Adverse Drug Reaction Risks in Genetically-Defined Subpopulations

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Introduction

- Adverse Drug Reactions (ADR) can reduce or eliminate therapeutic drug benefit, cause morbidity and occasionally mortality. Some Adverse Drug Reactions may be due to genetic factors.

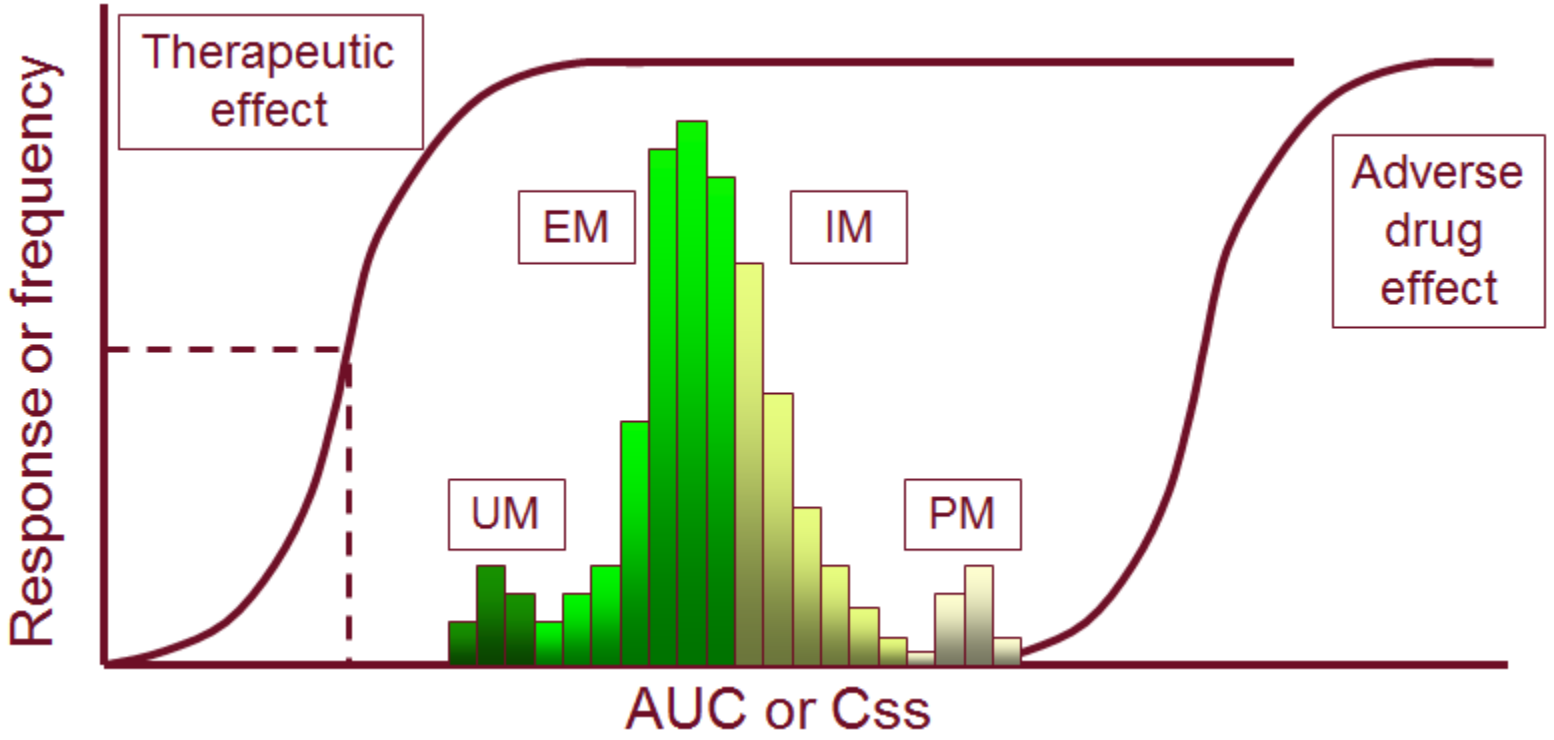
- Determinants of susceptibility to ADR include pharmacokinetic factors, such as gene polymorphisms in cytochrome P450 enzymes, other drug metabolizing enzymes, and pharmacodynamic factors, such as polymorphisms in drug targets (*e.g.* VKORC1).

- ADR that are based on known genetic variants are preventable.

Pharmacogenomic guidance documents

- FDA - Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling, 2013
- EMA - Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products, 2011
  - 4.2.1. *In vitro* studies prior to human exposure
    - Identification of the enzymes catalyzing the *in vitro* metabolism
    - Characterization of metabolites formed through candidate major metabolic pathways
    - Together the involvement of known functionally polymorphic enzymes can be established

Drug interaction and ADR mechanism



Structural basis of genetic polymorphisms

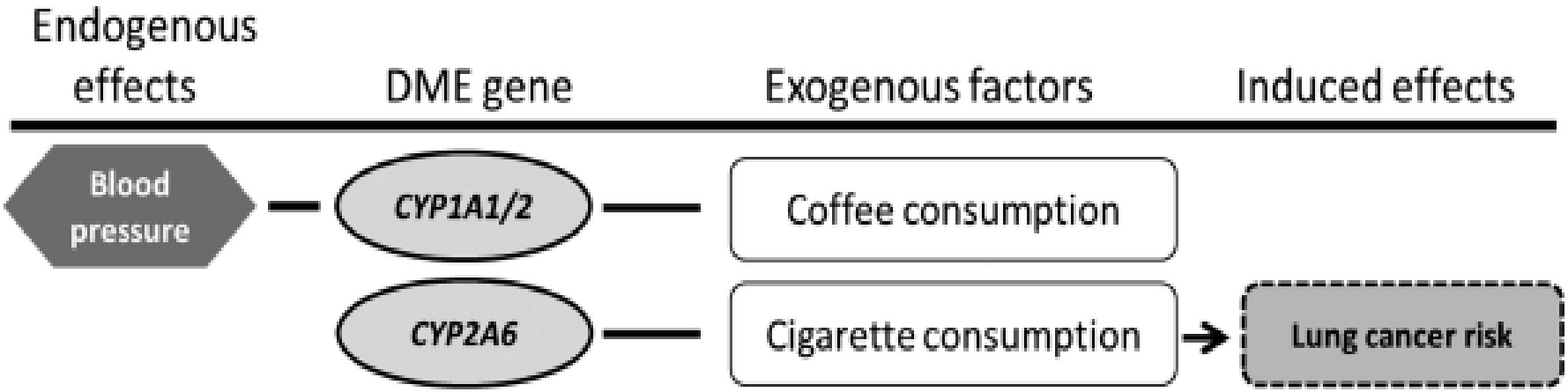
- Effective SNPs can be located in 5'- or 3'-untranslated region but non-synonymous amino acid are most frequent (~400, www.cypalleles.ki.se)
- SNPs interfering with splicing - no-activity *CYP2C19*2*, *CYP2D6*4*, decreased activity *CYP2B6*6*, *CYP2D6*41*, *CYP3A5*3*
- In the promoter region - extra TA in *UGT1A1*28* reduces transcription, -806C>G in *CYP2C19*17* increases gene expression
- Gene copy number polymorphism, *e.g. CYP2D6*

Drugs affected by major polymorphisms of DME

Drug	*Clinical use*	*Impacting DME alleles*	*Type of clinical parameter affected*
Warfarin	Cardiovascular disorders	*CYP2C9*2* and **3*	Bleeding
Clopidogrel	Cardiovascular disorders	*CYP2C19*2, *3, *17*	Stent thrombosis and bleeding
Tamoxifen	Breast cancer	*CYP2D6 (various)*	Breast cancer recurrence
Tacrolimus	Organ transplantation	*CYP3A5*3*	Graft rejection
Antidepressants	Depression	*CYP2D6 (various)*	Non-response
Escitalopram	Depression	*CYP2C19*17*	Non-response
NSAIDs	Pain relief	*CYP2C9*2* and **3*	GI bleeding
Irinotecan	Colorectal cancer	*UGT1A1*28*	Myelotoxicity
6-MP and AZA	Leukemia and chronic inflammation	*TPMT*2, TPMT*3A* and *TPMT*3C*	Myelotoxicity
Codeine	Pain relief	*CYP2D6 (various)*	Response or CNS depression

The Pharmacogenomics Journal (2013) 13, 1–11

Other major effects of polymorphism of DME



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Prevalence of PMs for enzymes implicated with ADR

Enzyme	Prevalence of Poor Metabolizers (%)
CYP1A2	12 Caucasian
CYP2C9	2 – 6 Caucasian
CYP2C19	2 – 6 Caucasian, 15 – 17 Chinese, 18 – 23 Japanese
CYP2D6	3 – 10 Caucasian, < 2 Chinese, Japanese, African American
NAT2	50 – 59 Caucasian, 41 African American, 20 Chinese, 8 – 10 Japanese, 92 Egyptian

JAMA, November 14, 2001—Vol 286, No. 18

PMs associated with ADRs

- Drugs implicated in adverse reactions metabolized by enzymes with variant alleles associated with poor metabolism.

Enzymes	Drugs
CYP1A2	Carbamazepine, diltiazem, erythromycin, fluoxetine, imipramine,† isoniazid, naproxen, nortriptyline hydrochloride, phenytoin, rifampin, theophylline,† verapamil
CYP2C9	Fluoxetine,† ibuprofen sodium,† imipramine, isoniazid, naproxen, phenytoin,† piroxicam,† rifampin, verapamil, warfarin sodium
CYP2C18	Fluoxetine, imipramine, piroxicam, rifampin
CYP2C19	Fluoxetine, imipramine,† isoniazid, nortriptyline, phenytoin, rifampin, warfarin
CYP2D6	Diltiazem, fluoxetine,† imipramine,† metoprolol,† nortriptyline, theophylline
CYP2E1	Fluoxetine, isoniazid, theophylline, verapamil
UGT2	Ibuprofen, naproxen
NAT2	Isoniazid†

*Drugs appear more than once because of multiple metabolic pathways.
†Indicates enzymes with major metabolic pathways that are more likely to determine ADR susceptibility than minor enzymes.

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Genetically-defined tools for metabolism studies

- Human liver microsomes characterized for allelic variants of enzymes: CYP2C9, CYP2C19, CYP2D6, CYP3A5, UGT1A1, UGT1A9. Microsomes from multiple donors with polymorphisms coding for high, moderate or no enzyme activity are available.
- Geneknown™ hepatocytes are pools of cells from donors with different polymorphism having the same phenotypic effect, e.g. CYP2D6.HA pool comprises alleles *1 and *2 with varying gene copy number and other alleles.

Application of genotyped hepatocytes

- Geneknown™ hepatocytes are formulated for confirmation of identity of a polymorphically expressed enzyme suspected of catalyzing formation of a given metabolite. Initially such information may be obtained from pooled microsome study with specific enzyme inhibitors. When possible cells are grouped into high, moderate and no activity pools.
- The cells can also be used to study metabolic pathways of a given drug in a genetically deficient population.
- Taken together these studies can identify patient populations with an increase risk for ADR.

Geneknown™ Hepatocytes Characterization

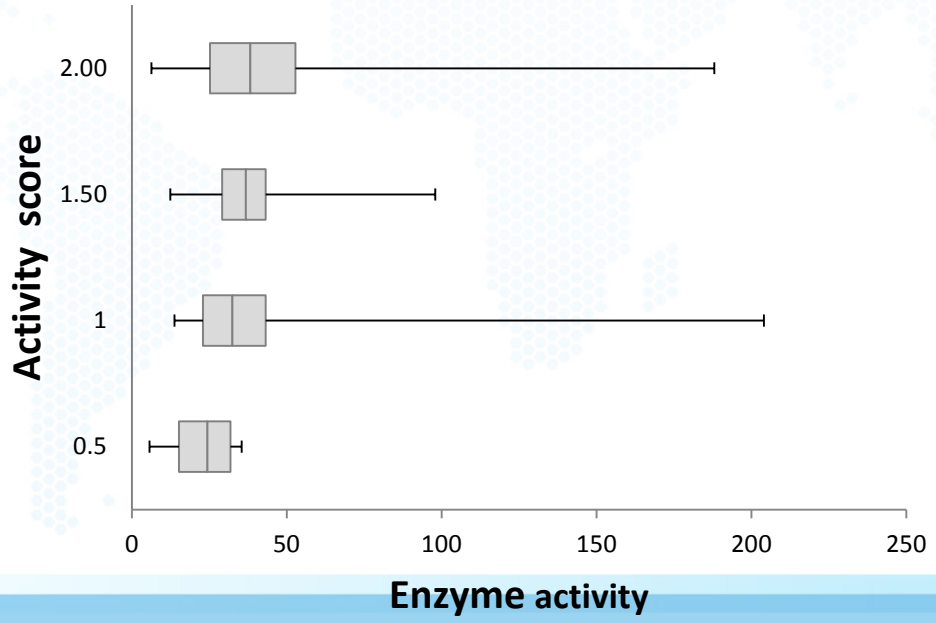
Phase I	Phase II	Transporters	Pharmacodynamic
CYP1A1	DPYD	MDR1	VKORC1
CYP1A2	GSTP1	MRP2	
CYP2A6	TPMT	BCRP	
CYP2B6	UGT1A1	PEPT2	
CYP2D6	UGT2B7	OCT1	
CYP2C8	UGT2B15	OCT2	
CYP2C9	NAT1	OAT1	
CYP2C19	NAT2	OATP1B1	
CYP2E1		OATP1B3	
CYP3A4		OATP2B1	
CYP3A5			

Gene symbols, SNP ID, PharmaADME names and assay IDs (Applied Biosciences) can be found at www.xenotech.com

CYP2D6 characterization and activity score

Allele	Enzyme activity	Activity score
*1, *2, *35	normal	1
*9, *10, *17, *29, *41	decreased	0.5
*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *36, *44, *68, *76	none	0

Hepatocyte CYP2D6 enzyme activity and activity score



Geneknown™ pools

CYP2C8, CYP2C9 - high and moderate activity

CYP2C19, CYP2D6, CYP3A5 - high, moderate and no-activity

OATP1B1 – high (*5) and moderate activity

UGT1A1 - high and moderate (*27, *60) activity

Future products -

Thiopurine methyl transferase and N-acetyl transferase

CYP3A5 – no activity pool

CryostaX Geneknown™

Single Freeze Pooled Cryopreserved Human Hepatocytes

HPCH.3A5.NA Lot No. 1510230

Pool of 3

Assured Minimum Yield: 4.5 x 10^6 cells per vial
Viability: >70.0%

Individual Donor Genotype Information:

Donor	CYP1A1	CYP1A2	CYP2A6	CYP2B6	CYP2D6	CYP2C8	CYP2C9	CYP2C19	CYP2E1	CYP3A4	CYP3A5
1196	*1/*1	*1F/*1K	*1/*1	*1/*6	*1/*4	*1/*1	*1/*1	*17/*17	*1/*1	*1/*22	*3/*3
1207	*1/*1	*1C/*1C	*1/*1	*1/*6	*2/*2	*1/*1	*1/*1	*1/*1	*1/*1	*1/*1	*3/*3
1211	*1/*1	*1F/*1F	*1/*1	*1/*1	*2x2/*4	*1/*1	*1/*2	*1/*1	*1/*1	*1/*1	*3/*3

CYP2C19 – no activity pool

CryostaX Geneknown™

Single Freeze Pooled Cryopreserved Human Hepatocytes

HPCH.2C19.NA Lot No. 1510236

Pool of 3

Assured Minimum Yield: 4.5 x 10^6 cells per vial
Viability: >70.0%

Individual Donor Genotype Information:

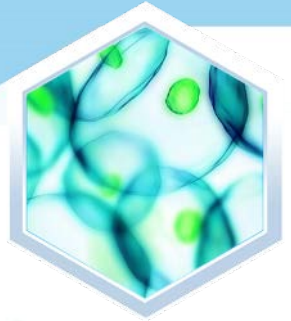
Donor	CYP1A1	CYP1A2	CYP2A6	CYP2B6	CYP2D6	CYP2C8	CYP2C9	CYP2C19	CYP2E1	CYP3A4	CYP3A5
960	*1/*2	*1C/*1F	*9/*9	*1/*16	*1/*36x2+*10	*1/*1	*1/*1	*2/*2	*1/*1	*1/*1	*3/*7
1205	*1/*1	*1/*1	*1/*1	*1/*1	*1/*4	*1/*1	*1/*1	*2/*2	*1/*1	*1/*1	*3/*3
1209	*1/*4	*1/*1	*1/*1	*1/*6	*1/*1	*1/*4	*1/*1	*2/*2	*1/*1	*1/*1	*3/*3

CYP2C19 – no activity pool

Enzyme	Marker Substrate Reaction	[S] (µM)	Rate (pmol/million cells/min)
CYP1A2	Phenacetin *O*-dealkylation	100	57.1
CYP2A6	Coumarin 7-hydroxylation	50	14.2
CYP2B6	Bupropion hydroxylation	500	27.1
CYP2C8	Amodiaquine *N*-dealkylation	20	126
CYP2C9	Diclofenac 4'-hydroxylation	100	258
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	400	0.57
CYP2D6	Dextromethorphan *O*-demethylation	80	37.3
CYP2E1	Chlorzoxazone 6-hydroxylation	500	90.6
CYP3A4/5	Testosterone 6β-hydroxylation	250	73.9
CYP3A4/5	Midazolam 1'-hydroxylation	30	13.7

Donor Information

Gender:	Male (3)
Age:	57-60 years of age
Race:	Caucasian (2), African American (1)
Cause of Death:	Cerebrovascular accident (3)
Cytomegalovirus (CMV):	Positive (2), Negative (1)
Human Immunodeficiency Virus (HIV):	Negative (3)
Hepatitis B Surface Antigen (HBsAg):	Negative (3)
Antibody to Hepatitis C Virus (HCV):	Negative (3)



Available Genotyped Products

Human Liver Microsomes

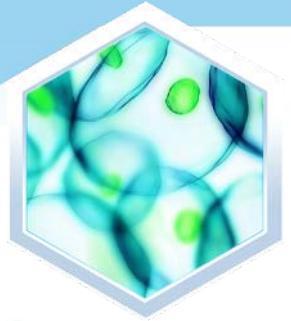
- High, moderate and no activity available
- CYP2C9, 2C19, 2D6, 3A5, UGT1A1, 1A9
- Used to study influence of allelic variance on the safety & efficacy of new compounds

0.5mL @ 20 mg/mL

Geneknown™ Hepatocytes

- High, moderate and no activity available
- CYP2C8, 2C9, 2C19, 2D6, 3A5, OATP1B1, UGT1A1
- Used to study genetic variants effect on DM & DT to identify risks of adverse interactions in genetically-defined subpopulations

Pooled, AMY > 4.5×10^6 cells



WHY?

Committed to furthering the knowledge surrounding hepatic diseases

WHAT?

Collection of high-quality human tissue specimens representing the early stages of alcoholic or non-alcoholic fatty liver disease. Tissue collected in a timely manner with precise care taken to minimize downtime and preserve tissue viability.

- Tissues allow for the analysis of expression of drug targets and early markers of fatty liver disease
- All samples come with pathologic diagnosis, demographic, BMI, history of diabetes and alcohol use data.
- Multiple photomicrographs available for each specimen.
- H&E slides prepared for each lot to illustrate tissue conditions.

Available Products

- Normal Liver Pre-Lysate
- Steatohepatitis Liver Pre-Lysate
- Steatosis Liver Pre-Lysate
- Hepatocytes (Select Donors)

Full Donor List Available Online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Thank You!