Pharmacogenetics of (Newer) Anticonvulsants

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Disclosures

- **Relevant Financial Relationships**
  - **Nothing to disclose**

- **Off Label Use**
  - **None**

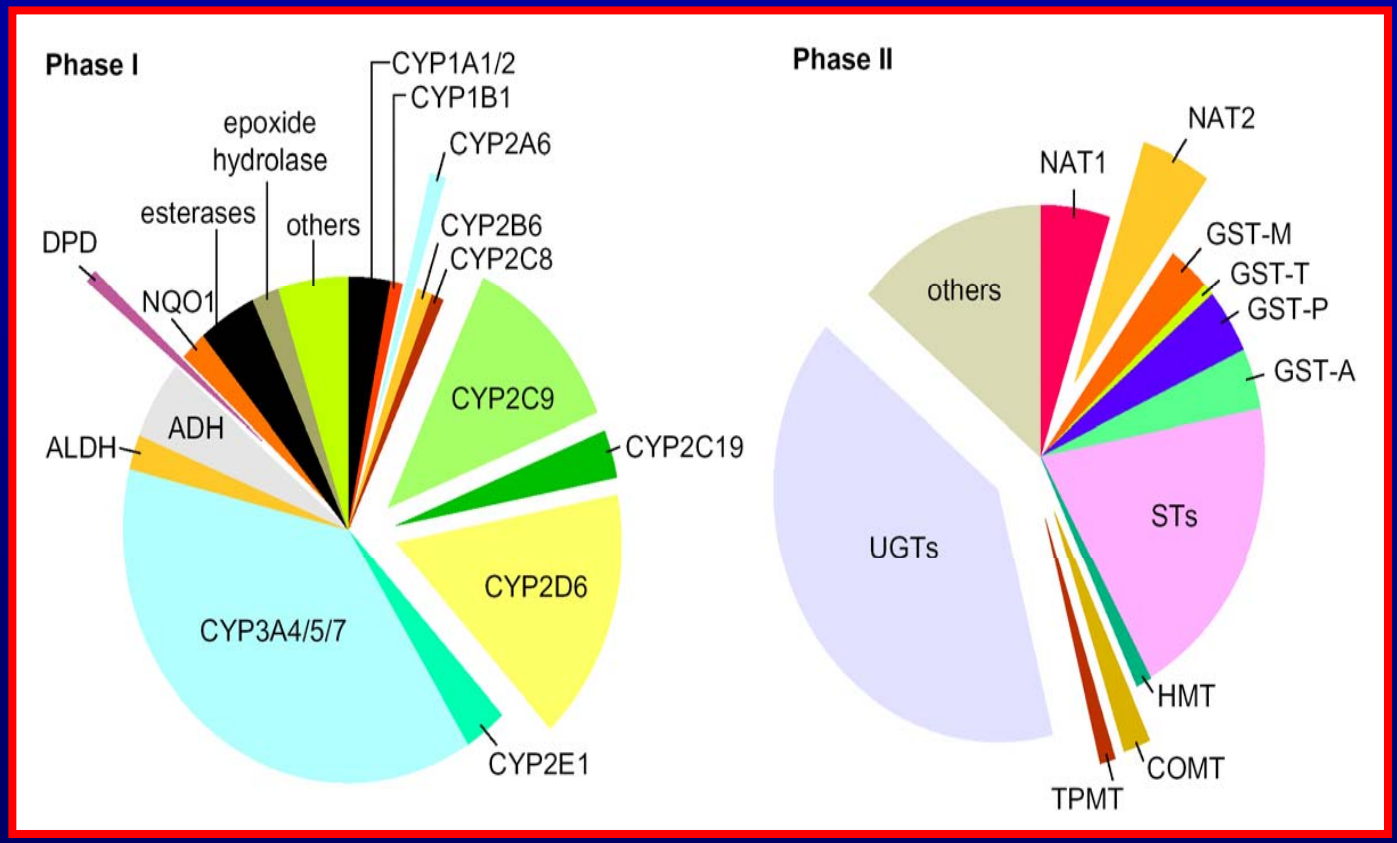
What is Pharmacogenetics?

- **Study of the genetic basis for individuality in drug metabolism and response**
  - **Drug-metabolizing enzymes/ drug transport molecules (Pharmacokinetics)**
  - **Drug targets (Pharmacodynamics)**
  - **Change in DNA sequence of the gene (*i.e.* an allele) results in a difference in the protein expressed**
    - **Amount of enzyme synthesized may be altered**
    - **Activity of the enzyme may be altered**
- **Application of this information to individualize therapeutic management**
  - **Enhance pharmacotherapy**
  - **Minimize adverse drug events (ADEs)**

Pharmacogenetics

- Testing is performed using DNA
- DNA is generally extracted from white cells in whole blood
- Alterations in the DNA are tested involving some means of amplification (target or signal)
  - Single nucleotide polymorphisms
    - Coding (amino acid changes in proteins)
    - Non-coding (may affect mRNA splicing or the amount of mRNA made)
  - Small insertions or deletions
  - Expansion or contraction of nucleotide repeats

Drug Metabolizing Enzymes



Evans, Relling, Science, 286:487-91, 1999

FDA Statement

21 CFR 201.57

"…if evidence is available to support the safety and effectiveness of the drug only in selected subgroups of the larger population with a disease, the labeling shall describe the evidence and identify specific tests needed for selection or monitoring of patients who need the drug."

Drug Label Changes

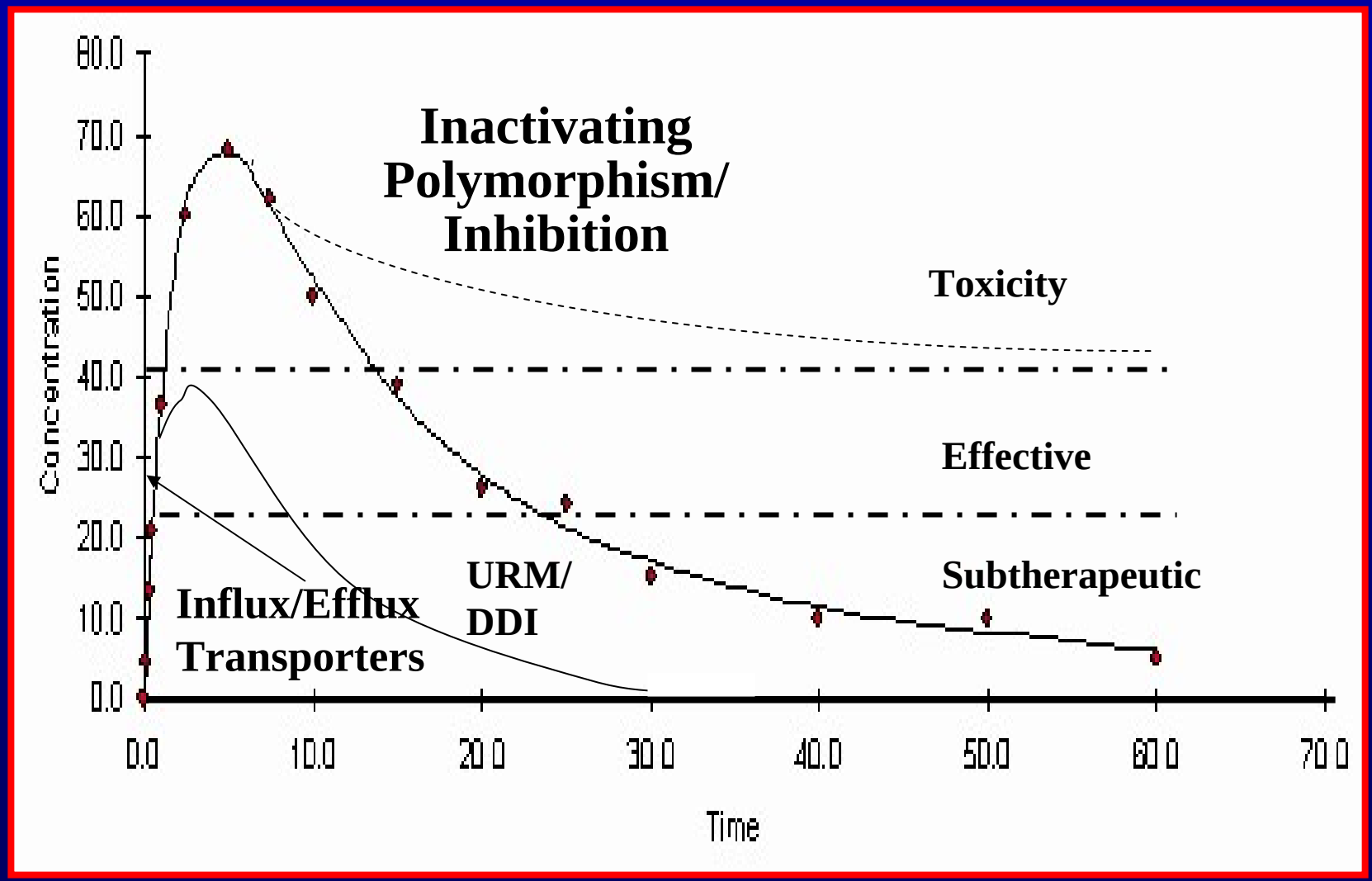
Drug	Testing	Recommendation
Imantinib	C-Kit Expression	Required
Thiopurines	TPMT genotype	Recommended
Irinotecan	UGT1A1 TA repeat	Recommended
Abacavir	HLA-B*5701	Recommended
Carbamazepine	HLA-B*1502	Recommended
Phenytoin	HLA-B*1502	Recommended
Warfarin	CYP2C9 genotype	Suggested
Atomoxetine	CYP2D6 genotype	Available
Tamoxifen	CYP2D6 genotype	Considering
Clopidogrel	CYP2C19 genotype	Considering

FDA Drug Label Guidance

- Description of polymorphic enzymes (for example, genetic-based differences in enzyme activity such as reduced cytochrome P450 enzyme activity due to polymorphisms in DMEs – ex. CYP2C9, CYP2C19)
- Pharmacogenomic studies performed that provide evidence of genetically based differences in drug metabolism (changes in dose based on genotype of DMEs or transporters)
- Clearance of the drug in relationship to genotype (AUC)
- Subpopulation-based information on the prevalence or frequencies of alleles, genotypes, haplotypes, or other genomic markers (related to DME polymorphisms)
- Predictive values associated with the use of the genomic marker for safety and/or efficacy purposes (HLA-B alleles)

http://www.fda.gov/cder/guidance/1923dft.pdf

AUC Examples



Older Anticonvulsant Drugs

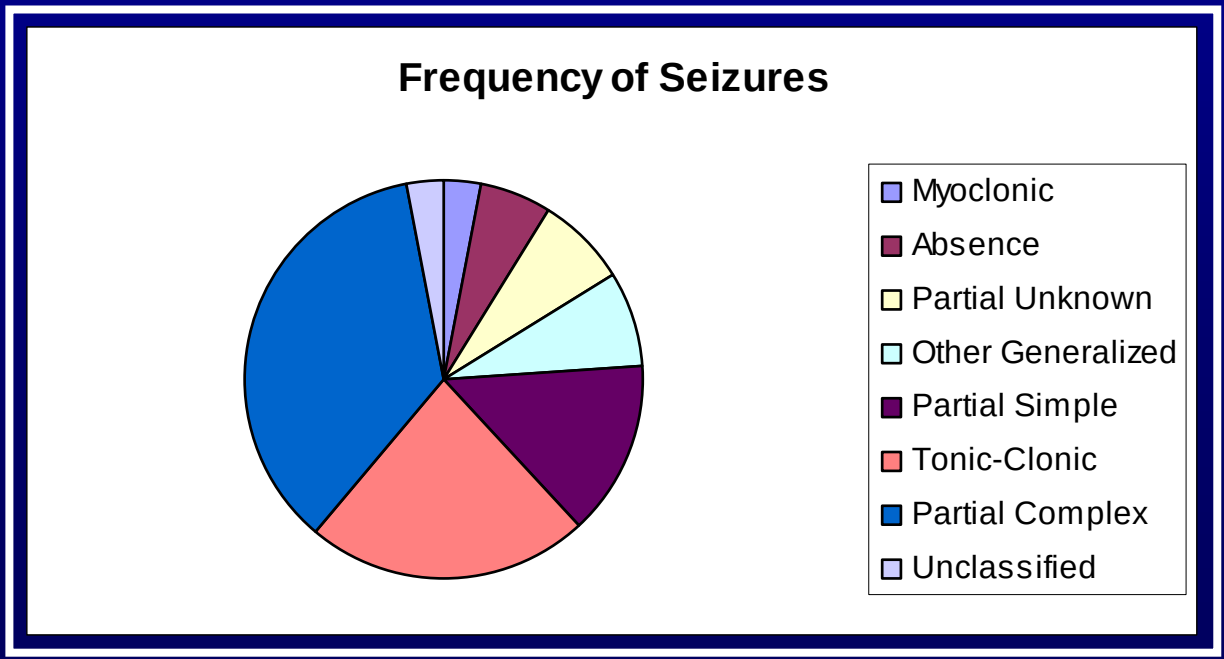
Drug	Year	Target
Phenobarbital	1912	GABA receptor complex (Cl CHNL)
Phenytoin	1938	Na CHNL inhibition
Primidone	1954	GABA receptor complex (Cl CHNL)
Ethosuximide	1960	Na/Ca CHNL inhibition
Carbamazepine	1974	Na CHNL inhibition; Adenyl cyclase inhibition
Clonazepam	1975	BZD agonist
Valproic Acid	1978	Na/Ca CHNL inhibition ****

Newer Anticonvulsant Drugs

Drug	Year	Target
Vigabatrin	1989	GABA transaminase inhib.
Gabapentin	1993	Ca CHNL inhibit/GABA incr.
Lamotrigine	1994	Na CHNL inhibition; Glu decr.
Felbamate	1994	Na CHNL inhib.; NMDA antagonist
Topiramate	1996	Na/Ca CHNL inhib.; GABA Receptor A; Glu antagonist
Fosphenytoin	1996	Na/Ca CHNL inhibition
Tiagabine	1997	GABA reuptake inhibit.
Levetiracetam	1999	SV2A inhibitor(?)
Zonisamide	2000	Na/Ca CHNL inhib.; DA incr.
Oxcarbazepine	2000	Na (K?) CHNL inhibition
Pregabalin	2004	Ca CHNL inhib; GABA incr.

Epilepsy and Seizures

- Affects ~ 1% of the US population
- New cases are ~1/1000 population per year
- Variety of causes
  - ~2/3 affected have no cause identified
- Different types of seizures



Clinical Indications

ACD	Partial	GTC	Absence	Myoclonic	InfSpasm	LGS
Phenobarbital	+	+	-			
Phenytoin	+	+	-	-		
Ethosuximide	-	-	+			
Carbamazepine	+	+	-	+		
Benzodiazepines	+	+	+	+		(+)
Valproate	+	+	+	+	(+)	(+)
Vigabatrin	+	+	-	-	+	(+)
Lamotrigine	+	+	-	+/-		
Tiagabine	+	-	-			
Levetiracetam	+	(+)	(+)	(+)		
Gabapentin	+	+	-			
Topiramate	+	+	(+)	(+)	(+)	+
Felbamate	+	+	(+)			+
Zonisamide	+	+				
Pregabalin	+	-	-	-		

Indications: Older ACDs

Drug	Anticonvulsant Indications
Phenobarbital	Seizures (tonic-clonic/cortical local/status epilepticus) except Absence
Phenytoin	Tonic-clonic, partial, seizures due to neurosurgery
Primidone	Same as phenobarbital
Ethosuximide	Absence seizures
Carbamazepine	Seizures except Absence
Clonazepam	Lennox-Gastaut (L-G) seizures myoclonic seizures. absence seizures unresponsive to succinimides
Valproic Acid	Complex partial, absence seizures

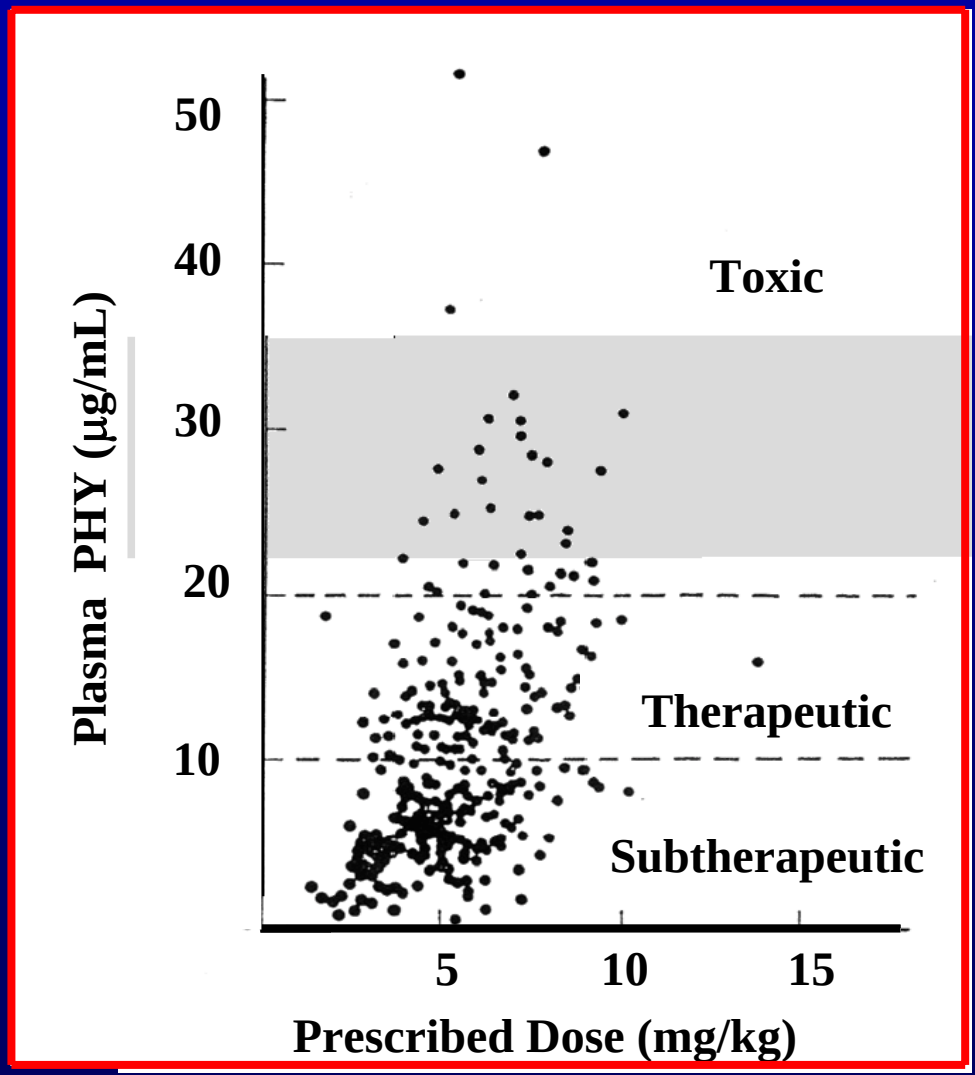
Indications: Newer ACDs

Drug	Anticonvulsant Indications
Vigabatrin	Adjunct for Resistant Epilepsy, partial seizures
Lamotrigine	Epilepsy (partial & tonic-clonic seizures)
Fosphenytoin	Epilepsy (when other options are not feasible)
Tiagabine	Adjunct for partial seizures
Levetiracetam	Monotherapy for epilepsy
Gabapentin	Clonic-tonic; Adjunct for partial seizures
Topiramate	Epilepsy in peds. and adults; L-G in peds.
Felbamate	Partial seizures adults; L-G in peds.
Zonisamide	Adjunct partial onset seizures in adults
Oxcarbazepine	Partial seizures in adults and peds. (>2yo)
Pregabalin	Adjunct for partial seizures

Metabolism of Older ACDs

Drug	Enzyme(s)
Phenobarbital	CYP2C19
Phenytoin	CYP2C19/CYP2C9 (predominant) glucuronidation
Primidone	CYP2C19
Ethosuccimide	CYPs/Glucuronidation
Clonazepam	Reduction; acetylation; glucuronidation
Valproic Acid	CYP2C9; UGT1A3,6,9; UGT2B7; β-oxidation (mitochondrial)
Carbamazepine	CYP3A4

Phenytoin Variability



Richens,A. (1979) Clin. Pharmacokin. 4:153

CYP2C9 and Phenytoin Toxicity

- CYP2C9 has been reported to be associated with phenytoin toxicity
- However, one association study systematically assessed the low-activity alleles CYP2C9*2 and CYP2C9*3 in relation to ADRs on phenytoin and found no correlation.
  - However, CYP2C9 is inducible so effects on individuals with a single deficient copy may be difficult to demonstrate
- Several case studies found CYP2C9 deficiency associated with severe phenytoin intoxication.
- CYP2C9*3 allele is most often implicated
  - Allele frequency is ~6% in North American population
  - Approx. 1 in 350 individuals tested are homozygous
  - CYP2C9 activity is reduced to 5 to 15 % of normal

CYP2C9 Polymorphisms

- CYP2C9 involved in the metabolism of NSAIDS, oral hypoglycemic agents, warfarin and a few ACDs
- Only 2 common deficiency polymorphisms

Allele		Changes	Activity	Allele Freq.
*1	Normal	None	100 %	75 - 85
*2	430 C → T	Arg > Cys	~ 50 %	10 - 15 %
*3	1075 A → C	Ile > Leu	5 - 10 %	5 - 10 %

- Several other infrequent or rare alterations
  - *4 - only reported twice in individuals from Asia
  - *5 - has slight decrease in activity 12 to 18%
  - *6 - single base deletion creates a null allele (<0.5% frequency)

CYP2C19 Polymorphisms

- CYP2C19 involved in the metabolism of proton pump inhibitors, antidepressants and a few ACDs
- Only 2 common polymorphisms; produce null activity alleles

Allele		Changes	Activity	Allele Freq.
*1	Normal	None	100 %	75 - 85
*2	681 G → A	Splicing	0 %	15 %
*3	636 G → A	Trp > Stop	0 %	0.2 - 75 %

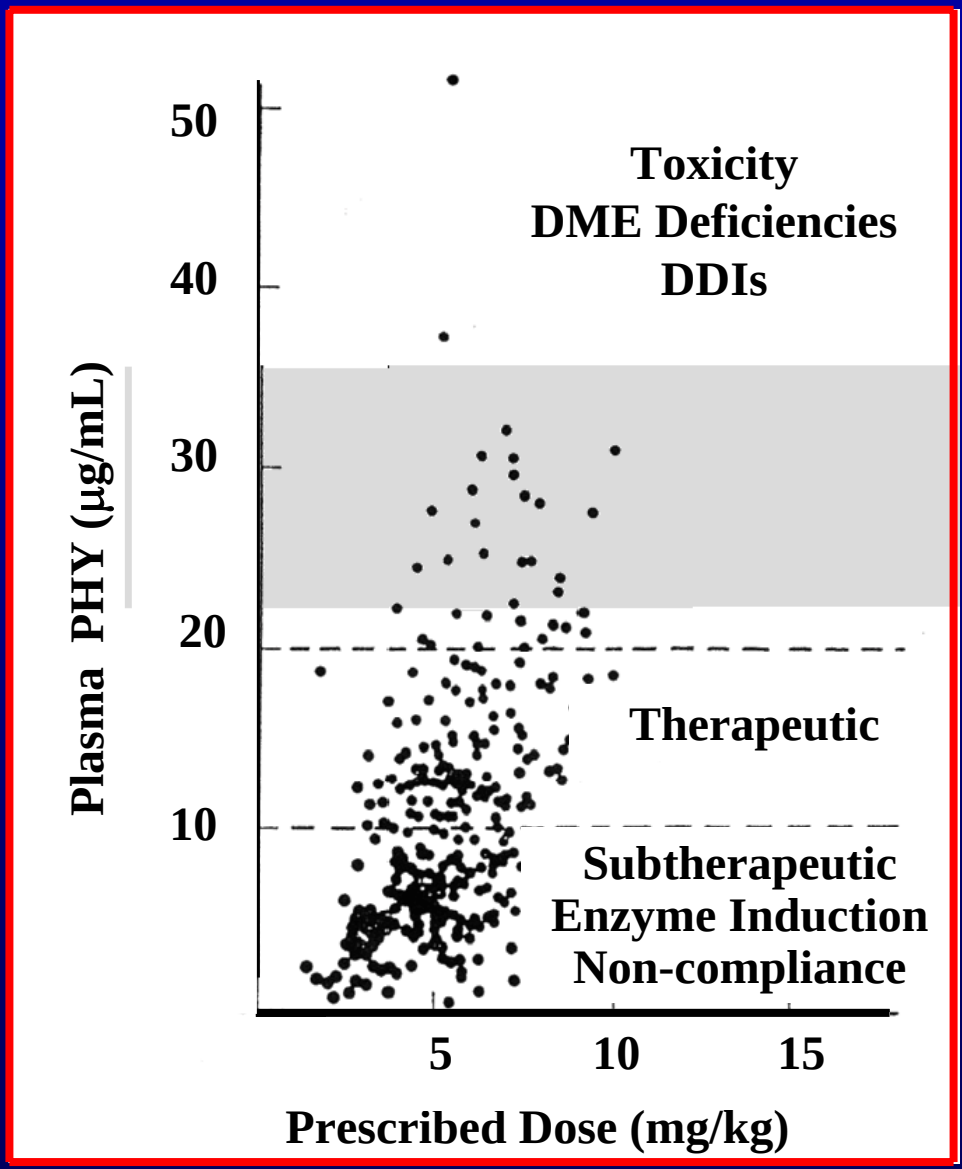
  - *3 polymorphism found predominately in Asian and Oceanian populations
- Several other infrequent or rare alterations

CYP2C9 and Phenytoin Toxicity

	Em9/Em19	Em9/Het19	Em9/Pm19	Het9/Het19	Het9/PM19
Km mg./kg/d	8.2	9.0	9.4	10.4	15.6
Vmax mg/kg/d	10	9.8	9.2	6.3	5.4
Clint mg/kg/d	1230	1081	976	607	348
Dose Range mg/kg/d	5.5-7	5-7	5-6	3-4	2-3

Hung et al. 2004

Phenytoin Variability



Metabolism of Newer ACDs

Drug	Enzyme(s)
Vigabatrin	CYP2E1
Lamotrigine	Glucuronidation
Fosphenytoin	CYP2C19/CYP2C9 (predominant)
Tiagabine	CYP3A4/5
Levetiracetam	Minimal hepatic metabolism (nonCYP)
Gabapentin	No significant metabolism
Topiramate	70% excreted unchanged; some hydroxylation and glucuronidation
Felbamate	CYP3A4/CYP2E1
Zonisamide	CYP3A4/5; CYP2C19
Oxcarbazepine	Cytosolic enzymes (nonCyp)
Pregabalin	Negligible metabolism

Inhibition/Induction Issues

Drug	Elimination	T1/2 (h)	Comments
Phenobarbital	Renal/hepatic	100	Induces CYP2C, CYP3A, UGT
Phenytoin	Hepatic	13-69	Induces CYP2C, CYP3A, UGT; inhibits CYP2C9
Carbamazepine	Hepatic	12-17	Induces CYP2C, CYP3A, UGT
Valproic acid	Hepatic	9-16	Inhibits epoxide hydrolase, CYP2C9,UGT
Felbamate	Renal/hepatic	20	Induces CYP3A4; inhibits CYP2C19, β-Oxidation
Gabapentin	Renal	?????	No induction/inhibition
Lamotrigine	Hepatic	25	No induction (?)/inhibition
Topiramate	Renal/hepatic	23	Induces β-oxidation; inhibits CYP2C19
Tiagabine	Hepatic	7-9	No induction/inhibition
Levetiracetam	Renal	7	No induction/inhibition
Oxcarbazepine	Hepatic/renal	8 to 10	Induces CYP3A4/5; inhibits CYP2C19
Zonisamide	Renal/hepatic	60	No induction/inhibition

Summary of Induction

Weak Inducers	Strong Inducers	Non-Inducing Drugs
Felbamate (CYP3A4)	Carbamazepine	Gabapentin
Lamotrigine (UGT?)	Phenobarbital	Levetiracetam
Oxcarbazepine (CYP3A4)	Phenytoin	Pregabalin
Topiramate (CYP3A4)		Tiagabine
		Valproic Acid
		Zonisamide

Variability with Newer ACDs

- Large individual variations in relationship between serum concentrations and dose have been reported for:
  - Felbamate – CYP3A4/2E1; induces CYP3A4/5
  - Oxcarbazepine – nonCYP; induces CYP3A4/5
  - Tiagabine – CYP3A4/5; no induction
  - Levetiracetam – minimal nonCYP; no induction
  - Zonisamide – CYP3A4/5, CYP2C19; no induction
- Some of the variability may be due to CYP3A4 variability which cannot be readily predicted by pharmacogenetics
  - Polymorphisms in CYP3A4 do not correlate with enzymatic variability which is reported to range 60- to 100-fold between individuals

Why is there so little PGx evidence?

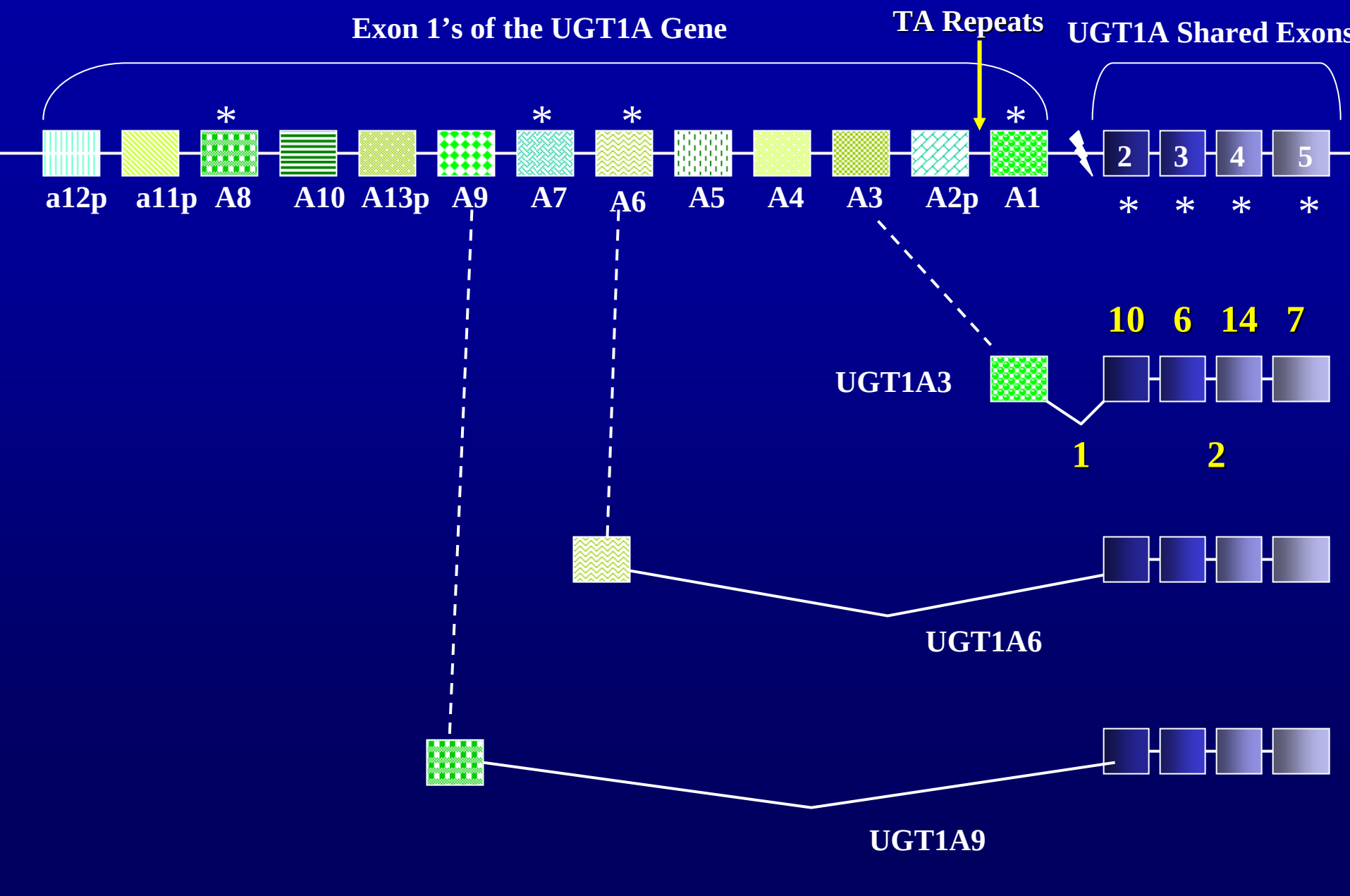
- Metabolism of the ACDs is often complicated
  - Several enzymes may be involved
  - Multiple polymorphisms
  - Polypharmacy
  - Hard to get a study of sufficient size to have adequate statistical power (small studies often have contradictory results)
- Limited funding
  - Many of the drugs are not under patent, so no incentive to put funding behind studies

Example - Valproic Acid

- Valproic acid metabolism is complex
  - Glucuronidation (4 genes)
  - CYP2C9 - polymorphic
  - β-Oxidation in mitochondria – issue in elderly
- Inhibits CYP2C9 and UGTs at physiologic concentrations
- Metabolism varies widely in hepatocytes
- Toxicities are well established and variable
  - CNS (cerebral edema)
  - CV (hypotension)
  - GI (pancreatitis, hyperammonemia)
  - Metabolic (hypernatremia, metabolic acidosis)
  - Hematologic (thrombocytopenia, leucopenia)

Clin Toxicol 2002;40:789-801

UGT1A Gene Complex



SNPs for Valproic Acid

- **UGT1A Exons 2-5 – 40 SNPs/Mutations**
- **UGT1A3 - 7 SNPs**
- **UGT1A6 - 4 SNPs**
- **UGT1A9 - 4 SNPs**
- **UGT2B7 - 1 SNP**
- **CYP2C9 - 5 SNPs (existing assay)**
- **61 SNPs total – a difficult, expensive assay**
  - **TDM is less expensive, but performed after toxicities may have occurred**

Summary: Valproic Acid PGx

- Genotyping single genes is not too informative
- To assess for specific drugs:
  - Need to genotype polymorphic genes in the metabolic pathway
  - Multiplex genotyping for more than one gene
  - Genotyping assay can ascertain if there is a genetic basis for the metabolizer phenotype
  - Drug monitoring can confirm metabolizer phenotype as well as verify compliance, easier and less costly

Age Related Changes in PGx

- Consideration of age-related changes in the activity of specific P450 isoenzymes involved in AED metabolism (e.g., CYP2C9, CYP2C19, CYP3A4) would be a more accurate approach to assessing alterations in drug metabolism in the elderly
- Antipyrine clearance declines by 0.34 mL/minute per year after age 40 and drug metabolism by the liver is reduced by 30% in persons over age 70
- Studies of certain drugs (i.e. propranolol) have suggested that oxidative metabolism in the liver declines with age while conjugative metabolism remains virtually unchanged
- Has significant implications for ACDs that are metabolized by the CYP microsomal enzymes

Transporters

- Required to get drugs into/out of cells
- Mixed Results – evenly divided 5 pro/5 con
  - ABC Cassette Transporters (use ATP)
    - P-Glycoprotein (MDR1 or ABCB1)
    - Non-coding SNP 3435C>T associated with changes in plasma concentrations of AEDs
    - 3435C>T is predicted to cause a stem/loop structure in the mRNA decreasing transcription of the gene
- Non-ABC transporters – 1 pro/2 con
  - RLIP76 (RALBP1) found over-expressed in neuronal tissues of epilepsy patient and localized to the blood-brain barrier: carbamazepine and phenytoin
  - Localization and role disputed by 2 subsequent studies

http://www.biomedcentral.com/1471-2202/6/61

Targets: SCN1A

- *SCN1A* encodes the α–subunit of the voltage-gated neuronal sodium channel
- A SNP rs3812718 (IVS5N+5G>A) is highly associated with maximum daily dose of phenytoin
- The IVS5N+5G>A polymorphism disrupts the consensus sequence of the 5´ splice donor site of a highly conserved alternative exon 5N
- Significant correlation was established between IVS5N+5G>A genotype and the proportion of SCN1A transcripts containing exon 5N

Pharmacogenet. Genomics 16(10), 721–726 (2006).

IVS5N+5G>A

- **IVS5N+5G>A is significantly associated with phenytoin serum levels at maintenance dose**
- **Patients with the AA genotype taking on average 50 mg more than those carrying the GG genotype**
- **When correlated with CYP2C9 genotype, phenytoin doses ranged from a mean of 250 mg in individuals carrying CYP2C9*3*3_GG to 377 mg in individuals carrying CYP2C9*1*1_AA**
- **The *SCN1A* SNP was also correlated with maximum daily dose of carbamazepine, which also binds to the voltage-gated neuronal sodium channel**

***Am. J. Hum. Genet.* 80, 876–883 (2007)**

Carbamazepine Warning

- SERIOUS AND SOMETIMES FATAL DERMATOLOGIC REACTIONS, INCLUDING TOXIC EPIDERMAL NECROLYSIS (TEN) AND STEVENS-JOHNSON SYNDROME (SJS), HAVE BEEN REPORTED DURING TREATMENT WITH TEGRETOL. .....
- A STRONG ASSOCIATION BETWEEN THE RISK OF DEVELOPING SJS/TEN AND THE PRESENCE OF HLA-B*1502, AN INHERITED ALLELIC VARIANT OF THE HLA-B GENE. HLA-B*1502 IS FOUND ALMOST EXCLUSIVELY IN PATIENTS WITH ANCESTRY ACROSS BROAD AREAS OF ASIA .....
- PATIENTS WITH ANCESTRY IN GENETICALLY AT-RISK POPULATIONS SHOULD BE SCREENED FOR THE PRESENCE OF HLA-B*1502 PRIOR TO INITIATING TREATMENT WITH TEGRETOL .....

SJS and TEN

- SJS is a serious disorder of the skin and mucous membranes
  - SJS occurs with an estimate of 1 to 6 per 10,000 in patients treated with various drugs
  - SJS occurs at 10-fold greater frequency in Asian populations
  - Flu-like symptoms, followed by inflammation of the mucous membranes and a painful rash that spreads and blisters, eventually causing the top layer of your skin to die and shed
- TEN is more serious and life-threatening
  - Detachment of the epidermis from the lower layers of the skin all over the body
  - Treatment is similar to that for severe burns

Drugs Associated with SJS/TEN

- Sulfonamides
- Penicillins
- Barbiturates
- Allopurinol
- Anticonvulsants
  - carbamazepine
  - phenytoin and fosphenytoin
  - lamotrigine
  - lamotrigine with sodium valproate (increased risk)
  - phenobarbital

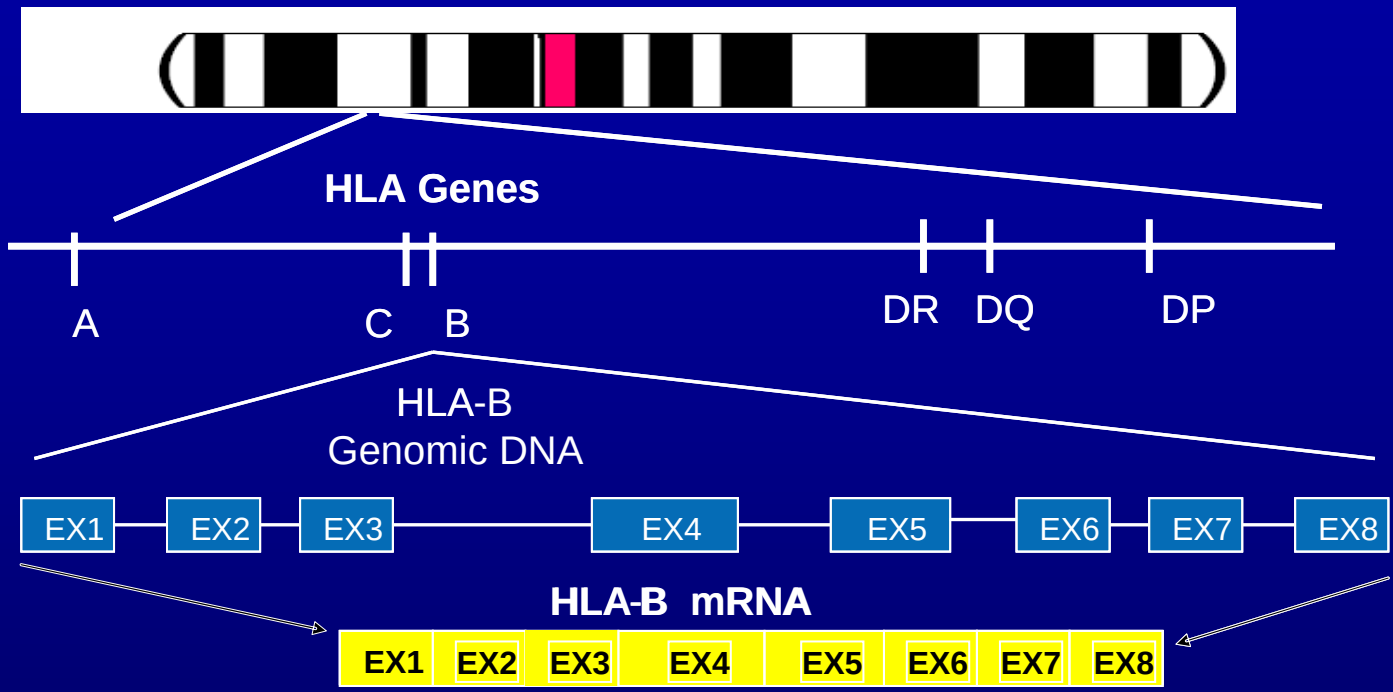
Carbamazepine and HLA-B Gene

- Carbamazepine-SJS is significantly more common in patients with the specific human leukocyte antigen (HLA) allele, HLA-B*1502
- 100% of Han Chinese patients who developed Stevens–Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) on carbamazepine therapy had the HLA-B*1502 allele, versus 9% of unaffected controls
- HLA genes are located on the short arm of chromosome 6
- HLA genes encode for cell surface-expressed proteins
  - Class I expressed on most nucleated cells
    - Encoded by HLA-A, HLA-B, and HLA-C genes
  - Class II found on antigen-presenting cells
    - Encoded by HLA-DR, HLA-DQ, and HLA-DP genes

HLA-B Antigens and Alleles

- Class I HLA proteins (including HLA-B) present peptides to CD8+ T lymphocytes
  - This may elicit an immune response
- HLA-B: 1 gene, but generates many protein antigens:
  - more than 500 allelic variants identified by molecular methods
  - not all allelic variants cause a change in amino acid sequence of the HLA-B antigen on the surface of cells
  - approximately 50 HLA-B protein antigens encoded by the HLA-B allelic variants identified serologically

HLA-B*1502



CCG	GAG	UAU	UGG	GAC	CGG	AAC	ACA	CAG	AUC	UAC	AAG	GCC	CAG	GCA	CAG	ACU	GAC	CGA	GAG	Ref.
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Pro	Glu	Tyr	Trp	Asp	Arg	Asn	Thr	Gln	Ile	Tyr	Lys	Ala	Gln	Ala	Gln	Thr	Asp	Arg	Glu	Ref.
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Summary

- Little clear cut evidence to recommend PGx screening testing for ACDs
- May be some benefit to identifying the cause for toxicities in a more limited number of patients for select drugs in order to make dosing adjustments
- Newer ACDs may still have plasma concentration/dose variability despite limited metabolism and absence of induction issues (absorption issues for example)
- Significance of polymorphic transporters not established
- Testing for prediction of CZ maximally-tolerated dose is available commercially
- Testing HLA-B*1502 is recommended for those of Asian ancestry who are to be initiated on CZ, PHY or Fos-PHY

Final Thought

- **Clinicians caring for children who have epilepsy anticipate further advances in the pharmacogenetics and molecular pathophysiology of epilepsy, leading to individually tailored, effective, and safe therapy.**

Bergin and Connolly (2002) New Antiepileptic Drug Therapies. Neurologic Clinics of North America

Thanks to

- **Thomas P. Moyer, Ph.D. – providing support for development of the PGx program**
- **Loralie Langman, Ph.D. – Associate Director of the Nucleotide Polymorphism Laboratory**
- **Staff of the NPL - for the opportunities to work with them and for them**

Further Reading

- **Shorvon, S. (2005) Handbook of Epilepsy Treatment.**
- **Rogawski and Loscher (2004). The neurobiology of antiepileptic drugs. *Nat. Neurosci. Rev.* 5: 553-64**
- **Depondt, C. (2008) Pharmacogenetics in Epilepsy Treatment: Sense or Nonsense? *Personalized Med.* 5:123-131**
- **Hung et al. (2004) Dosage recommendation of phenytoin for patients with epilepsy with different CYP2C9/CYP2C19 polymorphisms. *Ther. Drug Monit.* 26:534–540**
- **Collins et al. (2006) Antiepileptic Agents and Metabolic Drug-Drug Interactions: Focus on Induction. Millennium CME Institute, Hampton, NH**
- **Celeste et al. (2007) Association between HLA-B*1502 allele and anti-epileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48:1015-18**