Slide 1

MBChB221B

Variability due to genetic differences

Dr Nuala Helsby
Molecular Medicine and Pathology

Slide 2

Objectives

- Understand how inherited differences in drug metabolising enzymes may influence
- Plasma pharmacokinetics (clearance)
- Drug safety (adverse reactions)
- Drug effectiveness

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Variability

- Many factors can influence the variable disposition of drugs & pharmacogenetics is only one of these factors
  - Sex
  - Age
  - Organ function
  - Pregnancy
  - Co-morbidities
  - Polypharmacy & Drug Interactions
  - **Genetic variation** (Pharmacogenetics/genomics)

Slide 4	**Inherited differences in the disposition of drugs** • Inherited differences can occur in any of the proteins which influence the absorption, distribution, metabolism & excretion of drugs (ADME) and also in drug receptors ("targets") • The focus of this lecture will be inherited variation in drug metabolism • the concepts that I will introduce also apply to the other proteins involved in drug disposition	
Slide 5	**Heredity and phenotype** • Heredity is the passing of traits from parents to their offspring • a trait is a distinct variant of a phenotypic characteristic • A phenotype results from the expression of genes *as well as* the influence of environmental factors • This lecture will focus on variant phenotypes of drug metabolism that are due to inherited (genetic) differences	
Slide 6	**Drug metaboliser traits (phenotypes)** • **Poor metaboliser (PM)** • Intermediate metaboliser (IM) • Extensive metaboliser (EM) • Reference "normal" trait • **Ultra-rapid metaboliser (UM)** All of these traits will be discussed in this lecture	

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Genetic polymorphism

- When two or more clearly different phenotypes exist in the same population it is called **polymorphism**
  - i.e. At least two distinct forms or 'morphs' exist
- Genetic polymorphism is when **gene mutations** result in an altered protein and **altered phenotype**
- When the distinct form of the gene occurs in **at least 1% of the population** it is a genetic polymorphism
  - if it occurs in <1% of a population it is considered a rare mutation
- Numerous genetic polymorphisms occur in drug metabolising enzymes and these can result in phenotypic changes in drug metabolism and potentially change plasma pharmacokinetics

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Variant genes

- The activity of drug metabolising enzymes can be altered due to any of the following inherited gene changes
  - Single nucleobase variants (SNP)
  - Insertions or deletions of nucleobases (indel)
    - Variable number tandem repeats (VNTR)
  - Whole gene deletions or gene duplications
    - Copy number variations, CNV
- Many of the clinically relevant genetic polymorphisms that influence drug metabolism are located either in the coding region of the gene (exons), or in the promoter region of the gene

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Pharmacogenetics nomenclature

Cytochrome **P**450 (CYP) as an example

- A *GENE* name is italicised to differentiate it from the PROTEIN it encodes
- Polymorphic forms of each *CYP* gene are denoted by ✱ ("star")
- The wild-type (reference) form of the gene is typically ***1**
- Individual gene polymorphisms (**variant alleles**) are then reported numerically in the order they are identified & characterised
  - E.g. *CYP2C9**2; *CYP2C9*3, CYP2C9*4* etc
- A variant (✱ 'star') allele may be due to
  - a single nucleotide change (SNP)
  - a combination (haplotype) of SNPs
  - an indel (VTNR)
  - a gene duplication
  - a gene deletion
- Each **variant allele** will have a characterised functional change
  - Note many star variants have no clinical relevance

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Poor metaboliser (PM) trait

- Inherited loss of function of a drug metabolising enzyme can result in no enzyme activity
- The poor metaboliser trait **may** lead to higher drug levels in the blood & this **might** result in increased risk of an adverse drug reaction compared with individuals with "normal" metabolism
  - Reference group of 'normal' metabolisers are called extensive metabolisers (EM)

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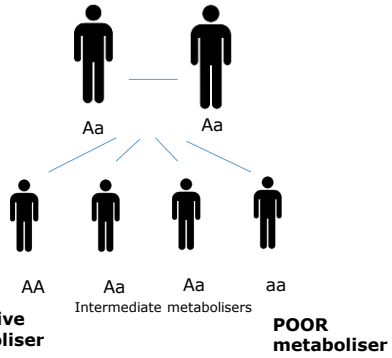
- The poor metaboliser (PM) phenotype is typically inherited in a autosomal recessive Mendelian manner co-dominant
- Remember humans are diploid & poor metabolisers are homozygous for null function variant (e.g. aa)



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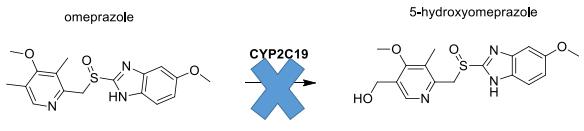
Omeprazole

- Proton pump inhibitor (PPI)
  - irreversible inhibitor of the H^+/K^+-ATPase in the gastric parietal cells
    - suppresses stomach acid secretion
  - Treatment of peptic ulcer, GERD
- The major route of elimination of omeprazole is hepatic clearance by the CYP2C19 enzyme



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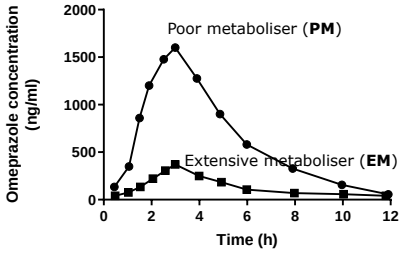
- Individuals who have inherited two variant copies of null function *CYP2C19* will be poor metabolisers of omeprazole
- Plasma omeprazole concentrations will be elevated due to decreased hepatic clearance



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Example PK of omeprazole

CYP2C19	**Omeprazole AUC $_{0-\infty}$** (ng·h/mL)	**Cl/F** (L/h/kg)
Extensive metaboliser (EM)	**1658.69**	**0.55**
Intermediate metaboliser (IM)	3887.72	0.22
Poor metaboliser (PM)	**9130.84**	**0.05**

- Poor metabolisers can also be detected by measuring the ratio of drug/metabolite in the plasma

Román, M., Ochoa, D., Sánchez-Rojas, S. D., Talegón, M., Prieto-Pérez, R., Rivas, Â., ... & Cabaleiro, T. (2014). *Pharmacogenomics, 15*(15), 1893-1901.

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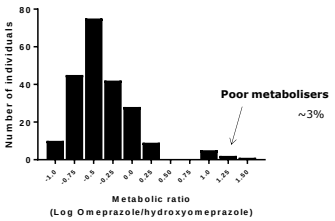
Metabolic ratio (MR)

- **Drug/metabolite** plasma concentration = **metabolic ratio**
  - **High ratio = poor metaboliser**
  - **Low ratio = extensive metaboliser**
- The **frequency** distribution of MR in a population can detect a **bimodal** distribution (i.e. a phenotypic polymorphism)



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CYP2C19 genetics

Allele		CYP2C19 activity
CYP2C19*1	wt (reference)	**Normal**
CYP2C19*2	SNP c.681**G>A** (rs 4244285) Splicing variant	**No CYP2C19 protein produced** (no enzyme activity)
CYP2C19*3	SNP c.636 G>**A** rs4986893 Premature stop codon	**No CYP2C19 protein produced** (no enzyme activity)

- Remember humans are diploid
- Poor metabolisers can be any of the following genotypes
  - *CYP2C19*2/CYP2C19*2*
  - *CYP2C19*2/CYP2C19*3*
  - *CYP2C19*3/CYP2C19*3*
- i.e. **homozygous for null function**
- **Approx 3%** of European ancestry populations are CYP2C19 PM phenotype
- **Approx 15-20%** of Asian ancestry populations are CYP2C19 PM phenotype

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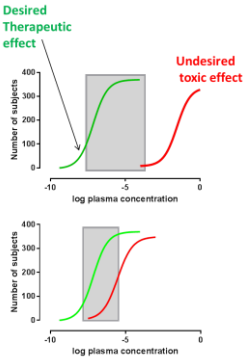
Omeprazole has a wide therapeutic index

- Elevated omeprazole concentrations in PM are *unlikely* to cause substantial toxicity
- A wide range of omeprazole concentrations can effectively supress gastric acid secretion
- There is conflicting data as to whether PM *CYP2C19* genotype influences response to treatment
- n.b. "cure" of a peptic ulcer requires combination of PPI with an effective antibiotic for *H. pylori* infection



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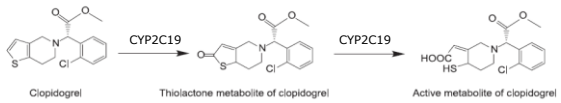
Clopidogrel

- Clopidogrel is used as antiplatelet therapy (combined with aspirin) after percutaneous coronary intervention (i.e. a stent) to minimise the risk of further ischemia due to thrombosis
- Clopidogrel is a **prodrug** that is bioactivated by **CYP2C19**



CYP2C19 poor metabolisers (PM) are likely to have very low bioactivation and therefore poor therapeutic response?

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CYP2C19 genetics

Allele		CYP2C19 activity	MAF (European ancestry)
CYP2C19*1	wt (reference)	**Normal**	**0.56**
CYP2C19*2	SNP c.681**G>A** rs4244285 Splicing variant	**No CYP2C19 protein produced** (no enzyme activity)	**0.145**
CYP2C19*3	SNP c.636 G>**A** rs4986893 Premature stop codon	**No CYP2C19 protein produced** (no enzyme activity)	**0**
CYP2C19*17	SNP g.-806C>T in the promoter region of gene **which is thought to increase the transcription of the gene**	**Slightly increased amount of the enzyme** (High normal)	**0.23**

MAF= Minor allele frequency

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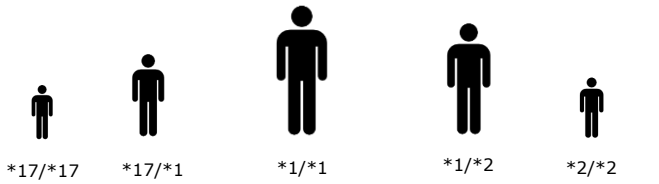

*17/*17 *17/*1 *1/*1 *1/*2 *2/*2

Rapid metaboliser (high normal) | **Extensive metaboliser** | **Intermediate metaboliser** | **POOR metaboliser**

*17 "carriers" *2 "carriers"

High formation of active metabolite

Low formation of active metabolite

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***CYP2C19*17* carriers**

Clopidogrel "high normal" activation

excessive pharmacodynamic response

Increased risk of excessive bleeding
N= 7,660 patients;
HR= 1.26 p=0.011

*CYP2C19*1/1* normal

LESS chance of another CVE
N= 6,584 patients;
HR= 0.75 p<0.001

***CYP2C19*2* carriers**

Clopidogrel "poor" activation

NO increased risk of another CVE
N= 16,360 patients;
HR= 1.23 p>0.05

attenuated pharmacodynamic response

Increased risk of a stent thrombosis
N= 8,686 patients;
HR= 2.24 p<0.001

meta-analysis of published studies (Zabalza et al 2012 *Heart* 98:100-108)

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Choices?

- Standard recommended dosage?
- Dose adjustment?
- Alternative drug?

Other P2Y12 inhibitors: prasugrel and ticagrelor, do not require metabolic conversion

- These are associated with higher bleeding risks than clopidogrel
- More effective than clopidogrel for preventing ischemic events

but these comparisons were undertaken in all pts receiving clopidogrel & a *substantial proportion will have been PM!*

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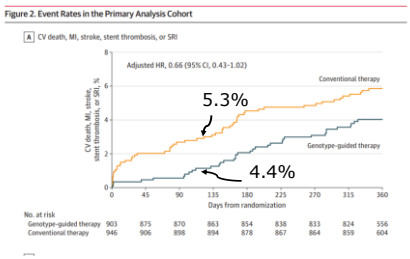
- **Editorial: August 25, 2020:** *JAMA.* 2020;324(8):747-749. doi:10.1001/jama.2020.13094

Genotype-guided dosing: RCT

- *CYP2C19* *2 carriers (IM & PM) were prescribed ticagrelor and non-carriers clopidogrel
- The primary end point was a **composite** of cardiovascular death, myocardial infarction, stroke, stent thrombosis, and severe recurrent ischemia at 12 months



p=0.16

JAMA. 2020;324(8):761-771. doi:10.1001/jama.2020.12443

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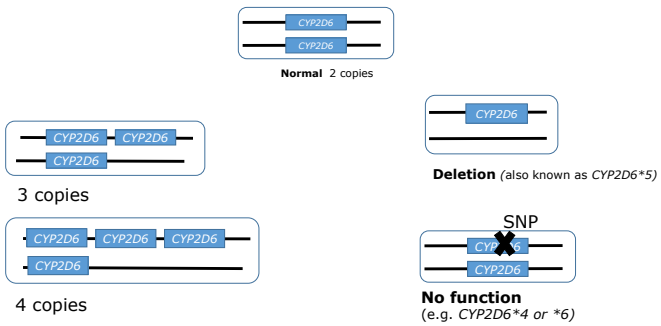
CYP2D6: Gene duplication or deletion and/or SNP



Ultra–rapid metabolisers
UM

Individuals with no functional alleles
i.e. a combination of SNPs or a deletion are
Poor metabolisers (**PM**)

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Ethnic variation in the prevalence of *CYP2D6* phenotypes

Ancestry	UM (gene duplication)	PM (homozygous null)
European	~ 4 %	~ 7 %
Asian	~ 2 %	< 1 %
African	~ 5 %	~ 1 %

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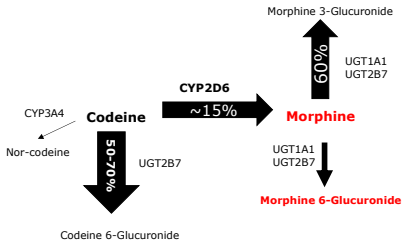
Codeine

Analgesic properties of codeine requires the conversion to **morphine by CYP2D6**
Morphine has 200-fold higher affinity for the μ-opioid receptor
Morphine 6-glucuronide is also active at this receptor



- **What is the risk if a patient is a *CYP2D6* UM?**
- **What is the risk if a patient is a *CYP2D6* PM?**
- **What could you choose to do *if you know* a patients *CYP2D6* genotype?**

Most of the dose of codeine is eliminated by these routes

Active metabolites with analgesic properties shown in red

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Pharmacogenetics based dosing guidelines

- Guidelines which assess and summarise scientific information for gene-drug combinations are regularly published in peer reviewed literature by the **Clinical Pharmacogenetics Implementation Consortium (CPIC)**
  - *Clinical Pharmacology and Therapeutics*
- All CPIC resources can be found at https://cpicpgx.org/ including the **dosing guidelines** https://cpicpgx.org/guidelines/

- Dutch Pharmacogenetics working group (DPWG) have pharmacogenomics guidelines for therapeutic (dose) recommendations
  - >80 drugs http://upgx.eu/guidelines/

- National Center for Biotechnology Information (NCBI) publish **drug-gene pair summary information**
  - Medical Genetics Summaries https://www.ncbi.nlm.nih.gov/books

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An example from CPIC

Phenotype	Implications for codeine metabolism	Recommendations for codeine therapy	Classification of recommendation for codeine therapy[a]
Ultrarapid metabolizer	Increased formation of morphine following codeine administration, leading to higher risk of toxicity	Avoid codeine use due to potential for toxicity.	Strong
Extensive metabolizer	Normal morphine formation	Use label-recommended age- or weight-specific dosing.	Strong
Intermediate metabolizer	Reduced morphine formation	Use label-recommended age- or weight-specific dosing. If no response, consider alternative analgesics such as morphine or a nonopioid.	Moderate
Poor metabolizer	Greatly reduced morphine formation following codeine administration, leading to insufficient pain relief	Avoid codeine use due to lack of efficacy.	Strong

Clin Pharmacol Ther 2014; 95:376–382

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Tramadol

- Tramadol is also a CYP2D6 substrate
- It is also a prodrug

- Prescribing the label recommended standard dose of tramadol to a *CYP2D6* poor metaboliser individual will result in:

- Sub-therapeutic concentrations?

Or

- Supra-therapeutic concentrations?

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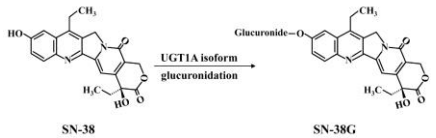
Irinotecan

- Irinotecan (SN-38) is used in treatment of colorectal cancer
  - Anti-neoplastic effect of SN-38 is due to the potent inhibition of DNA topoisomerase
- *This drug has a very narrow therapeutic index*
  - severe-life threatening neutropenia & GI damage occurs in some people
- SN-38 is eliminated by hepatic glucuronidation
  - catalysed by the **UGT1A1** enzyme



SN-38 SN-38G

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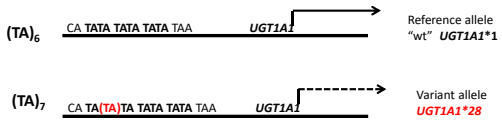
Irinotecan (SN-38) and *UGT1A1*28*

- 2bp (TA) insertion in the *UGT1A1* gene (rs8175347) is called ***UGT1A1*28***



- About 9% of Europeans are homozygous for *UGT1A1*28 & have Gilberts syndrome (intermittent mild hyperbilirubinemia)
- This variant **decreases** transcription by ~ 75%, i.e. = 25% **less** enzyme
- Individuals who are homozygous variant for *UGT1A1*28* are expected to have 50% **lower enzyme activity** than individuals who are *1/*1

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*UGT1A1*28* influences clearance of SN-38 in a predictable manner

*Summary of 11 clinical studies	*UGT1A1* genotype		
	*1*1	*1*28	*28*28
Relative changes observed in Clearance (Cl) of SN-38	**100%**	**80%** (73-86)	**62%** (44-79)

*Stingl et al. Pharmacology & Therapeutics 141 (2014) 92–116

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*UGT1A1*28* gene dose and risk of severe toxicity- grade 3-4 **neutropenia**

	n	Odds ratio (95%CI)	p
*1/*28 vs *1/*1 (heterozygotes vs wildtype)	1095	**1.90** (1.44-2.51)	0.00001
*28/*28 vs *1/*1 (homozygotes vs wildtype)	1035	**4.79** (3.28-7.01)	0.00001

Risk of neutropenia probably correlates with differences in CL_H of SN-38

Pharmacogenomics J. 2014 Apr;14(2):120-9.

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- The recommended dosage for irinotecan was based on the maximum tolerated dose in patients of **unknown** *UGT1A1* genotype
- *The* ~9% of individuals who were *UGT1A1**28/*28 will have influenced the apparent safe dose in the whole population
- This suggests that patients who are NOT *28 carriers may be underdosed!
- Consider:
  - Decrease the standard dose in patients with known *UGT1A1**28 homozygosity to improve safety?
  - Increase the standard dose in patients with known lack of *UGT1A1**28 to improve effectiveness?

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UGT1A1 genotype-directed dosing of irinotecan

- Patients stratified by **1/*1, *1/*28* and **28/*28* genotype
- All patients started at 400 mg dose (90 min IV infusion) & then dose escalated at next treatment to identify the maximum-tolerated dose for each genotype

UGT1A2 genotype	*1/*1	*1/*28	*28/*28
maximum-tolerated dose (mg)	**850**	**700**	**400**
Dose limiting toxicity (DLT)	G4 Neutropenia G3 Diarrhoea	G4 Neutropenia	G4 Neutropenia

~300 mg is standard dose used clinically to minimise excessive toxicity in the patient population

Data from J Clin Oncol. 2014 Aug 1;32(22):2328-34.

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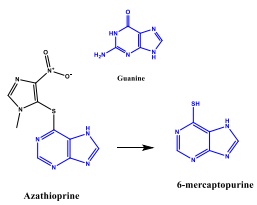
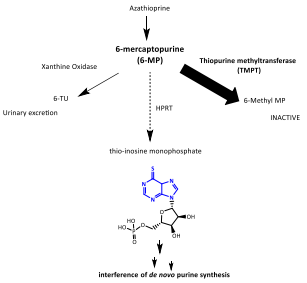
Azathioprine

- Immunosuppressive medication
- It is a guanine analog that acts as an 'antimetabolite'
  - interferes with *de novo* guanine synthesis for DNA and RNA
- Leucocytes rely on *de novo* synthesis of purines (rather than salvage)
- Azathioprine is a prodrug of 6-mercaptopurine
- 6-mercaptopurine is used in treatment of acute lymphocytic leukemia



Slide 37	**Inherited deficiency in *TMPT*** • Thiopurine methyl transferase (TMPT) is the major route of elimination of 6-MP • The non-functional variant alleles are called : *2, *3A, *3B, *3C and *4 • Individuals homozygous variant for *TMPT* are at increased risk of life- threatening myelosuppression at standard doses. • Individuals who are poor metabolisers (homozygous variant) should receive a **10-fold lower starting dosage**  For general interest only There is additional complexity due to a nucleoside enzyme (NUDT-15) which can convert one of the many downstream metabolites (thio-dGTP) into a less-toxic product. More detailed information can be found in this reference Relling, M. V., Schwab, M., Whirl-Carrillo, M., Suarez-Kurtz, G., Pui, C. H., Stein, C. M., ... & Yang, J. J. (2019). Clinical pharmacogenetics implementation consortium guideline for thiopurine dosing based on TPMT and NUDT 15 genotypes: 2018 update. *Clinical Pharmacology & Therapeutics*, *105*(5), 1095-1105.	
Slide 38	**When to consider the role of pharmacogenetic variability** • Is metabolic clearance an important/major route of elimination of the drug from the body? • Does the drug have one (or a limited number) of metabolic routes of elimination? • Is the drug biotransformed into an inactive or active metabolite? • Is there a narrow therapeutic index for the drug?	

Slide 39	Remember that pharmacokinetics can be affected by:-
	• Physiological differences
	  • body weight, fat distribution, liver blood flow etc
	• Pathological changes to organ function/ blood flow
	  • renal failure, hepatic failure, cardiovascular function etc
	• i.e. age, sex, pregnancy and disease can have **substantial effects on individual differences in pharmacokinetic parameters**
	• as well as **inherited (pharmacogenetic) differences** in the expression and activity of drug metabolising enzymes	

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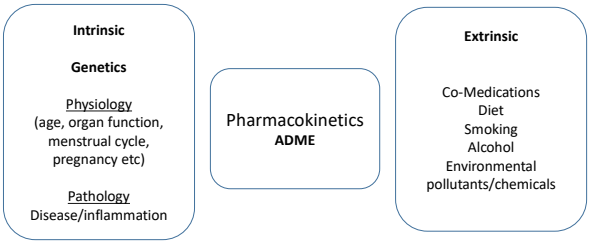
Remember there are many factors which can influence inter- and intra-individual variation in drug clearance



Expression/activity of drug metabolising enzymes is regulated by a complex combination of factors including

- inherited genetic polymorphisms
- induction/inhibition by xenobiotics
- regulation by hormones (e.g. pregnancy)
- cytokines (during disease states)
- age related maturation of expression of enzymes

} Genomics: gene-environment interactions

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Example short answer question

- Your patient has the following genotypes:-
  - *CYP2C19**17/*17 (high activity)
  - *CYP2D6**4/*4 (poor metaboliser)
  - *UGT1A1**1/*28 (intermediate metaboliser)
  - *TPMT* *1*1 (normal metaboliser)
- What decisions could you make with regard to prescribing the following drugs for this person?
  - Clopidogrel
  - Codeine
  - Omeprazole
  - Irinotecan
  - Azathioprine
  - Tramadol
- Briefly describe how you would justify each of these decisions

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For information

	Genetic polymorphism	indication	mechanism	Clinical scenario
Codeine	*CYP2D6*	diarrhoea, analgesia, drugs of abuse	mu-opiate agonist (prodrug)	[Dislocated joint]
Tramadol	*CYP2D6*	pain	mu-receptor opioid partial agonist (prodrug)	[Dislocated joint]
Omeprazole	*CYP2C19*	peptic ulcer disease	proton pump inhibitor	[Chronic upper abdominal symptoms], [Painful hands in the cold]
Clopidogrel	*CYP2C19*	ischaemic heart disease, cerebrovascular disease, peripheral vascular disease	ADP receptor (P2Y12) inhibitor	[Acute chest pain], [Acutely painful limb], [Chronic limb pain], [Epistaxis], [Pre-admission and surgical risk assessment], [Preoperative assessment and management]
Azathioprine	*TMPT*	inflammatory bowel disease, inflammatory arthritis	purine synthesis inhibitor (prodrug)	[Itching child]

The drugs mentioned in todays lecture appear in the following clinical scenarios as part of the MBChB programme of study.

You may find it useful to reflect on the potential role of inherited differences in drug metabolism when considering the use of these drugs in these contexts.