Advanced Pain Management – Pharmacogenomics Data to Complement Oral Fluid Compliance Testing

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Learning Objectives

- **Utilizing oral fluid as an alternative sample matrix**
- **Application of pharmacogenomics data in toxicology**
- **Implications of polypharmacy**
- **Integrating complementary data from compliance and pharmacogenomic testing**

Oral Fluid in Toxicology

- Cutoff levels in oral fluid are typically 10x lower compared with urine
  - Low ng/mL for many compounds
- Multi-analyte panels often include the following:
  - 6-monoacetylmorphine (6-MAM)
  - Amphetamines
  - Benzodiazepines
  - Cannabinoids
  - Cocaine
  - Opioids

J. Anal. Toxicol. 2009. 33(9):569-77.
Clin. Chem. 2009. 55:11, 1910-1931.

Advantages of Oral Fluid in Toxicology

- Simple, minimally invasive collection
- Collection may be observed
- Oral fluid is an ultra-filtrate of circulating blood
- Drug levels may be reflective of blood levels
- Provides an amenable medium for laboratory testing
- Conventional screening and confirmation methods are applicable

https://www.ncjrs.gov/pdffiles1/nij/grants/203569.pdf

Factors of Drug Concentrations in Oral Fluid

- Drug pKa
- Physical size of molecules
- Protein-binding
- pH of saliva
- Oral fluid production ~1000 mL/day

https://www.ncjrs.gov/pdffiles1/nij/grants/203569.pdf

Estimating Drug Concentrations in Oral Fluid

- S = concentration of drug in saliva
- P = concentration of drug in plasma
- pKa = pKa of drug
- pHs = pH of saliva
- pHp = pH of plasma
- fp=free (unbound) fraction of drug in plasma
- fs=free (unbound) fraction of drug in saliva

$$Acidic\ Drugs: \frac{S}{P} = \frac{1 + 10^{(pHs - pKa)} x f_p}{1 + 10^{(pHp - pKa)} x f_s}$$

$$Basic\ Drugs: \frac{S}{P} = \frac{1 + 10^{(pKa - pHs)} x f_p}{1 + 10^{(pKa - pHs)} x f_s}$$

https://www.ncjrs.gov/pdffiles1/nij/grants/203569.pdf

Collection of Oral Fluid

- Stimulated: may affect pH and deposition of drug into saliva or interfere with immunoassay
  - Chewing rubber bands, gum
  - Hard candy
- Unstimulated still has potential of oral contamination from drug administration
- Rinsing the oral fluid cavity has been suggested

https://www.ncjrs.gov/pdffiles1/nij/grants/203569.pdf

Sample Processing of Oral Fluid Specimens

- Sample collection: swab or neat saliva
- Sample preparation by extraction
- For example, liquid-liquid extraction
  - 1:4 sample+internal standard:hexane/ethyl acetate, followed by dry-down of organic layer
  - Reconstitute residue with mobile phase
- For example, solid phase extraction (SPE)
  - Mix sample with internal standard and phosphate buffer
  - Extract over SPE column; bind, wash, elute
  - Reconstitute residue with mobile phase
- Provides a 40+ drug and metabolite confirmatory analysis by liquid chromatography tandem mass spectrometry

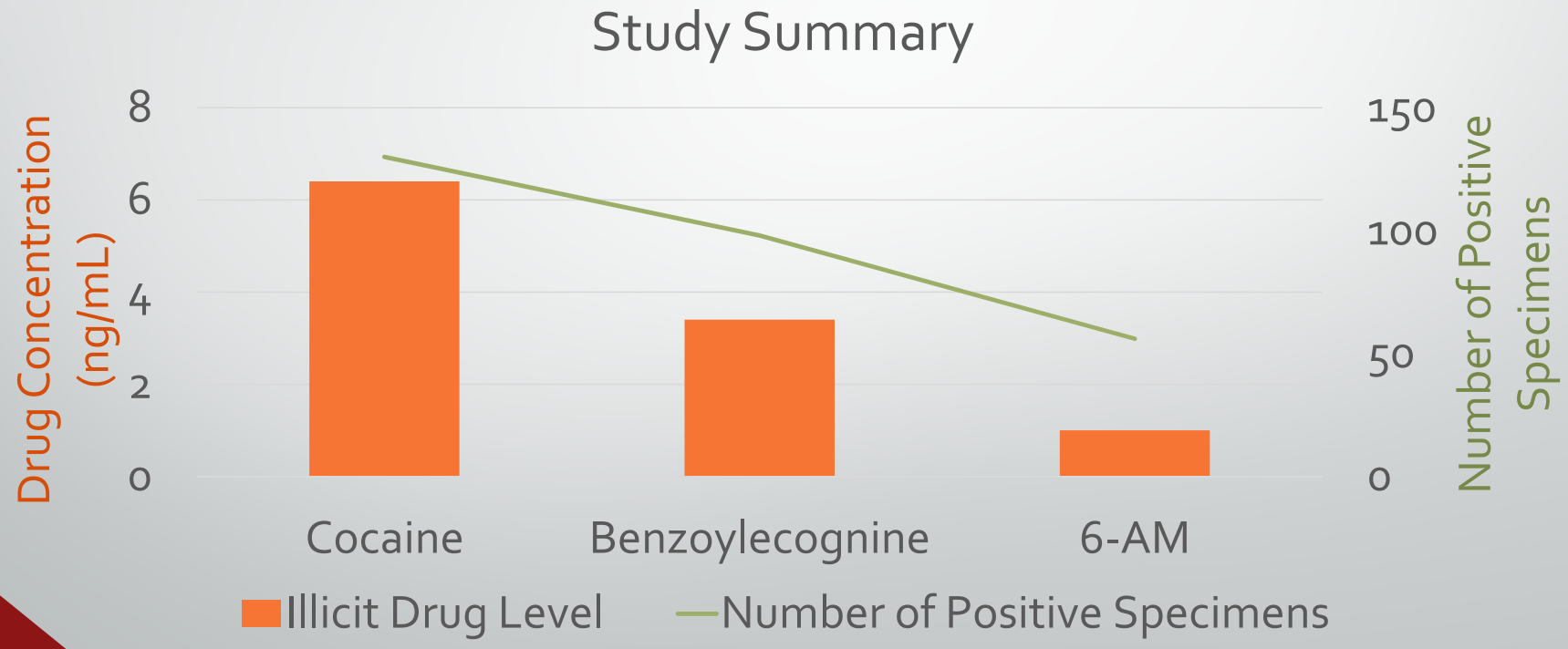
J. Anal. Tox. 2012, 36:75–80.

Application of Oral Fluid Testing for Substance Abuse Monitoring

- Disposition of illicit drugs in oral fluid requires low assay limits of detection
- Drug and metabolite cutoff levels in the study
  - Morphine/codeine: 40 ng/mL
  - 6-monoacetylmorphine: 4 ng/mL
  - Cocaine/benzoylecgonine: 8 ng/mL
- Over a 17 +/-5.8 week study, there were 28 opiate positive and 50 cocaine positive specimens from patients undergoing methadone treatment

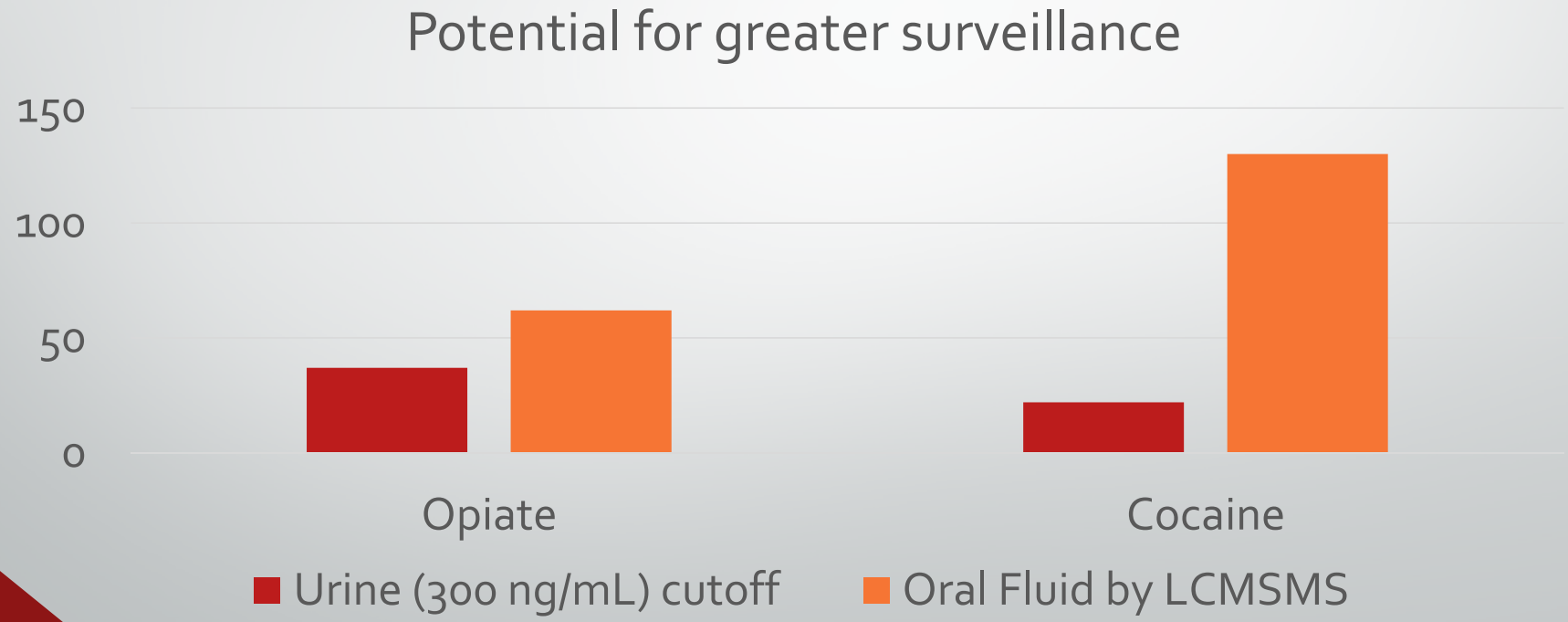
Drug Alcohol Depend. 2007, 87(2-3): 258–267.

Application of Oral Fluid Testing for Substance Abuse Monitoring



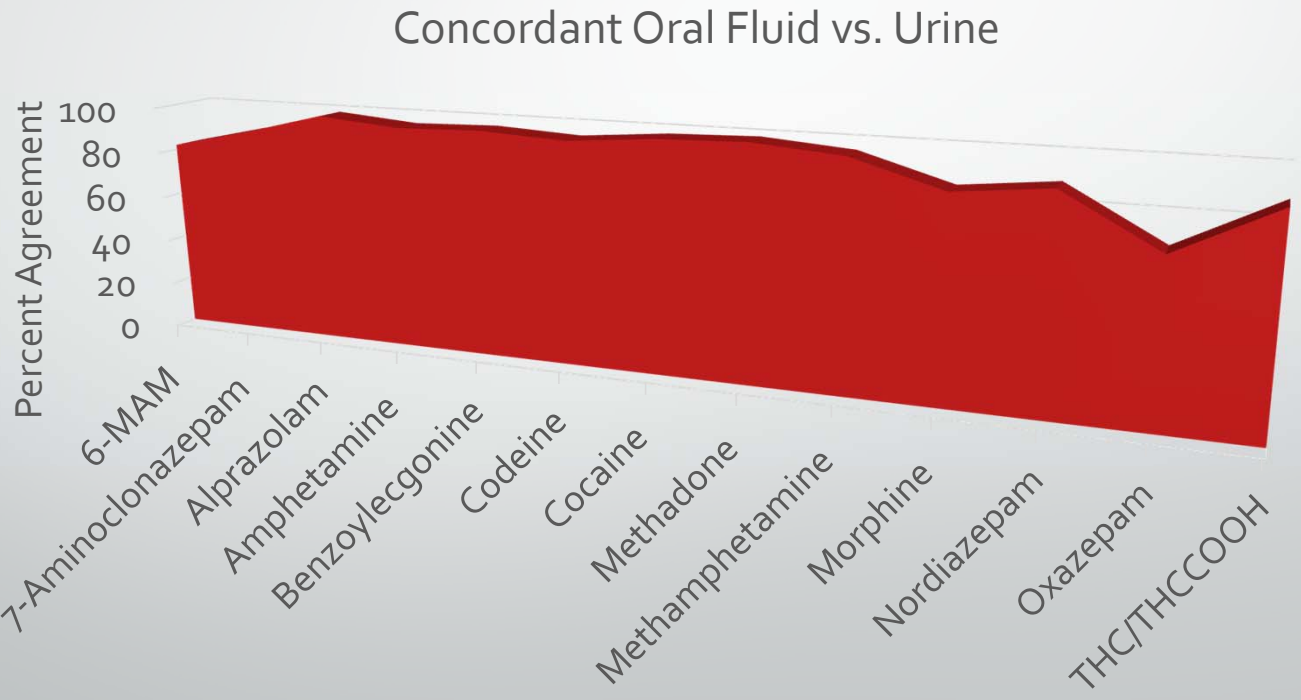
Drug Alcohol Depend. 2007, 87(2-3): 258–267.

Application of Oral Fluid Testing for Substance Abuse Monitoring



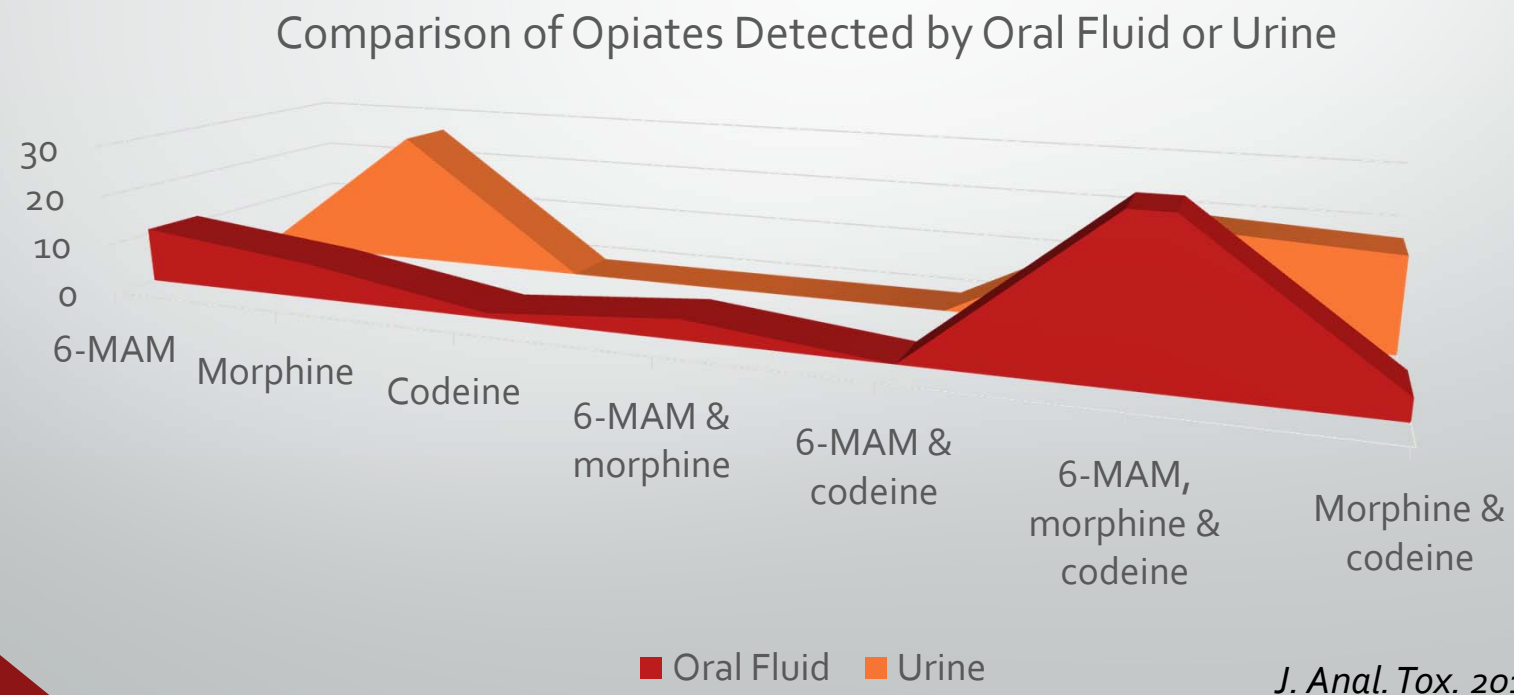
Drug Alcohol Depend. 2007, 87(2-3): 258–267.

Comparison of Oral Fluid Confirmation with Urine Confirmation



J. Anal. Tox. 2011, 35: 32-39.

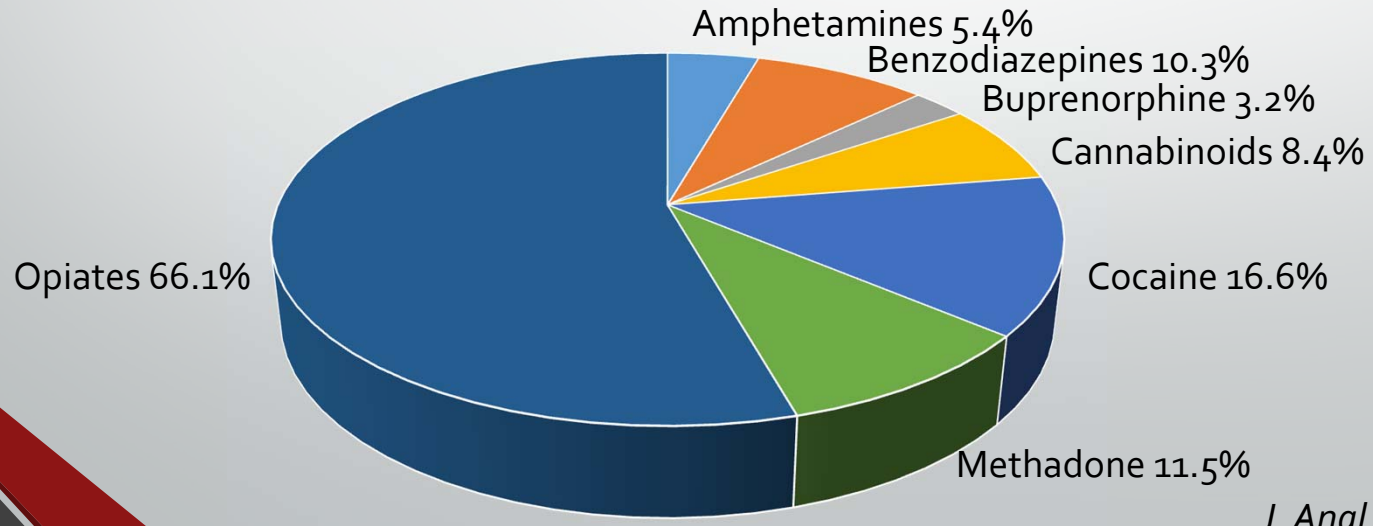
Presence of 6-Monoacetylmorphine in Oral Fluid and Comparison with Urine



J. Anal. Tox. 2011, 35: 32-39.

Cross Section of Positive Oral Fluid Specimens

- Specimens were collected over a ~28 month period
- Donors were from legal, treatment or workplace (5.5%) settings



J. Anal. Toxicol. 2007, 31(8):424-33.

Oral Fluid Phenotyping of CYP3A with Midazolam

- Midazolam and hydroxy midazolam metabolites may be quantified in saliva with correlation to plasma
- Enables phenotyping of CYP3A by monitoring elimination rate
- Rifampicin induction of CYP3A shows decreased concentrations of midazolam

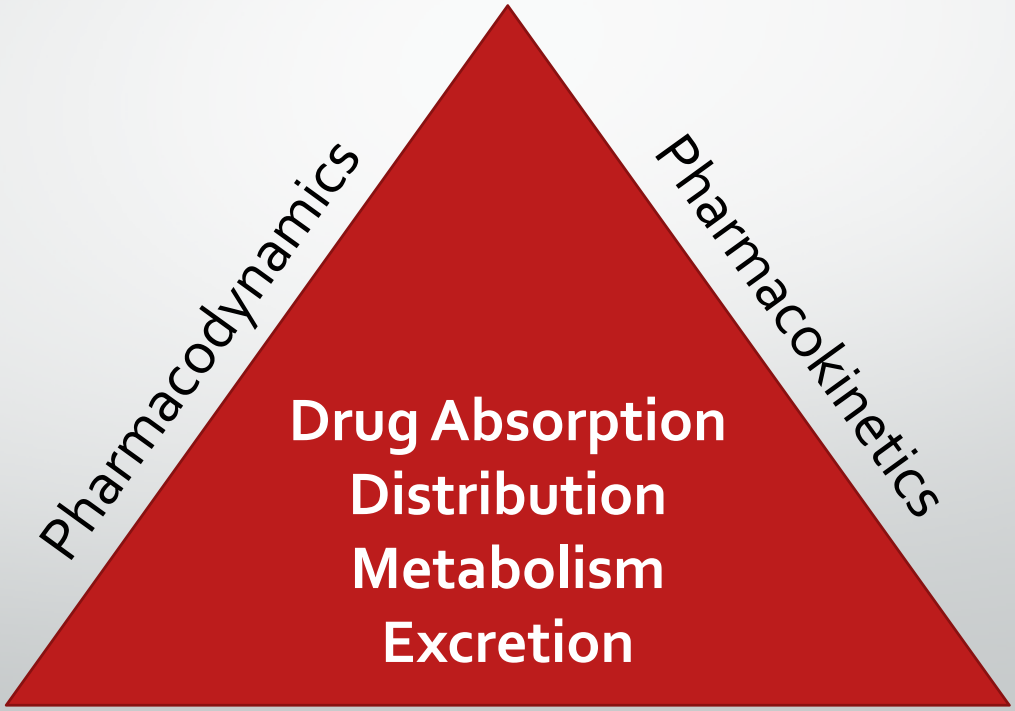
Br. J. Clin. Pharmacol. 2008, 66:4, 473-484.

Pharmacogenetics and Its Complementary Role with Compliance Monitoring

Factors Affecting Drug Metabolism

- Tissue distribution
- Genetics
- Co-administered substrates (inhibitors or inducers)
- Auto-induction
- Diet
- Disease (especially hepatic or renal)
- Protein-binding
- Age
- Gender
- Route of administration

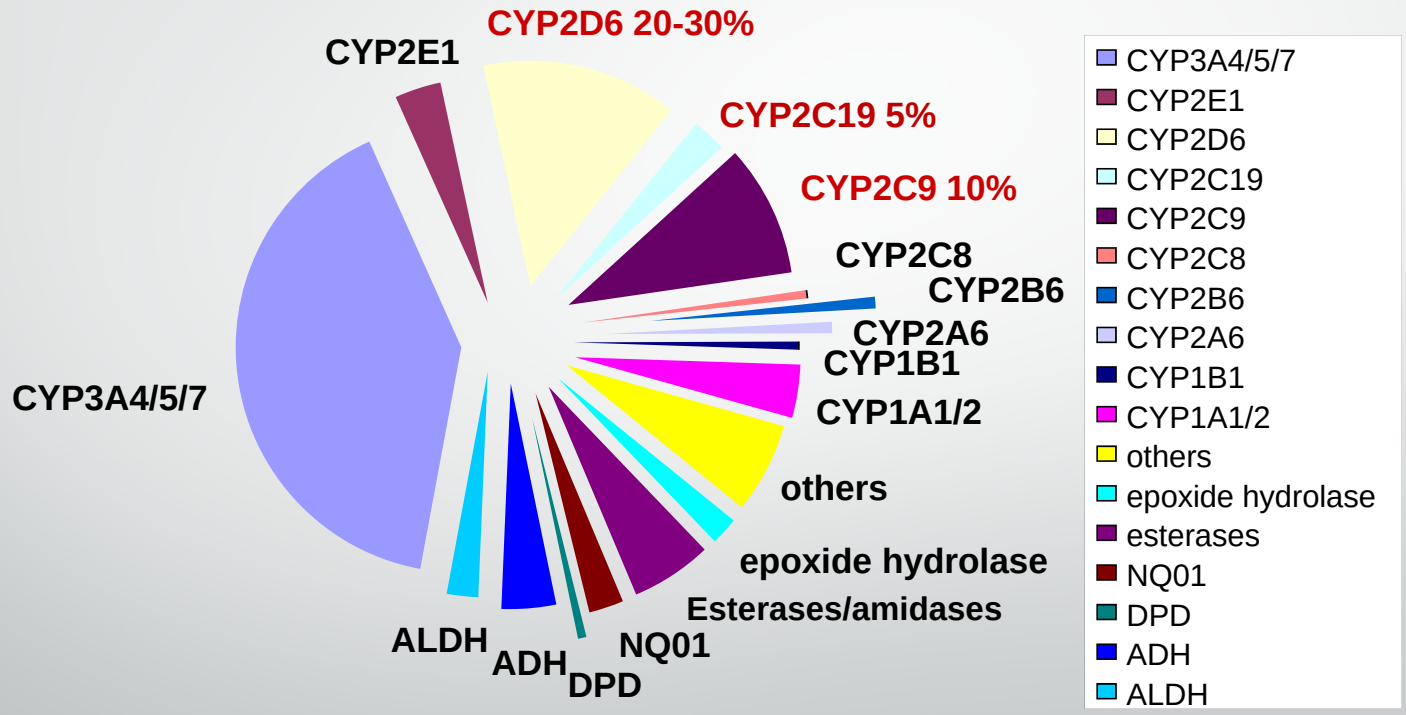
Integral Role in Drug Dynamics and Kinetics



Drug Metabolism

- The major drug metabolizing enzymes (DMEs) are expressed at the highest levels in the liver
- DMEs increase the polarity and aqueous to facilitate elimination from the body
- Impacts drug efficacy and detoxification

Human Phase I Enzymes of Drug Metabolism

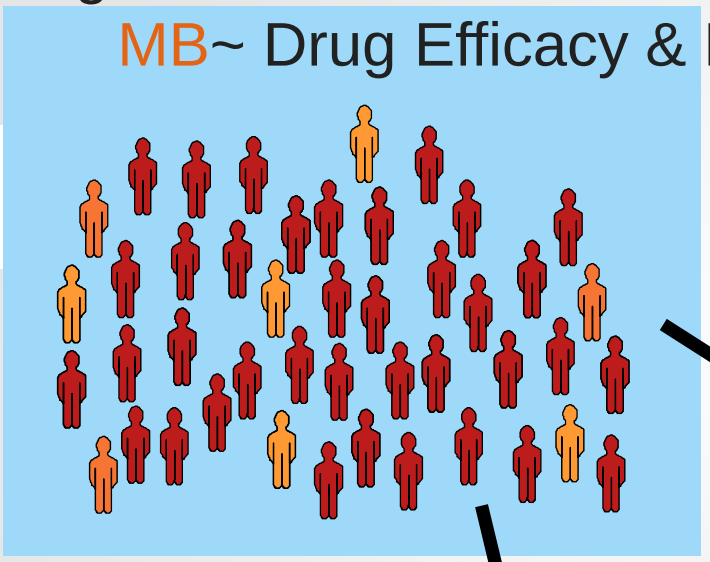


CYP: cytochrome P450, NQ01: NADPH:quinone oxidoreductase (DT diaphorase); DPD: dihydropyrimidine dehydrogenase; ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase

Evans and Relling, *Science* (1999)

Pharmacogenomics PG Proteomics PR Metabolomics

MB~ Drug Efficacy & Behavior



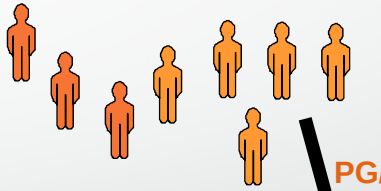
All patients with same diagnosis – pain, addiction

1

Remove, DUID / WUID

non-responders and toxic responders

PG/PR/MB



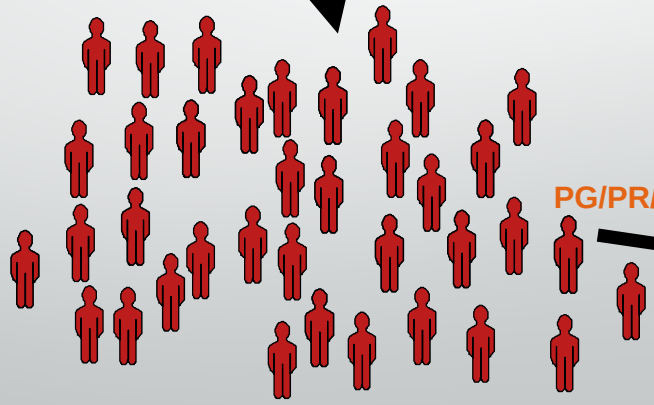
PG/PR/MB

2

Treat , DUID WUID

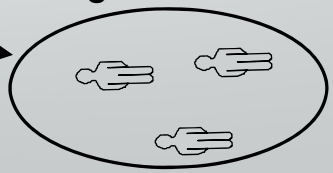
Responders and Patients Not Predisposed to Toxicity

PG/PR/MB



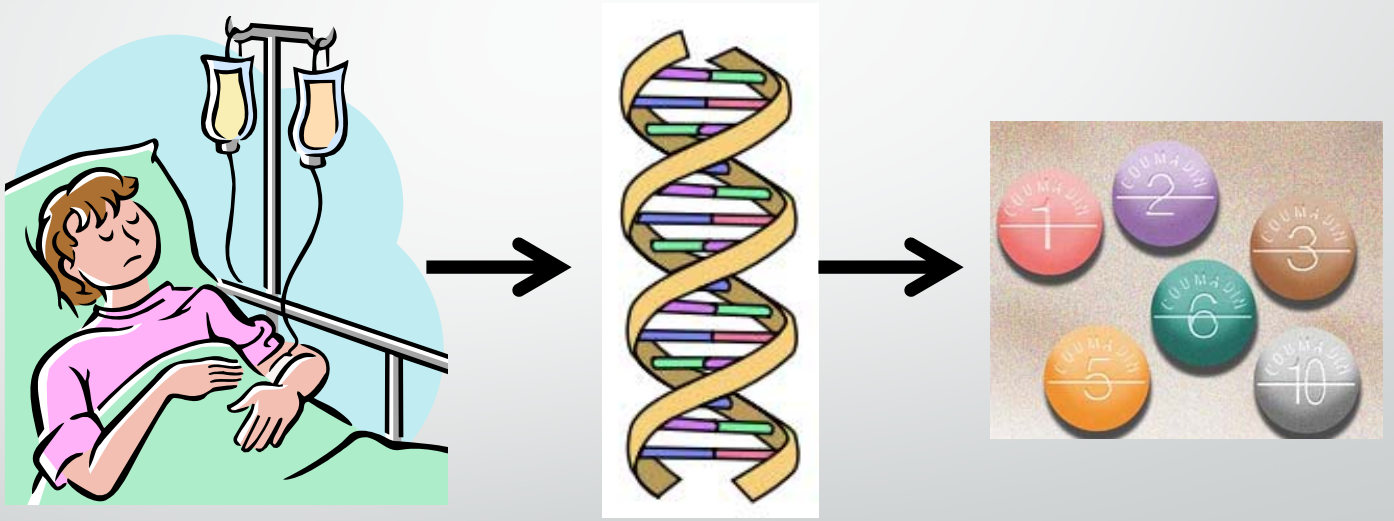
3 Death, DUID/WUID

Drug Induced/related



Evans and McLeod, modified by Wong 2013

Genotype Guided Dose Titration



Calculation of First Warfarin Dose

- Dose=Function (BSA, Age, Race, Goal INR, Amiodarone, Smoking Status)

$$Dose = e^{[0.613+(0.425*BSA)-(0.0075*Age)+(0.156*AA\ race)+(0.216*targetINR)-(0.257*amiodarone)+(0.108*smokes)+0.0784*DVT/PE]}$$

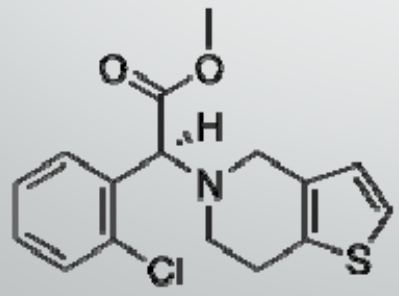
Clin Pharmacol Ther. 2008;84:326-31.

CYP2C9, VKORC1

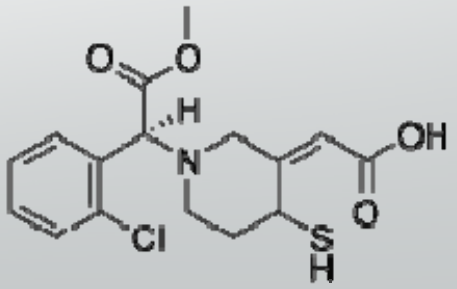
- Genotype information can assist in selecting starting dose of Warfarin
- The following polymorphisms have a recognized role in Warfarin metabolism
  - CYP2C9*1 is wild type
  - CYP2C9*2 (430C->T)– reduces metabolism by ~30%
  - CYP2C9*3 (1075A->C) – reduces metabolism by ~90%
  - VKORC1 1639G->A, produces less VKOR protein, lower doses needed to result in anticoagulation

Plavix (Clopidogrel)

- Clopidogrel is a prodrug
- Must be metabolized by CYP2C19 enzymes to produce the active metabolite (thiol) that inhibits platelet aggregation



Clopidogrel



Active metabolite of clopidogrel

CYP2C19 Genotyping for Clopidogrel (Plavix) Resistance

- Individuals with a personal or family history of adverse drug reactions to medications metabolized by CYP2C19
- Confirm presence of genotypes that affect the metabolism of drugs such as Plavix that are metabolized by cytochrome CYP2C19.
- CYP2C19*1 allele - fully functional metabolism
- CYP2C19*2 and *3 alleles - nonfunctional
- CYP2C19*2 and *3 - majority of reduced function alleles in white (85%) and Asian (99%) poor metabolizers

CYP2D6

- One of the only functional genes in the CYP2D subfamily genome.
- More than 80 *CYP2D6* alleles have been defined by the Cytochrome P450 Nomenclature Committee
- Large interindividual variation in the enzyme activity of CYP2D6.
- Enzyme is largely non-inducible and metabolizes ~25% of current drugs.
  - Opiates
  - β-Blockers
  - Anti-depressants
  - Tamoxifen

CYP3A Polymorphisms

- Broad substrate spectrum of 3A4, 3A5, 3A7
- Primarily expressed in liver and intestine
- CYP3A4 accounts for 30% of cytochrome P450 proteins
- Estimated to metabolize 45-60% of drugs, steroids and carcinogens
- Tacrolimus

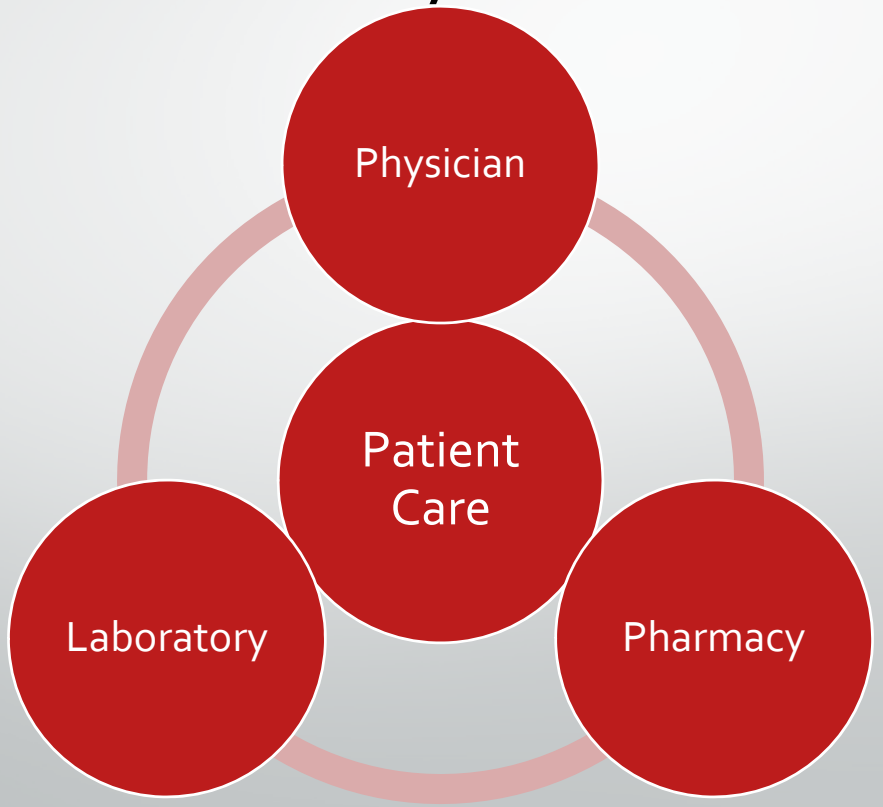
J. Natl. Cancer Inst., 2012, 104 (9): 657-669.
Ex. Op. on Drug Met. & Toxi., 2006, 2(2), 171-182.

Methadone – Chiral Pharmacology

- Methadone activity is almost solely to the drug itself rather than the metabolites
- Half life is variable 15-55 hrs
- R-methadone active form is 25-50 times more active than S
- However – CYP2B6 poor metabolizer and S-methadone cardiotoxicity

Courtesy of Dr. S.H. Wong

Pharmacogenetic Testing and Clinical Laboratory Consultation



Conclusions

- Oral fluid provides an alternative to urine for compliance monitoring toxicology
- Literature is growing to aid toxicologists and clinicians in the interpretation of oral fluid results
- The limits of detection are significantly lower than urine cutoff levels
- Pharmacogenetic testing provides insight to relevant metabolizing enzymes
- This information may supplement the toxicological evaluation
- Choice of effective medication may be aided with genotype

Thank you for your attention

- Acknowledgements: Dr. Qing Zhang and Dr. Steve Wong

- Contact: Brent.Dixon@PCLS.com 803-325-9823