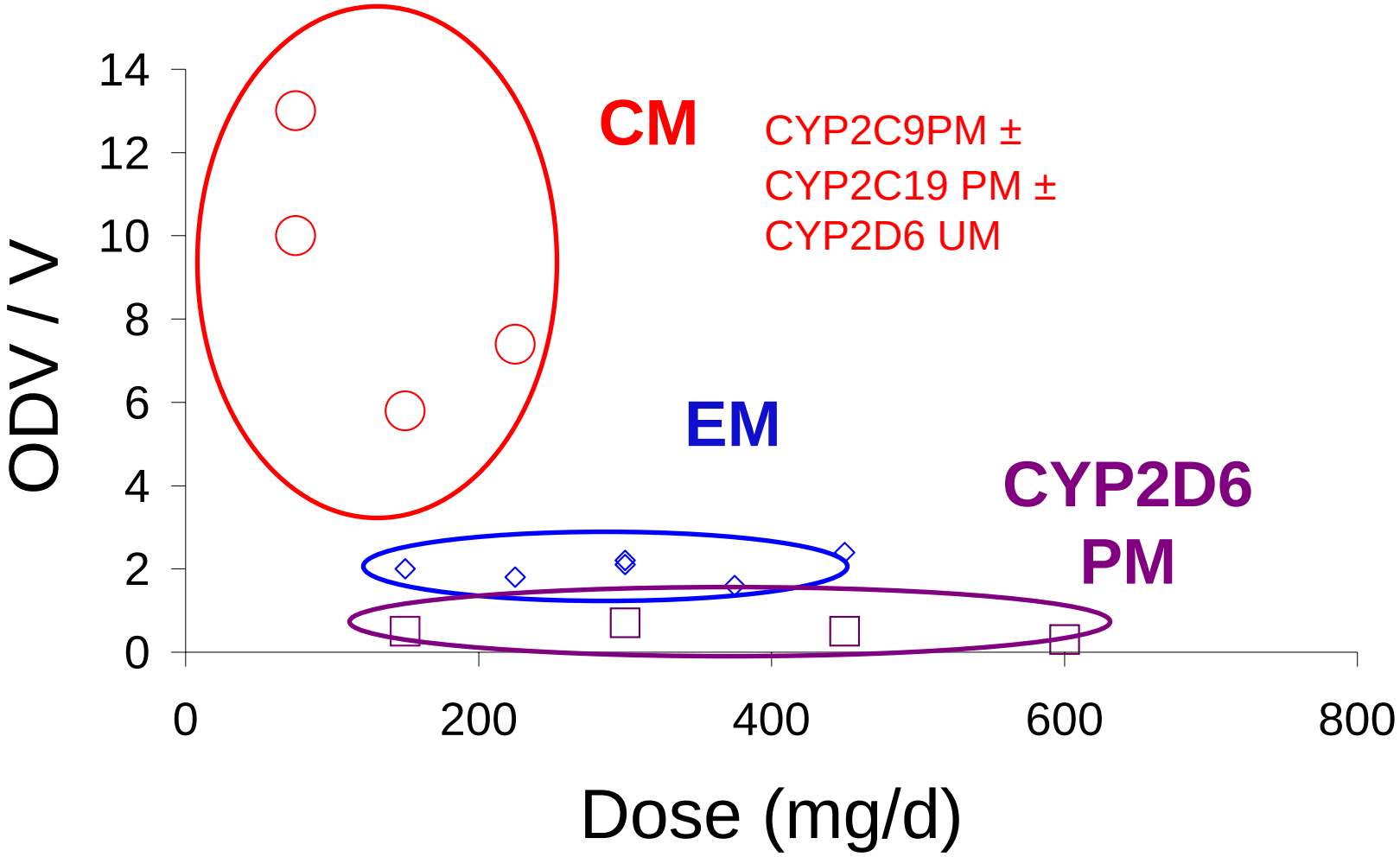


Lessard et al. 2001
J Clin Psychopharmacol

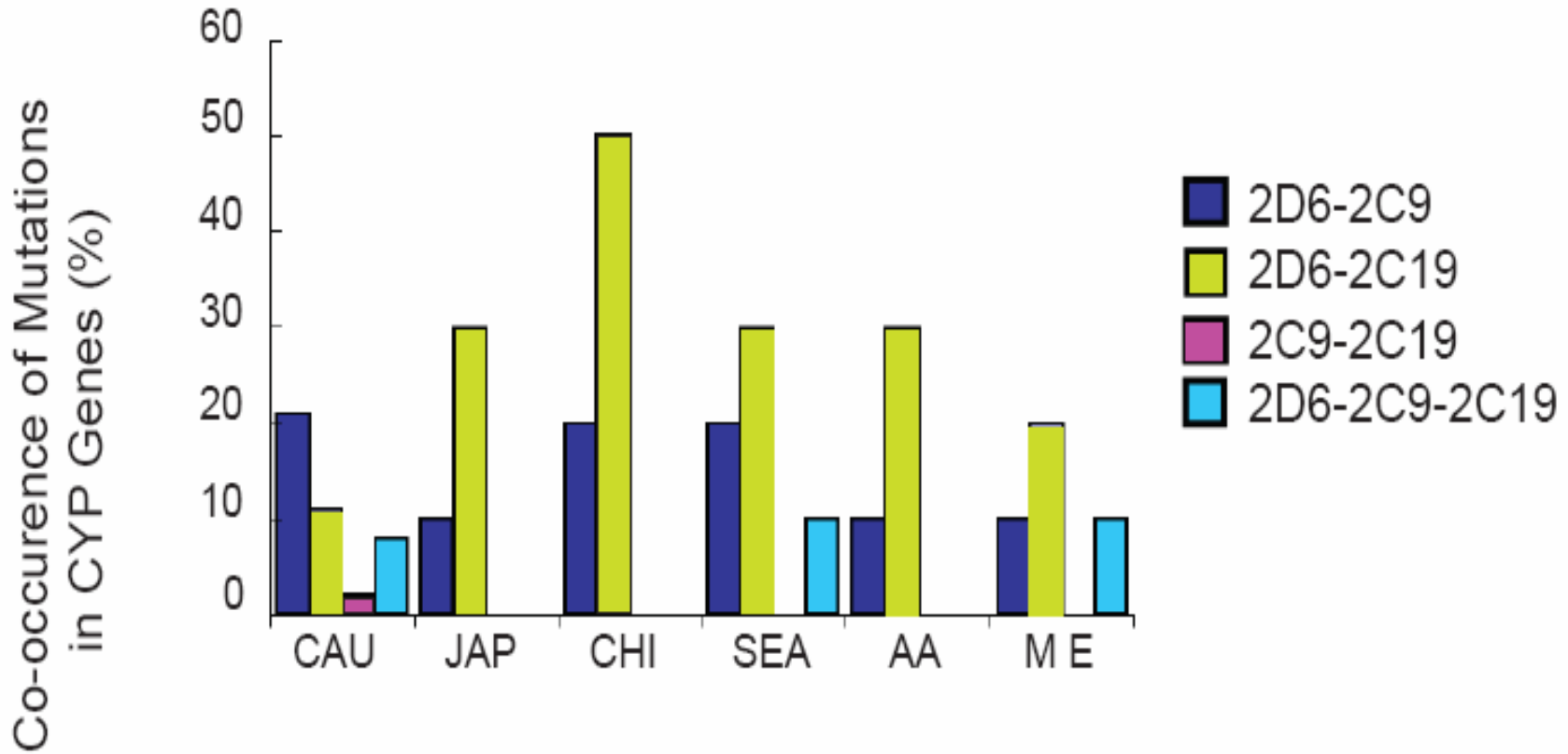
CYP effects on venlafaxine (V)

- CYP2D6
  - Converts V to active metabolite (ODV)
  - PM to have longer V $t_{1/2}$ and less active drug
  - UM have shorter V $t_{1/2}$ and more active drug
  - PM requires higher doses than EM
  - UM requires lower doses
- CYP2C9 or CYP2C19
  - Prevent V inactivation
  - PMs accumulate V and ODV
  - More active drug and higher ratio of ODV/V (complex phenotype)
  - PM may require lower doses than EM

Venlafaxine phenotypes: Parent/metabolite ratio



Co-occurrence of mutations in 2D6, 2C9, 2C19



Interpretation complexity

- Non-genetic factors
  - Diet, multiple drugs, nutraceutics
- Multiple genes (superfamily)
- Multiple pathways
- Alleles called based on presumed haplotypes
  - *4A (1846G>A; 100C>T)
  - *4K (1846G>A; 100C>T;2850C>T)
  - 2850C>T present in many alleles

Reporting: Information to the clinician

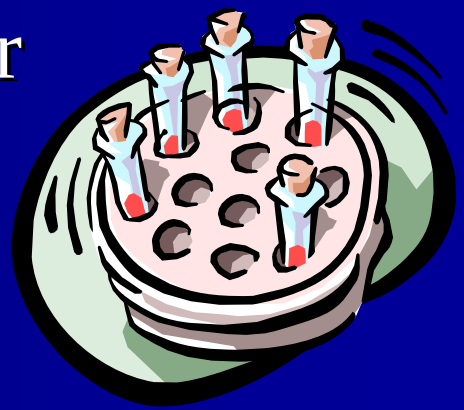
- Report alleles
  - (i.e. *3,*4)
- Include nucleotides
  - (i.e. 2549A>del,1846G>A/100C>T)
- Predicted phenotype
  - PM, IM, EM, UM
- Duplication
  - Functional alleles
  - Non-functional alleles
- Combined reports (2D6,2C9, 2C19)?

Reports

- Dosing adjustments not included in the report
  - Depends on medication or combination of medicines
- Recommend counseling with a clinical pharmacist

No pharmacogenomic test will replace the need to

- Monitor the patient clinically for response
- Monitor drug and/or metabolite levels, other biomarkers



Likely future directions

- Other genes
- More FDA-approved kits
- Application-based panels
- Additional “molecular autopsies”
- Changes to drug labeling
- More genotype-to-phenotype discovery of targets

Many thanks

Roberta Melis, Ph.D.

- Research scientist

- Cindy Meadows, B.S., CLSp(MB)
  - Clinical supervisor

- Jamie Williams, B.S., CLSp(MB)
  - Clinical technical specialist