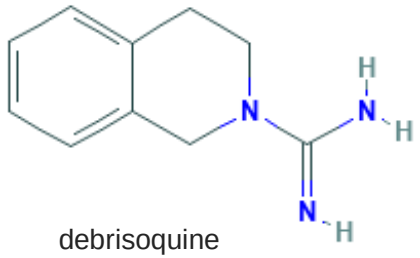
Substrate-specific metabolism of CYP2D6:
How can CYP2D6 phenotype prediction be improved?

Andrea Gaedigk, PhD

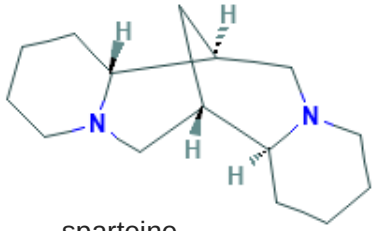
Children's Mercy Kansas City

Division of Clinical Pharmacology, Toxicology & Therapeutic Innovation

CYP2D6 (Debrisoquine-Sparteine Polymorphism)



debrisoquine



sparteine

- First discovered on debrisoquine and sparteine in the 1970s
- Metabolizes many clinically used drugs
- Highly polymorphic gene locus
- Large inter-individual variation of CYP2D6 activity
- Incidence of poor and ultrarapid metabolism varies widely across populations
- Risk of adverse events or treatment failure for extreme phenotypes
- Five CPIC guidelines (codeine, SSRIs, TCAs, ondansetron and tamoxifen)

Selection of drugs metabolized by CYP2D6

Antiarrythmics

encainide
flecainide
sparteine
perhexiline
propafenone
mexiletine

ß-blockers

Carvedilol
S-metoprolol
nebivolol\
Propafenone
timolol

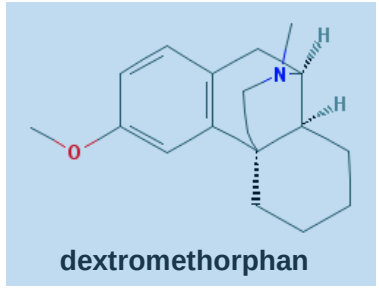
Others

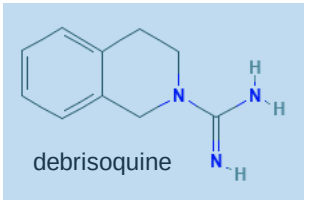
alprenolol
amphetamine
atomoxetine (Strattera)
bufuralol
chlorpheniramine
codeine
debrisoquine
dexfenfluramine
dextromethorphan
duloxetin
lidocaine
metoclopramide
methoxyamphetamine
ondansetron
oxycodon
perhexiline
phenacetin
phenformin
promethazine
tamoxifen
tramadol

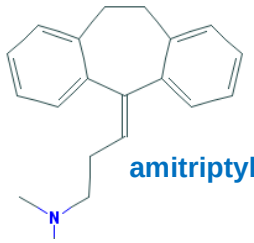
Antidepressants

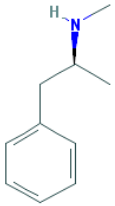
Antipsychotics

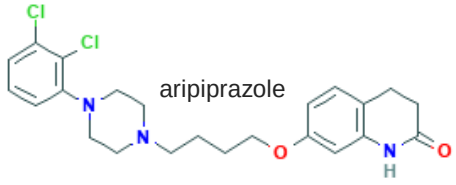
amitriptyline
aripiprazole
clomipramine
chlorpromazine
desipramine
duloxetin
fluoxetine (Prozac)
fluvoxamine
haloperidol
imipramine
minaprine
nortriptyline
paroxetine
perphenazine
risperidone
thioridazine
venlafaxine
zuclopenthixol

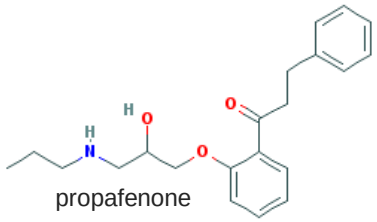

dextromethorphan

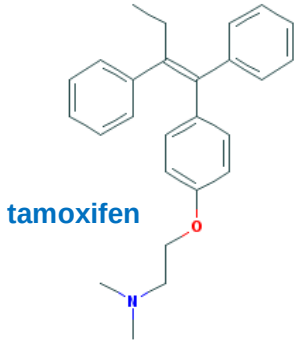

debrisoquine

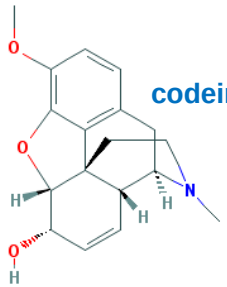

amitriptyline

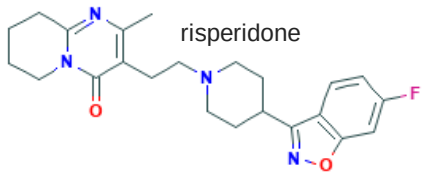

methamphetamine

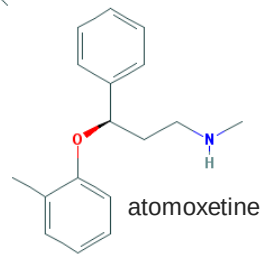

aripiprazole

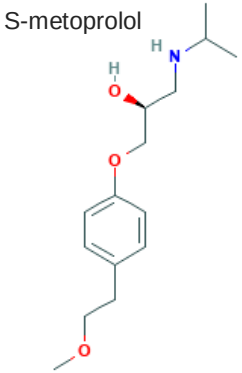

propafenone

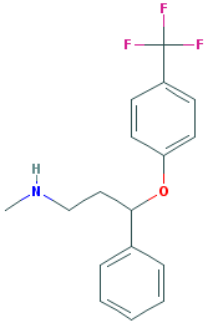

tamoxifen


codeine


risperidone


atomoxetine

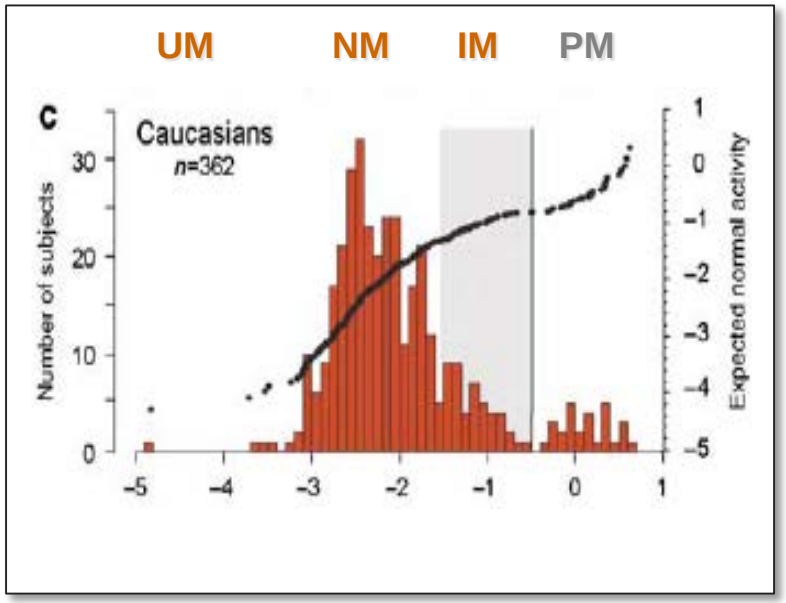

S-metoprolol

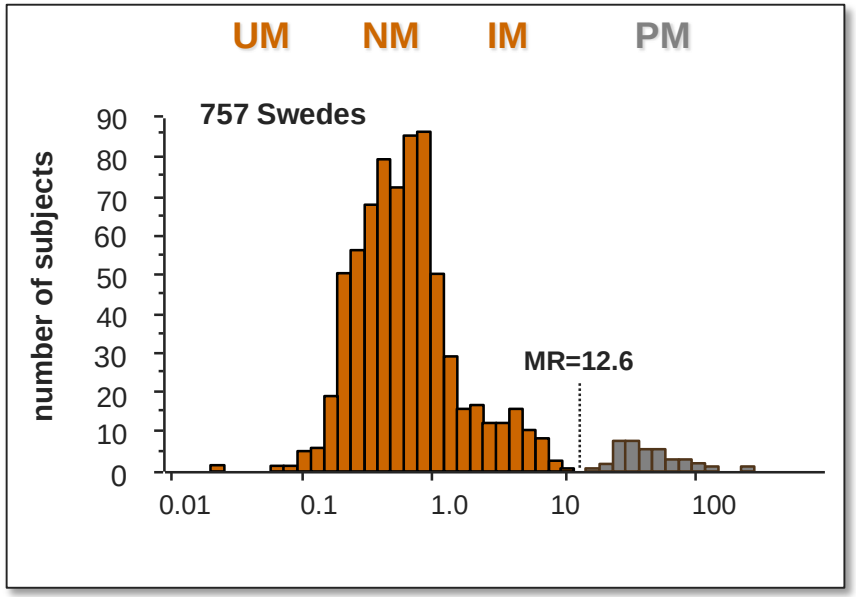

fluoxetine

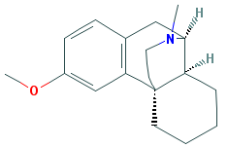
http://medicine.iupui.edu/flockhart
Zhou (2009) Clin Pharmacokinet 48:689-723 (Part 1) and 48:761-804 (Part 2)

CYP2D6 activity

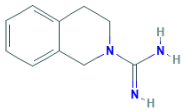
- Urinary metabolic ratios of a probe drug serve as a measure of CYP2D6 activity







DM/DX

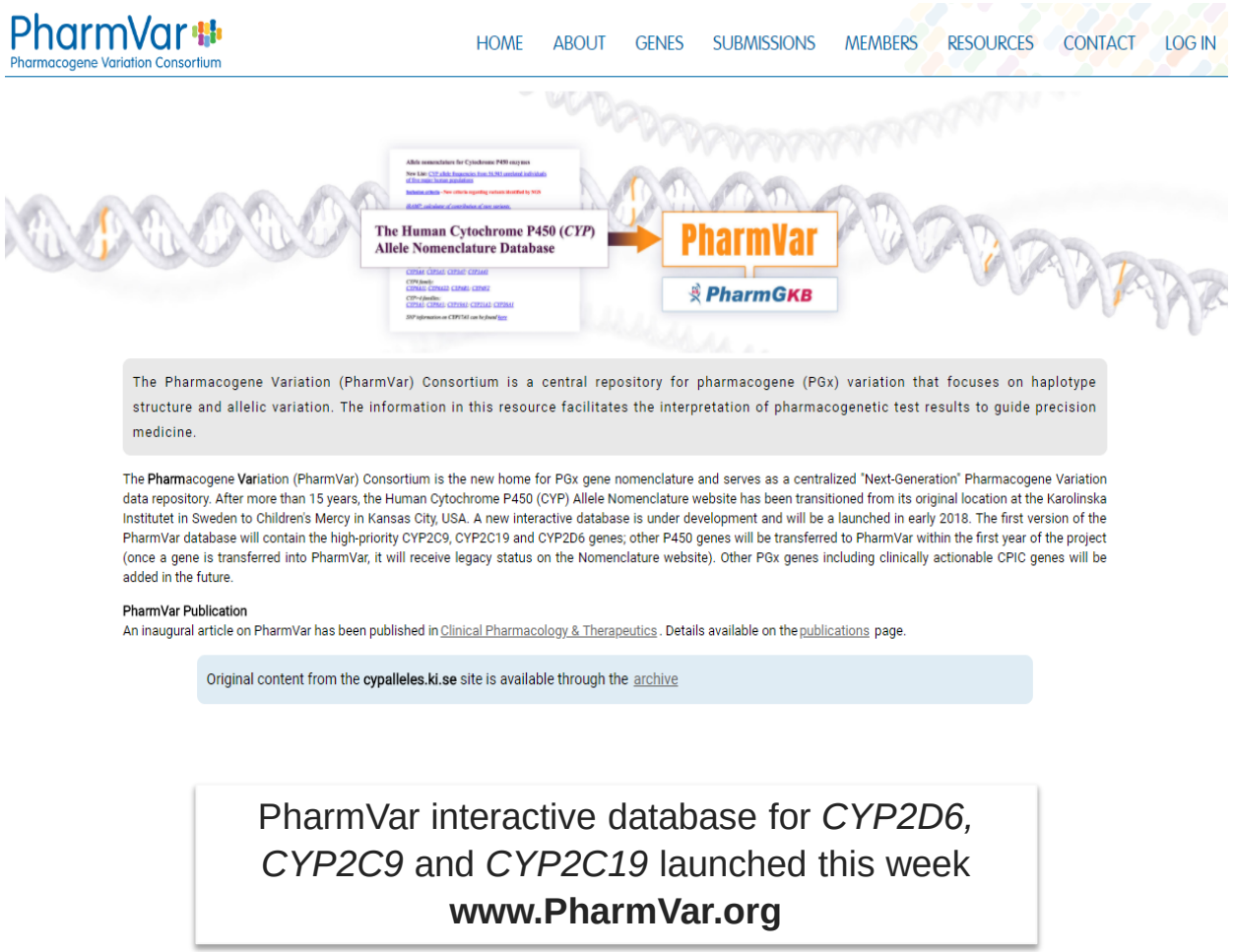


Debrisoquine/4-0H

Gaedigk et al (2008) CPT 83:234-242

Bertilsson et al (2002) BLCP 53:111-122

CYP2D6 allele definitions



PharmVar interactive database for *CYP2D6, CYP2C9* and *CYP2C19* launched this week
www.PharmVar.org

# of defined allelic variants: 105

# of possible diplotypes: 7752

Genetic variations comprise

- Single nucleotide polymorphisms (SNPs)
- Small deletions or insertions (indels)
- Large deletions (e.g. entire gene)
- Gene copy number variation (CNVs)
- Structural variation (hybrid genes, tandems)

Activity Score (AS)

- **To facilitate translation of diplotypes into phenotype**
  - Assigning a value to each allele reflecting its perceived activity towards a given substrate
  - AS = sum of the values of both alleles
  - Duplicated genes receive double the value of their single counterparts
  - Used in CPIC guidelines

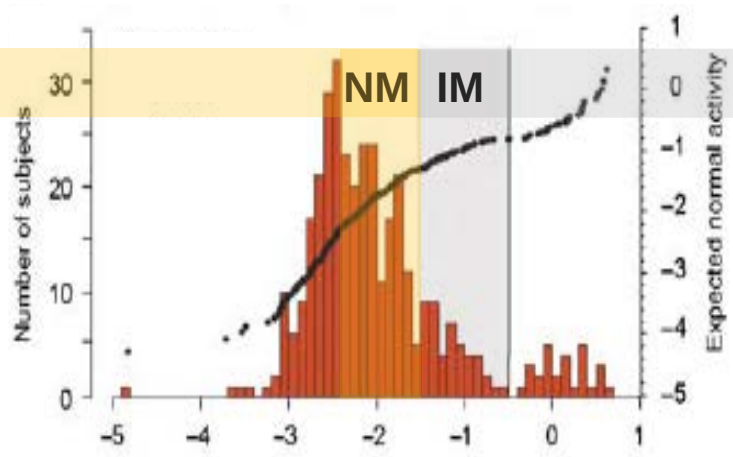
Functional Status	Value assigned	Alleles (selection)
Increased function	2 (3)	**1x2, *2x2, *35x2, *45x2 (x3)*
Normal function	1	**1, *2, *27, *33, *35, *39, *45, *46, *48, *53….. *9x2, *10x2, *17x2, *29x2…..*
Decreased function	0.5	**9, *10, *14B, *17, *29, *41, *49, etc*
No function	0	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14A, *15…..*

Gaedigk et al CPT 83:234-242 (2008)

Activity Score (AS)

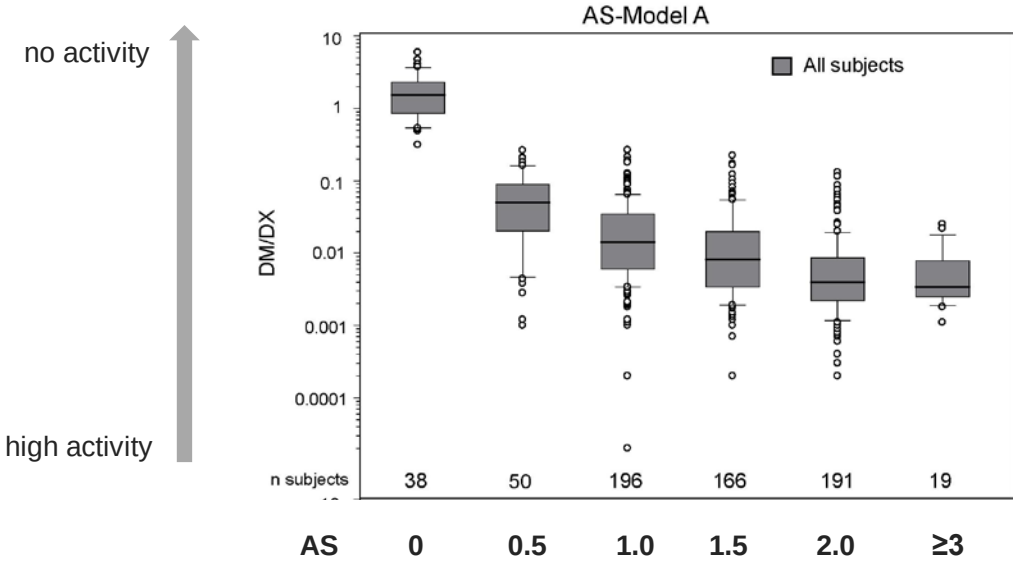
Metabolizer Status	Activity Score	Diplotypes (selection)
Ultrarapid metabolizer (UM)	≥2.5	**1/*2x2, *2x2/*35x2….*
Normal metabolizer (NM)	1.5-2 1	**1/*1, *1/*2, *1/*41, *2/*10…..* ****1/*4, *2/*5, *41/*41, *9/*17…..***
Intermediate metabolizer (IM)	1 0.5	**4/*10, *5/*17, *6/*41…..*
Poor metabolizers (PM)	0	**4/*5, *3/*6, *12/*84….*

AS = 1



AS = 0.5

Relationship between DM/DX and AS



Subjects: 672

Alleles tested: 21 + gene duplications

Genotypes: 94

Activity Score groups: 6

Variability explained by AS: 55%

Variability explained by genotype: 59%

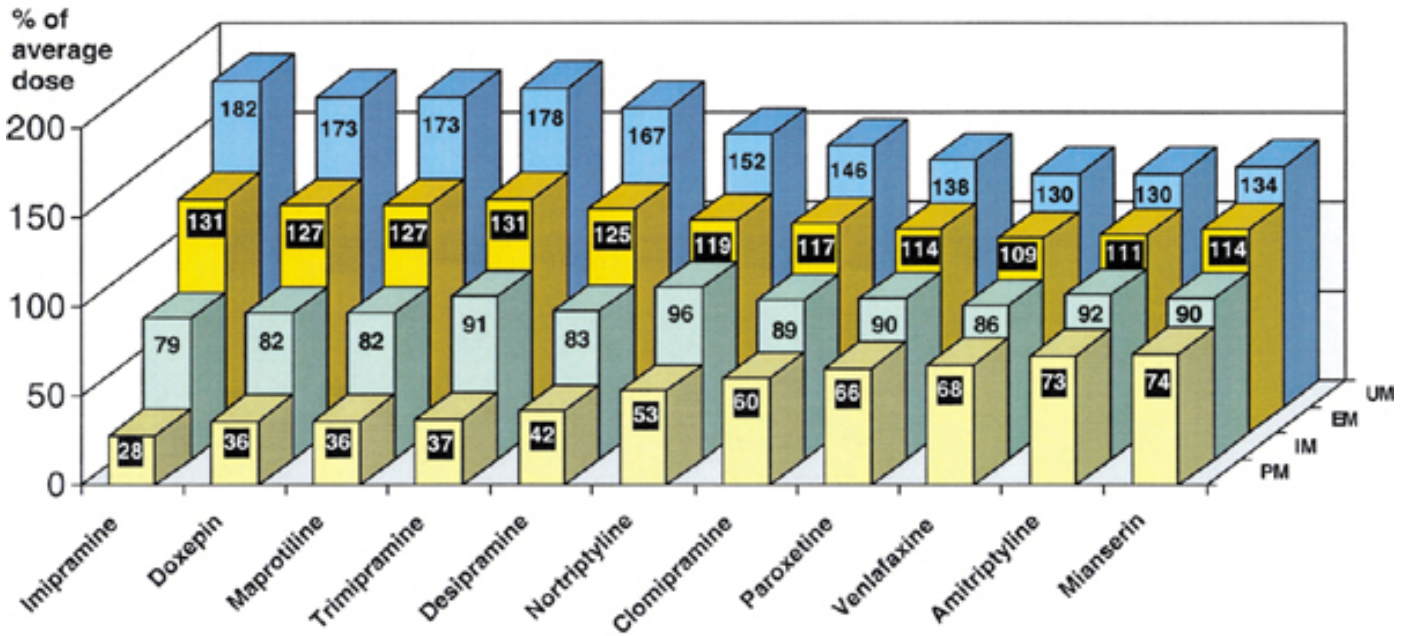
Gaedigk et al (2008) CPT 83: 234

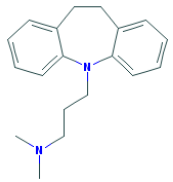
Limitations and challenges of the Activity Score

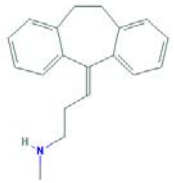
- Accuracy of the function/activity assigned to an allele
  - Categorical (no, decreased, normal and increased function)
  - No or limited data
- Different ways to measure “activity” or “metabolic capacity”
  - What is the best experimental approach to determine the activity of an allele or diplotype?
- Activity of an allele may be substrate-specific
  - Substrate may act as inhibitor

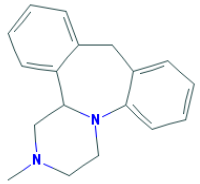
Genotype-based dose adaptations as % of recommended ‘usual‘ dose

PGx of antidepressants and antipsychotics: contribution of allelic variation to the phenotype of drug response – a meta analysis









Kirchheimer et al (2004) Mol Psych 9:442-472

*CYP2D6*17:* wide range of activity

Substrate	**CYP2D6.17** % of CYP2D6.1
chlomipramine	15
dextromethorphan	**29**
fluphenazine	34
bufuralol	38
timolol	46
metoprolol	58
propafenone	62
thioridazine	70
debrisoquine	**71**
sparteine	**87**

Bapiro et al (2002) Biochem Pharmacol 64:1387-1398

In-vitro systems

COS-7 cell expressed protein

Protein	DM	bufuralol
	V_{max}/K_m	V_{max}/K_m
	% CYP2D6.1	*% CYP2D6.1*
CYP2D6.1		
CYP2D6.2	71	72
CYP2D6.17	25	37

decreased function

Baculovirus expressed protein (Supersomes)

Protein	DM	bufuralol	debrisoquine
	V_{max}/K_m	V_{max}/K_m	V_{max}/K_m
	% CYP2D6.1	*% CYP2D6.1*	*% CYP2D6.1*
CYP2D6.1			
CYP2D6.2	109	117	101
CYP2D6.17	18	22	22

normal function

decreased function

Marcucci et al (2002) DMD 30: 595-601

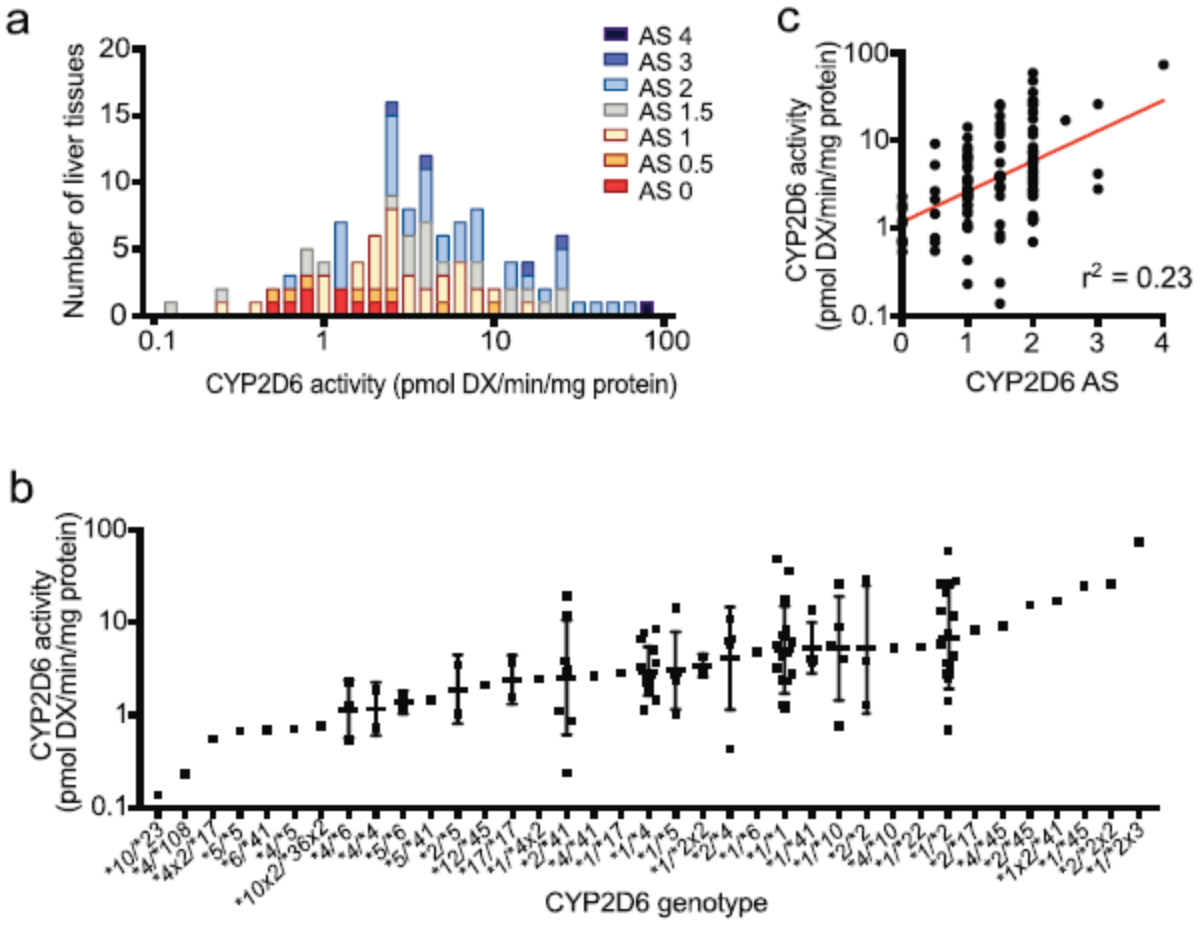
In-vitro systems

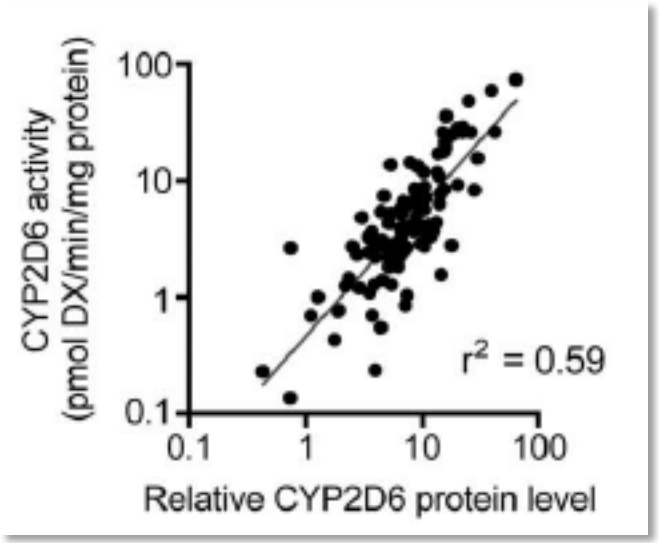
substrate	CYP2D6.10 % of CYP2D6.1	CYP2D6.17 % of CYP2D6.1
nortriptyline	1.3	7.3
bufuralol	3.7	22.8
dextromethorphan	**5.3**	**16.8**
tramadol	6.9	35.7
fluoxetine	7.5	8.17
atomoxetine	8.6	21.9
debrisoquine	**11.8**	**64.2**
codeine	27.9	80.4

Baculovirus-expressed protein (Supersomes)

Shen et al (2007) DMD 35: 1292-1300

CYP2D6 activity in human liver





- **Protein level is a better predictor than AS**
- Protein levels not an option to test patients
- Difficult to find tissues with genotypes of interest

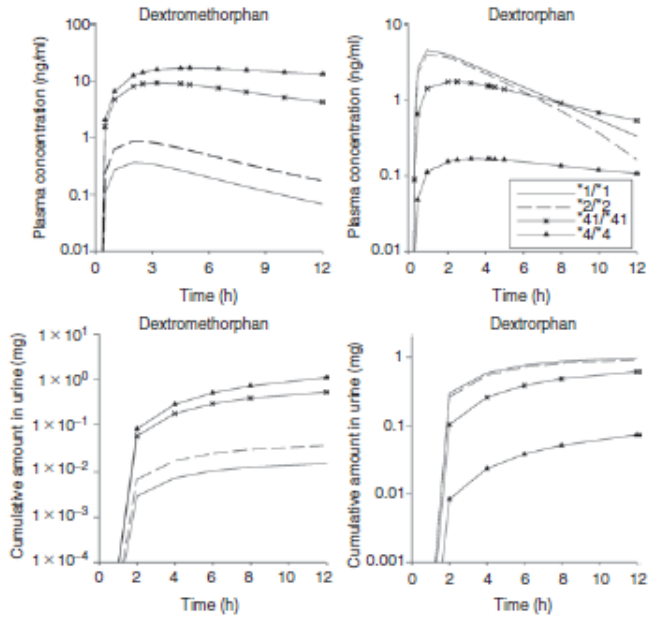
Ning et al (2018) CPT Epub ahead of print

Assessment of Activity Levels for *CYP2D6*1*, *CYP2D6*2*, and *CYP2D6*41* Genes by Population Pharmacokinetics of Dextromethorphan

K Abduljalil[1,2], D Frank[1], A Gaedigk[3], T Klaassen[1], D Tomalik-Scharte[1], A Jetter[1,4], U Jaehde[5], J Kirchheiner[6] and U Fuhr[1]

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 88 NUMBER 5 | NOVEMBER 2010

- 36 male subjects
- Plasma and urine
- Data modeled simultaneously using the population pharmacokinetics NONMEM software
- Five-compartment model adequately described the data
- Urinary pH was confirmed as a significant covariate for DM renal clearance
- ~55% of variability explained by genotype



- **Values of 1, 0.4 and 0.17 should be used for **1, *2* and **41*, respectively**
- No data for other alleles
- Many alleles are rare – difficult to find subjects

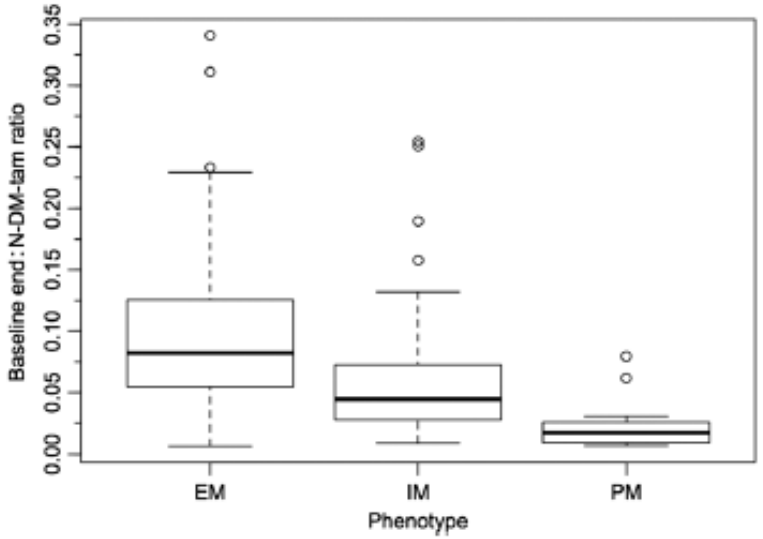
Model parameter[a]	Point estimate	95% CI
Components of apparent metabolic clearance CL_{23}		
*CYP2D6*1* (l/h, per gene copy)	5,010	3,579–6,441
*CYP2D6*2* (l/h, per gene copy)	2,020	624–3,416
*CYP2D6*41* (l/h, per gene copy)	85.0	63.8–106.2

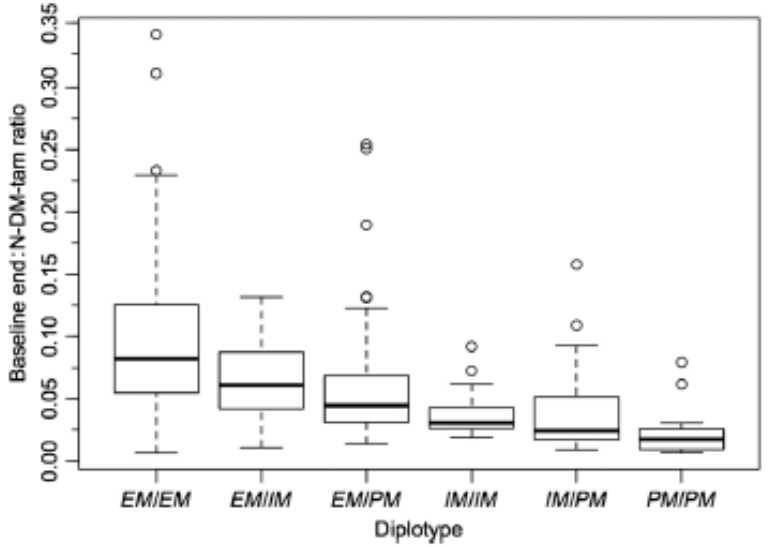
In vivo assessment of the metabolic activity of CYP2D6 diplotypes and alleles

Daniel L. Hertz,[1] Anna C. Snavely,[2] Howard L. McLeod,[3] Christine M. Walko,[3] Joseph G. Ibrahim,[4] Steven Anderson,[5] Karen E. Weck,[4] Gustav Magrinat,[6] Oludamilola Olajide,[7] Susan Moore,[7] Rachel Raab,[8] Daniel R. Carrizosa,[9] Steven Corso,[10] Garry Schwartz,[11] Jeffrey M. Peppercorn,[12] James P. Evans,[4] David R. Jones,[13] Zeruesenay Desta,[13] David A. Flockhart,[13] Lisa A. Carey[4] & William J. Irvin Jr[4,14]

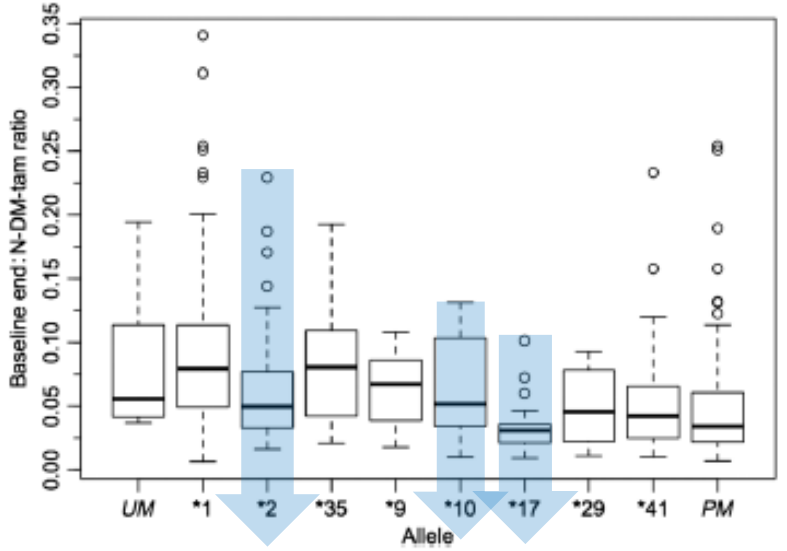
Substrate specificity - tamoxifen

Plasma metabolic ration of endoxifen/N-desmethyl-tamoxifen





AS	2	1.5	1.0	1.0	0.5	0



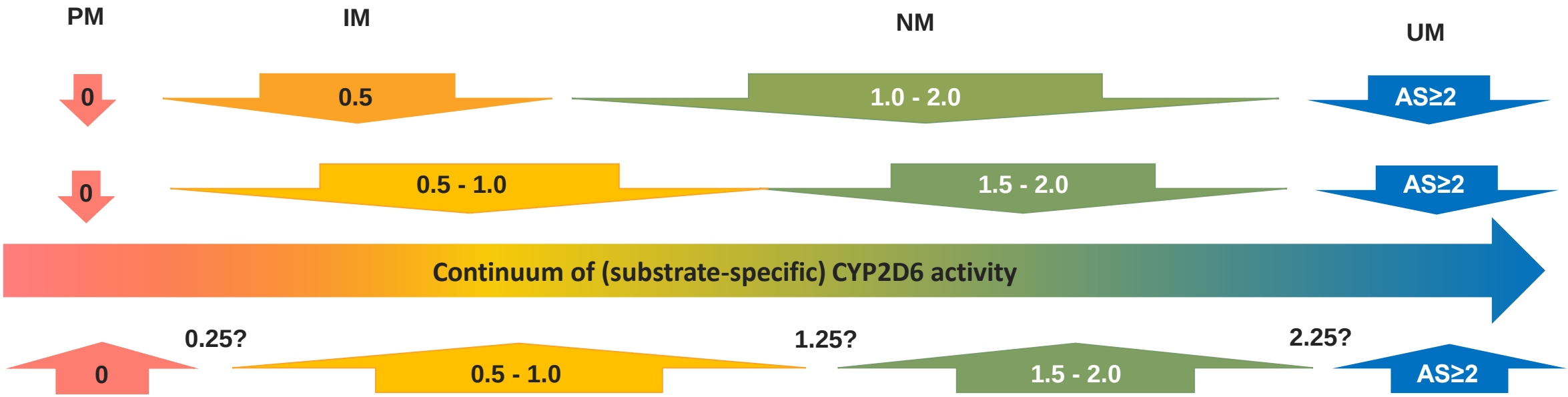
How can the Activity Score system be improved?

- How should function be determined to best inform the translation of genotype to phenotype?
  - A standardized approach to assign function is needed
  - Function currently assigned based on varied knowledge/evidence
  - A single activity label does likely not fit all substrates
  - Need a better understanding of other factors impacting metabolic capacity of an individual

Genotype translation standardization project

- **CPIC expert working group**

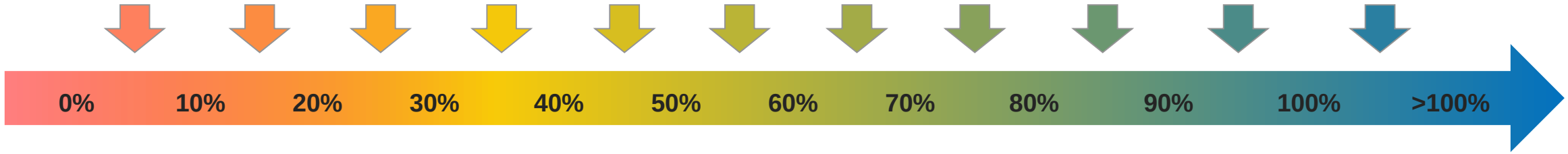
  Find consensus of how to translate genotype into phenotype groups



Tweak the system by ‘downgrading’ *CYP2D6*10*, a severely decreased activity allele and introduce a value of 0.25 for Activity Score assignments

Percent Activity (PA) system

- Conceived from the discussions of the CPIC working group
  - System proposed by Daniel Hertz
  - Average of 2 alleles on a 0-1 scale vs adding 2 alleles on a 0-2 scale
  - Minimize information loss during allelic activity and phenotype assignment
  - More flexible, precise and intuitive meaning, e.g. 80%
  - Translate PA% into phenotype
  - Reanalyze existing large datasets (e.g. endoxifen/N-desmethyl-tamoxifen ratios as proof of concept



GOLDILOKS

Genomic- and

Ontogeny-

Linked

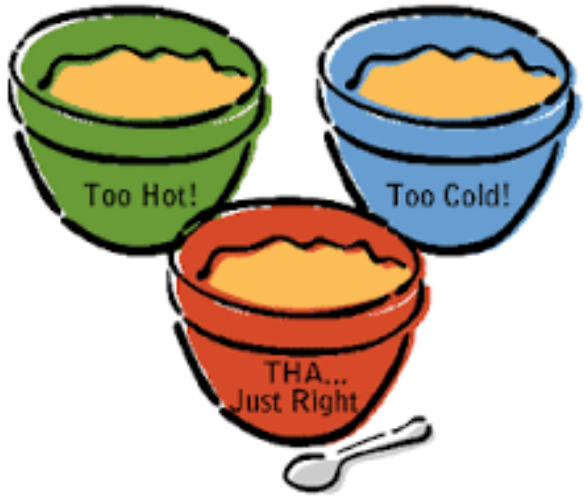
Dose

Individualization and

c**L**inical

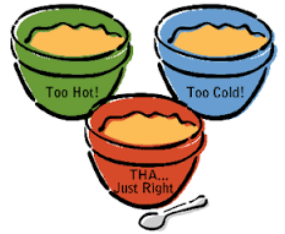
Optimization for

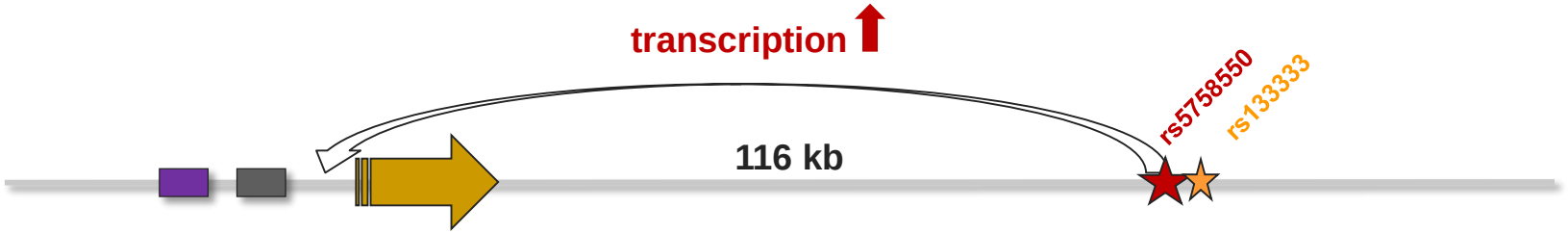
Kid**s**



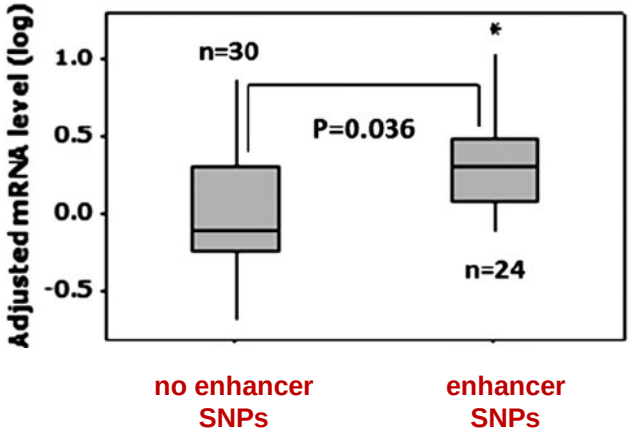
“Not too big, not too small … the dose of medication that is **‘just right’** for your child”

U54 translational study

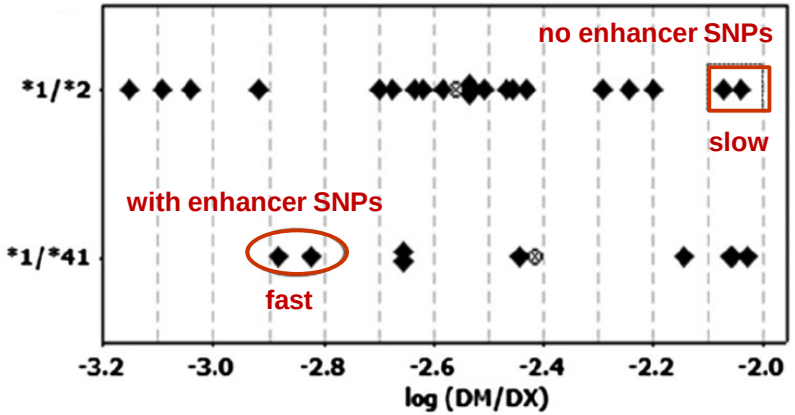




mRNA expression



In vivo phenotype (DM/DX)

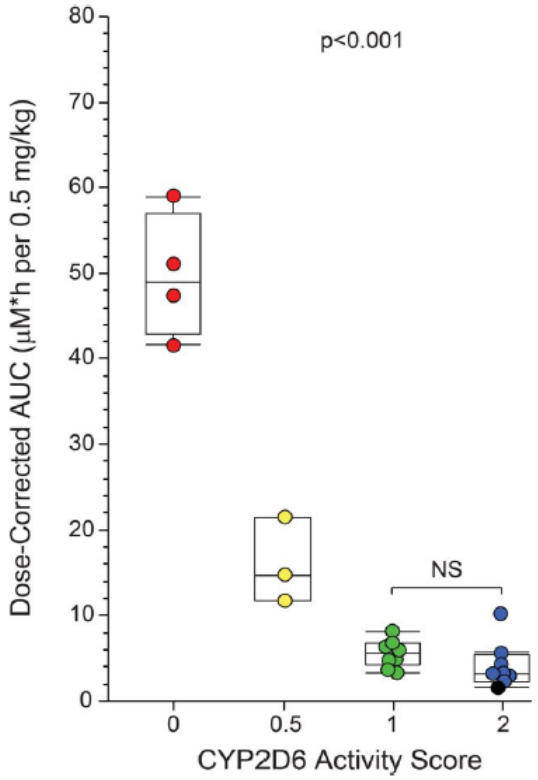


- Enhancer SNP 116 kb downstream of gene locus
- Increases transcription levels
- How much of the variability does the enhancer SNP explain?
- Which alleles have the enhancer SNP?
- Clinically relevant?
- Need to incorporate into the AS?

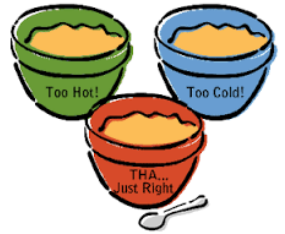
Wang et al (2014) HMG 23: 268-278

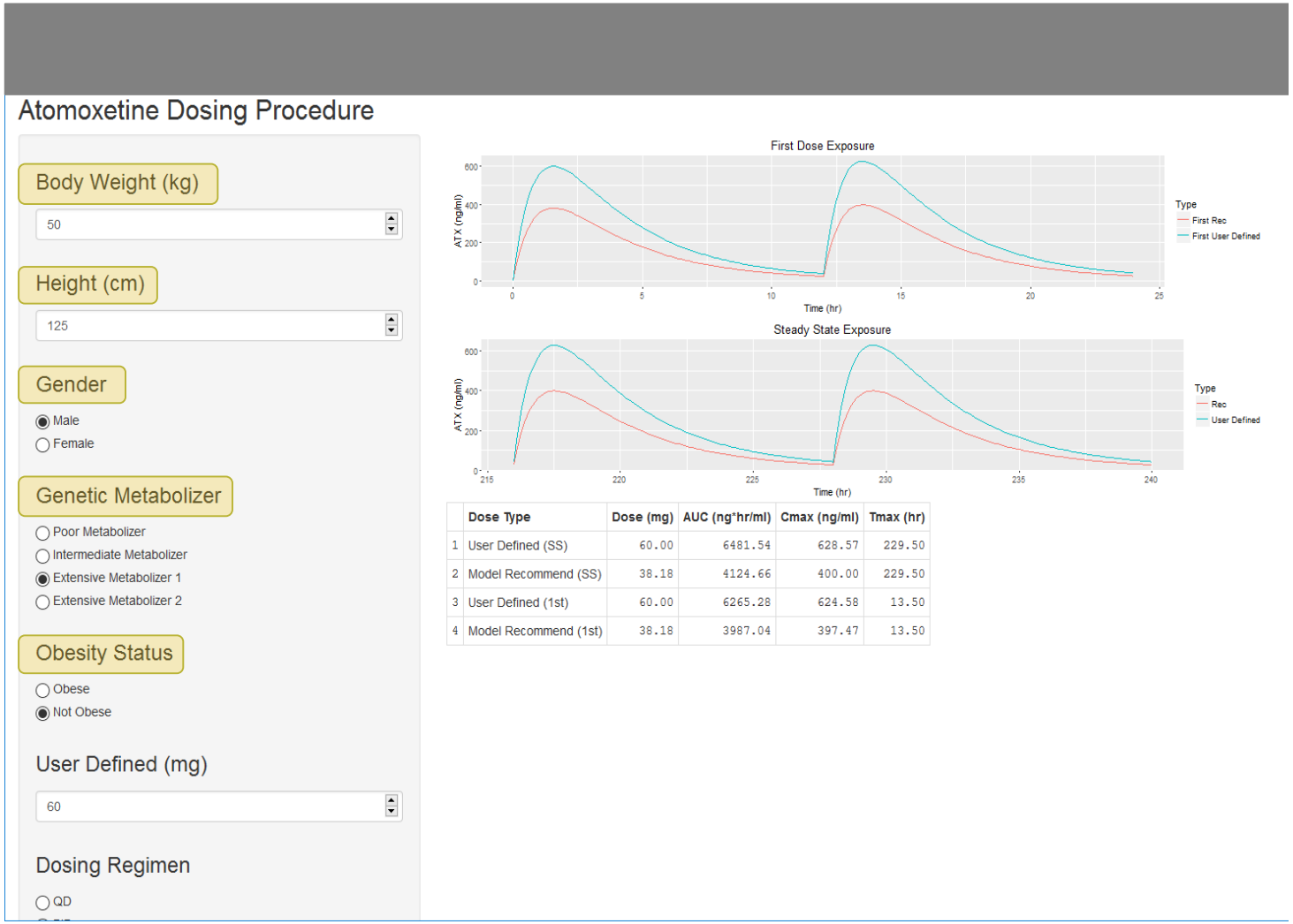
U54 clinical study

- Improve current PBPK model for atomoxetine
  - Published dataset (n=24); Validation study (n=24) nearly completed
  - Does the enhancer SNP improve phenotype prediction?
- Clinical Study
  - 120 subjects over 2 years (first patients enrolled)
  - Extensive clinical testing for ADHD diagnosis and response
  - Patients dosed to target exposure based on *CYP2D*6 genotype
  - PK study on 2 occasions
  - Genotyped for other PGx genes that may affect PK and PD
- Refine model
- Metabolomics
  - to identify biomarker predictive of response



Atomoxetine-specific dosing algorithm





	Dose Type	Dose (mg)	AUC (ng*hr/ml)	Cmax (ng/ml)	Tmax (hr)
1	User Defined (SS)	60.00	6481.54	628.57	229.50
2	Model Recommend (SS)	38.18	4124.66	400.00	229.50
3	User Defined (1st)	60.00	6265.28	624.58	13.50
4	Model Recommend (1st)	38.18	3987.04	397.47	13.50

What a **GOLDILOKs atomoxetine interface** might look like to guide precision drug therapy

Acknowledgements

- Division of Clinical Pharmacology at Children's Mercy
  - Steven Leeder
  - Susan Abdel-Rahman
  - Jean Dinh
  - Leon Van Haandel
  - Robin Pearce
  - Chengpeng "Charlie" Bi
  - Vincent Staggs
  - Carla Allan
  - Jaylene Weigel
  - Technical support staff

- Center for Pediatric Genomics at CMH
  - Sarah Soden
  - Neil Miller
  - Greyson Twist
  - Scott Casey
  - Emily Farrow

FUNDING

GOLDILOKS: U54 HD090258-01

PharmVar: R24 GM123930-01

PharmVar
Pharmacogene Variation Consortium