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# Pharmacogenetics today:

# What we don‘t know

# Pharmacogenomics and Drug Therapy

Selection of appropriate drug and dose for the individual patient in order to

- achieve optimal therapeutic response
- avoid therapeutic failure
- minimize side effects and toxicity

# Pharmacogenetics and Drug Therapy

**Ideal:** Candidate genes associated with response and side effects/toxicity are known

Value of pharmacogenetic test in predicting response and selection of appropriate and dose has been established in prospective clinical trials in two independent study cohorts

# Pharmacogenetics and Drug Therapy

**Reality:** Focus on one gene, limited number of mutations tested

Predictions based on case reports only

Retrospective studies with poor description of patient characteristics, clinical outcome & confounders

# Prerequisites for Pharmacogenetic Testing

- Clear definition of phenotype (confounders)
- Genotype-phenotype relationship
- Sufficient sample size to identify all relevant mutations associated with phenotype
- Association studies:  Plausible biological hypothesis

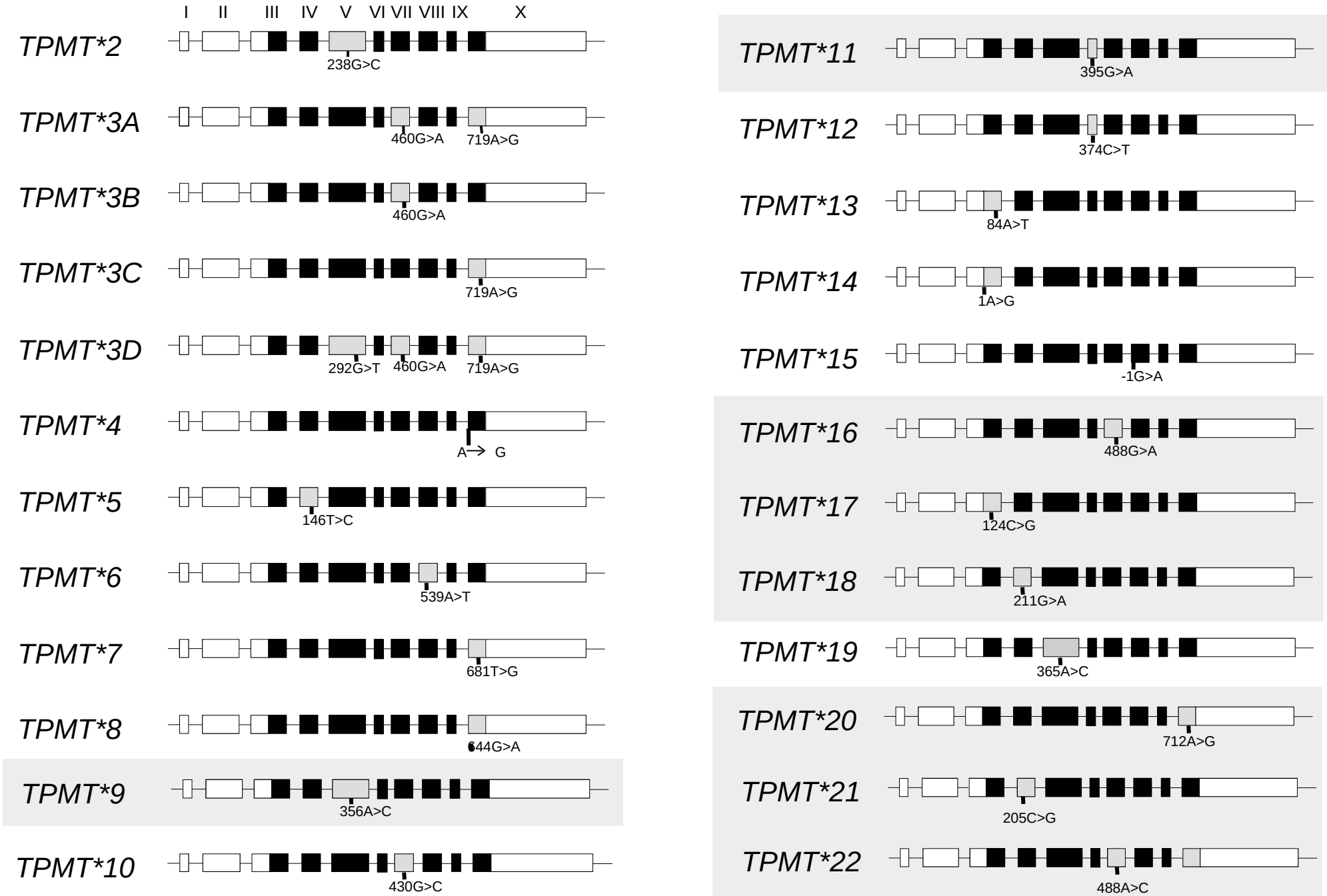
# Pharmacogenetics and Drug Therapy

How is the phenotype drug concentration related to drug response, side effects?

How predictive is the drug effect for efficacy, clinical endpoint?

# Pitfalls in Pharmacogenetic Testing

- Of the known functionally important mutations only a limited number are tested
- Presence of unknown mutations
- Penetrance of gene
- Phenotype studied is only in part caused by candidate gene
- Other genetic and nongenetic factors contribute to phenotype




Schaeffeler *et al.* 2003, 2004, 2005

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# Requirements for Pharmacogenetic Testing

How many mutations should be tested?

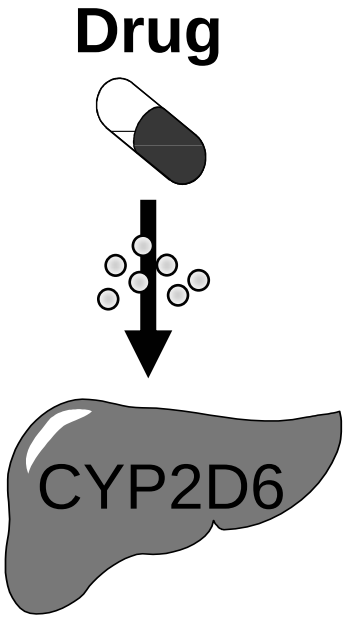
Restricted to most common mutations or as complete as possible?

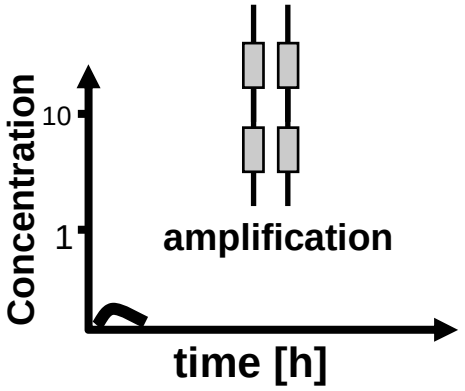
Example of TPMT: 22 loss of function mutations identified

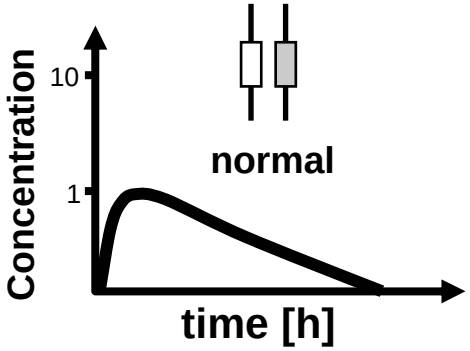
Cohort of ~ 15000 patients: 10 new mutations identified.

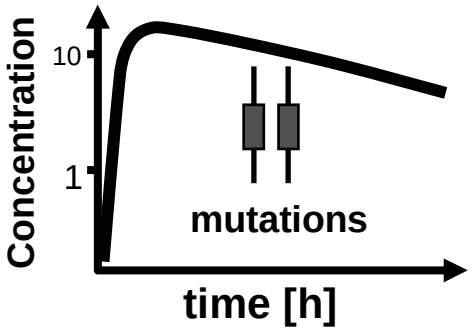
1 patient with complete deficiency was classified as heterozygous based on genotyping which was restricted to 3 most common SNPs

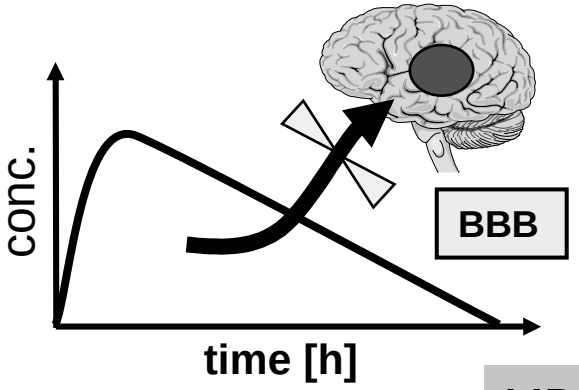
# Polygenic Nature of Drug Response: Antidepressants

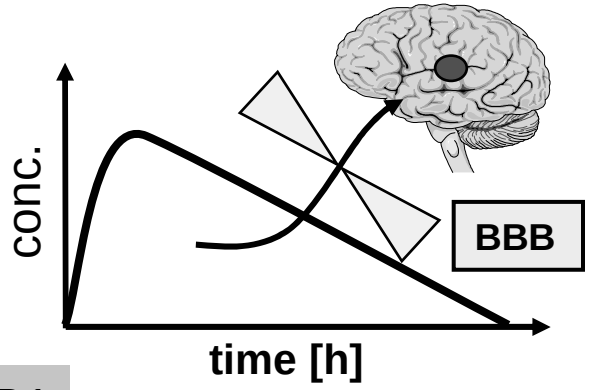


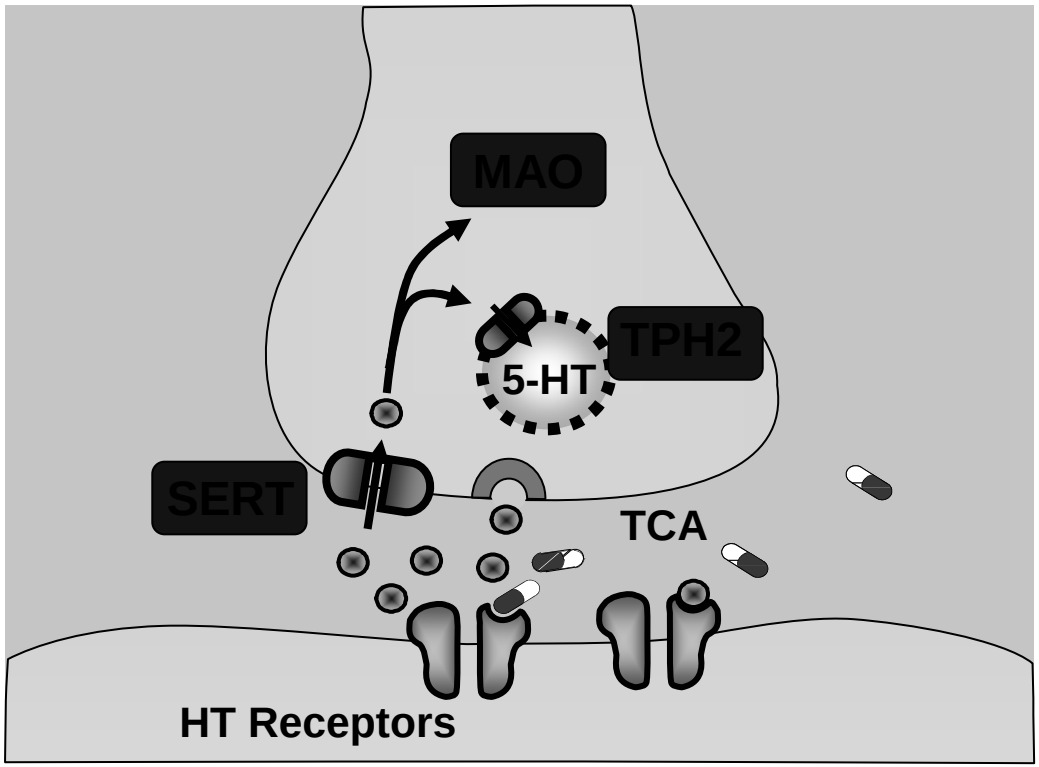




MDR1



# Pharmacogenetics and Prediction of Response and Toxicity

## The example of TPMT

- Of the known functionally important mutations only a limited number are tested
- Presence of unknown mutations
- Penetrance of gene
- Phenotype studied is only in part caused by candidate gene
- Other genetic and nongenetic factors contribute to phenotype

## Dose dependent ADRs

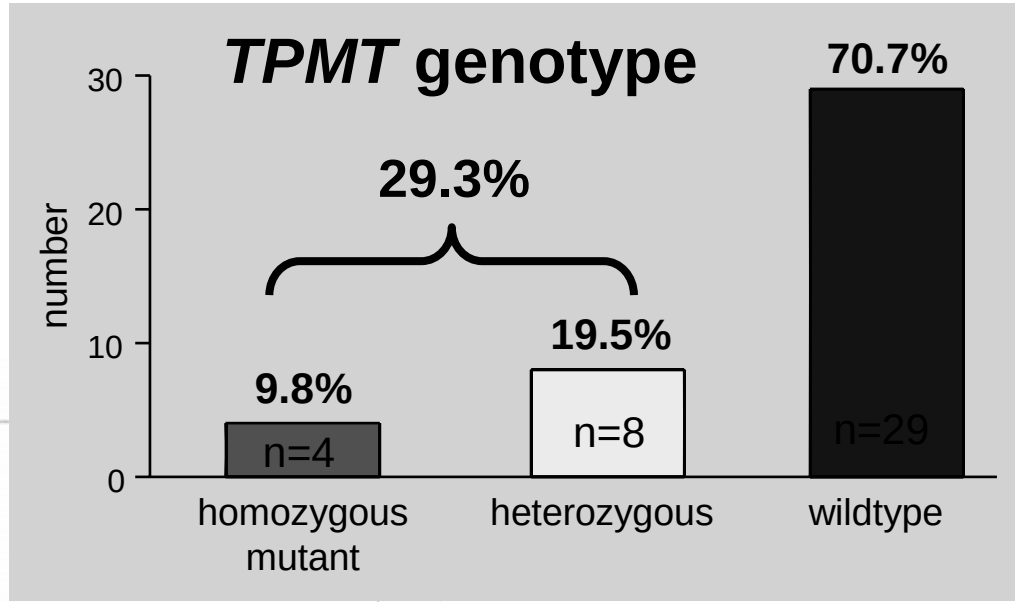
- hematotoxicity (leukopenia, pancytopenia)
- hepatotoxicity (most cases)

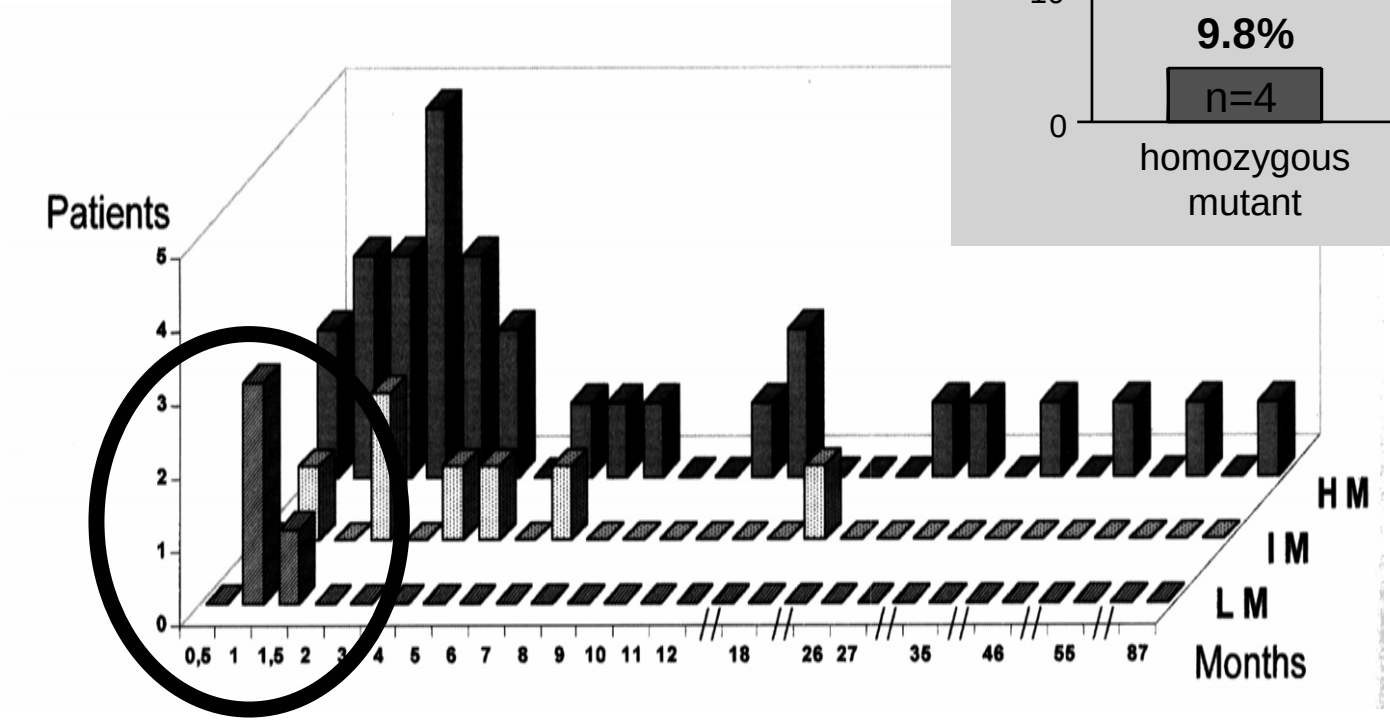
## Dose independent ADRs

- pancreatitis
- gastrointestinal disturbancies* (eg. nausea, vomiting, diarrhoea)
- flu-like symptoms (such as fever*, headache)
- rash*
- arthralgia*, myalgia*

* commonly termed also as *Azathioprine intolerance*

# Patients (n= 41) with Crohn‘s Disease and Severe Myelosuppression during Azathioprine Therapy




Colombel *et al*.
Gastroenterology 2000

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# Results of *TPMT* diagnostics in Stuttgart

**Spontaneous reporting of cases (n=14 066); 92 % IBD**

**Patients on thiopurine therapy (n=4525)**

**95 patients with severe myelosuppression (pancytopenia)**

**42 mutant (44%)**

**53 wildtype (56%)**

**12** homozygous

onset within 3 - 8 weeks

**28** heterozygous **2** het+allopurinol

**13 (14%)** **c**omedication **allopurinol**

**1** CMV Infection

**39** (36% received ASA ≥12 mo )

Schwab *et al.* unpublished data (Mai 2005)

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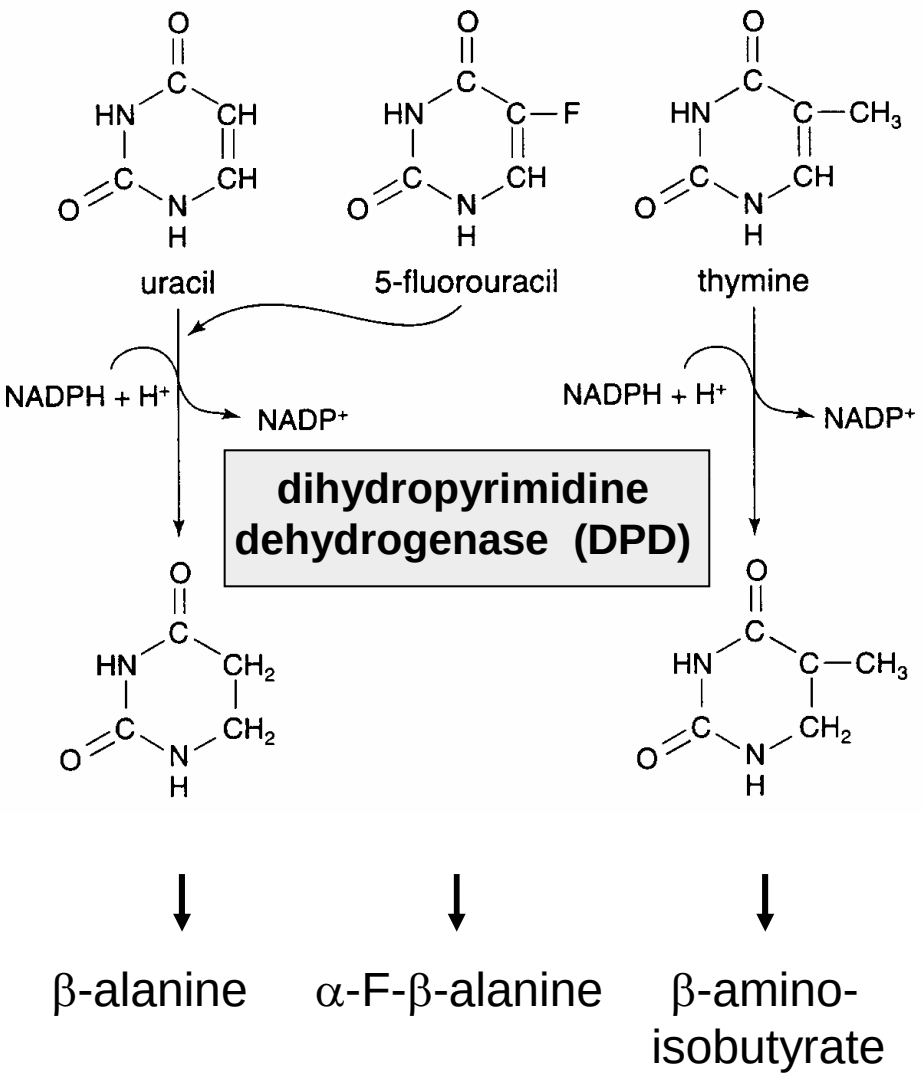
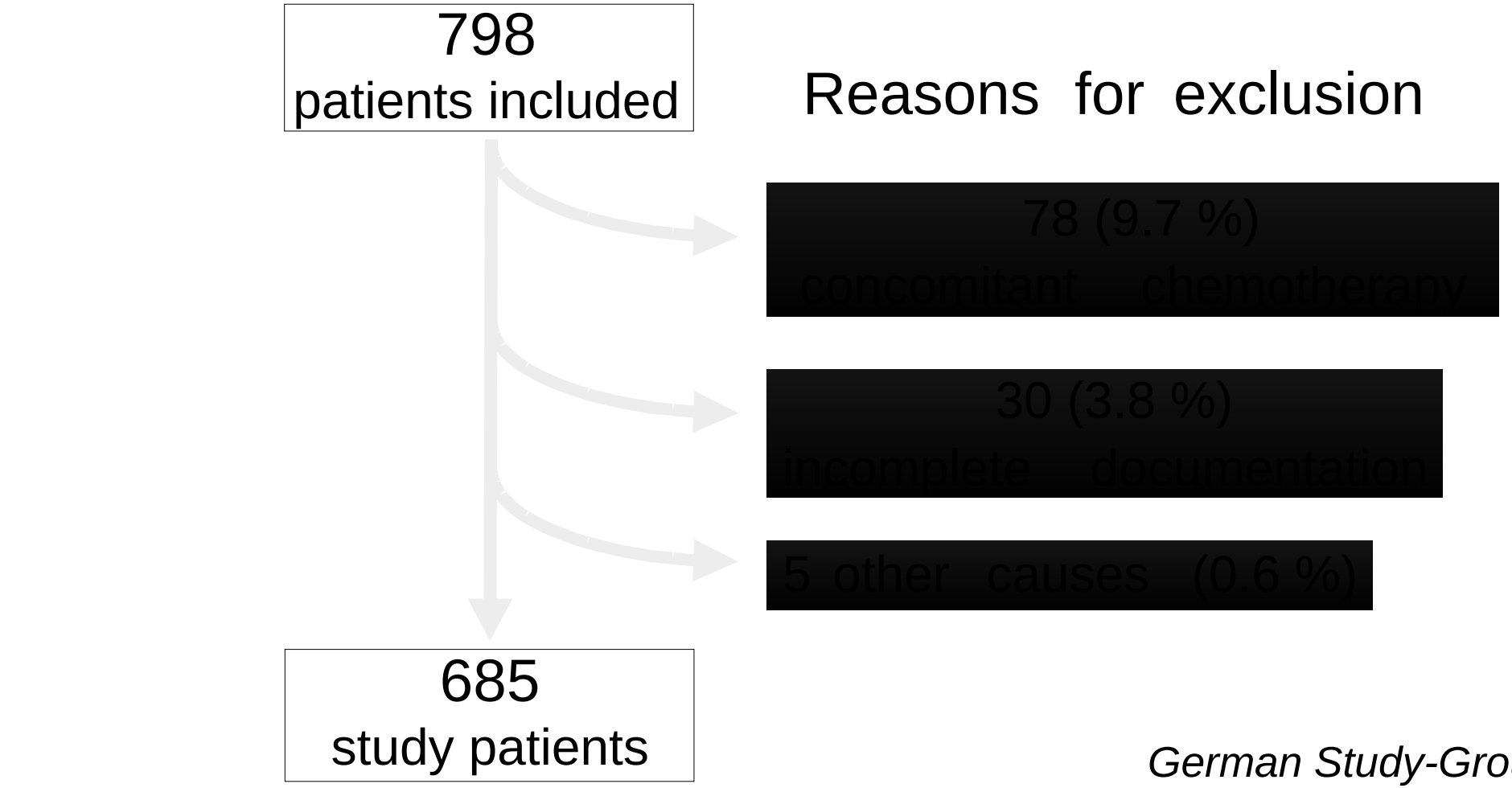
# Dihydropyrimidine Dehydrogenase (DPD)




- DPD catalyzes 1st and rate limiting step
- commonly expressed Fe-S protein (predominately in human liver)
- cytosolic enzyme
- endogenous substrates known
- association to inborn error (**familial pyrimidinemia**) and
- **severe 5-FU toxicity** (Diasio *et al.,* 1988)

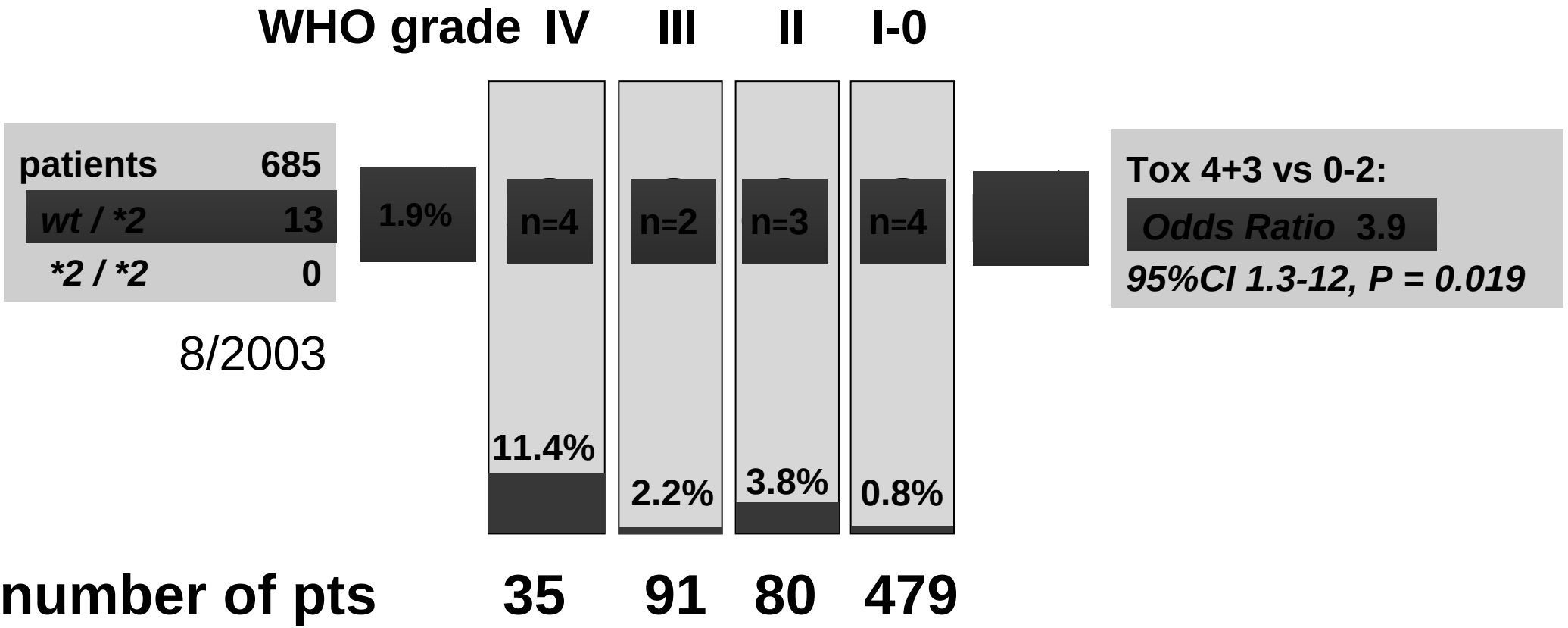
# Pitfalls in Pharmacogenetic Testing:
## DPYD Mutations and 5-FU Toxicity




*German Study-Group on 5-FU Toxicity*

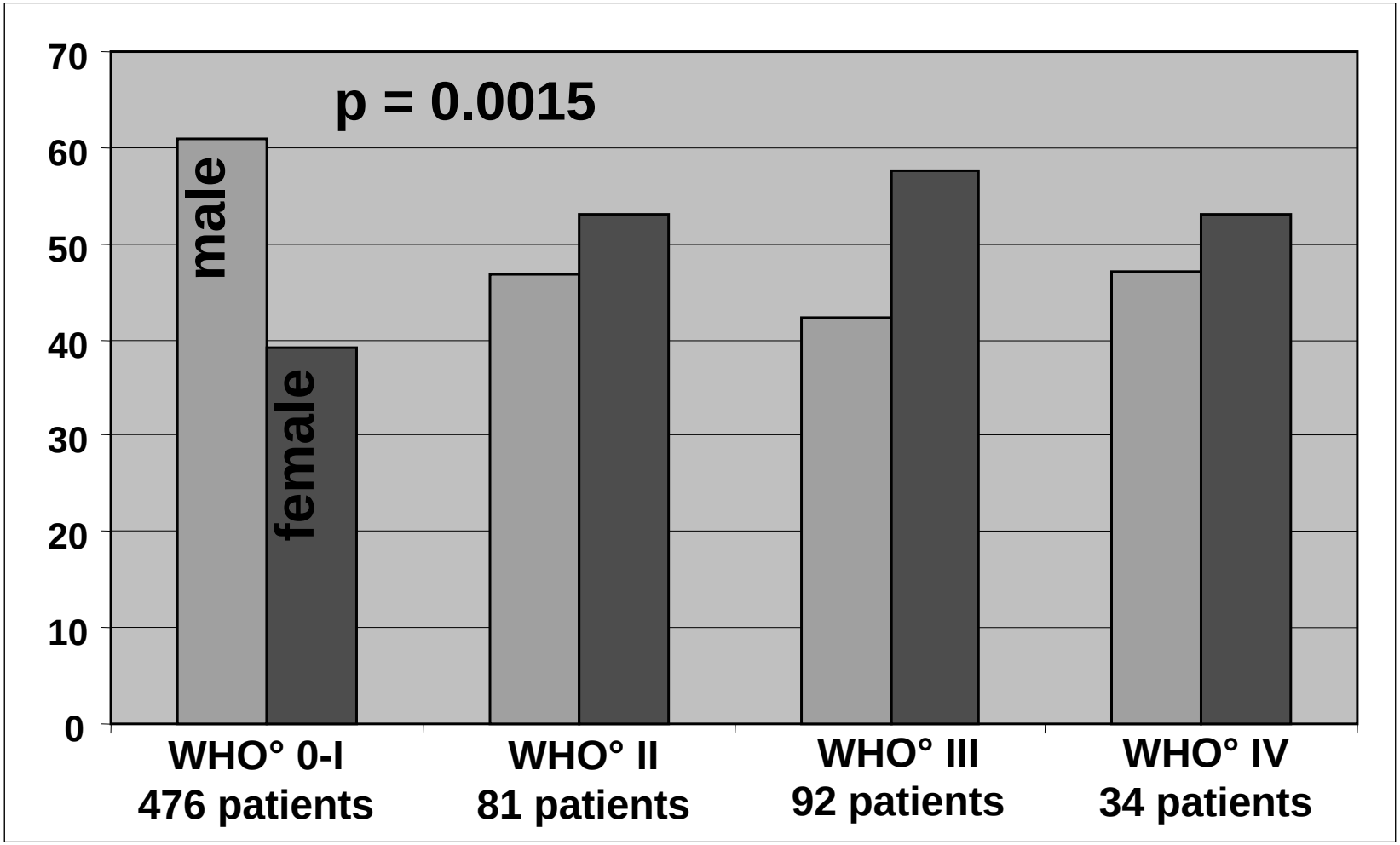
# Phenotype is only in part caused by candidate gene

DPYD Exon 14 Skipping Mutation explains only ~ 15 % of 5-FU toxicity




*German Study-Group on 5-FU Toxicity*

# Female Sex is a Risk Factor for 5FU Toxicity




| | | | | | |
|---|---|---|---|---|---|
| Sex ratio [%] | ♂:♀ | 61:39 | 47:53 | 42:58 | 47:53 |
| Total | 56:44 | | | | |

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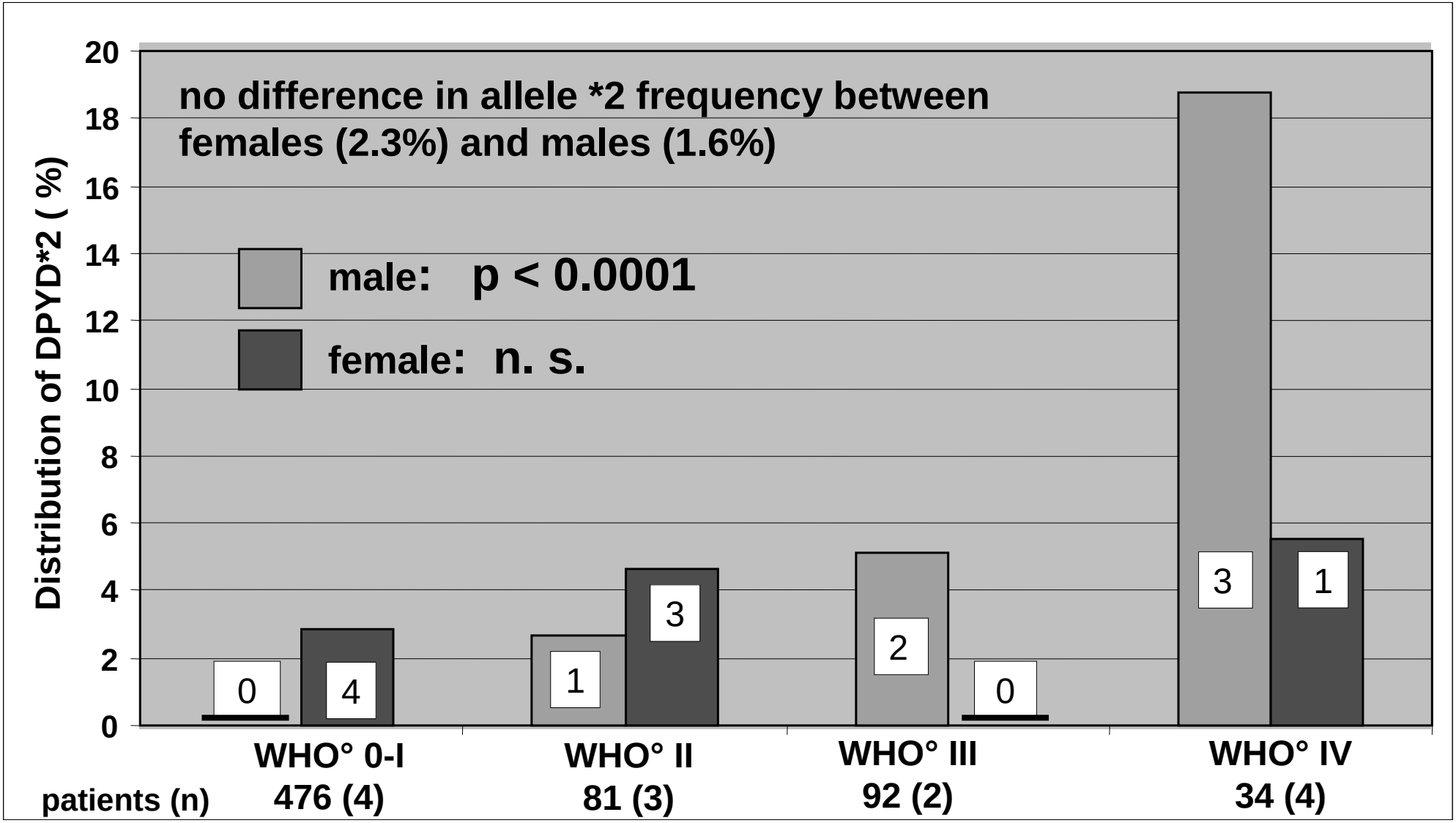
# Sex and DPYD*2 Allele associated 5-FU Toxicity

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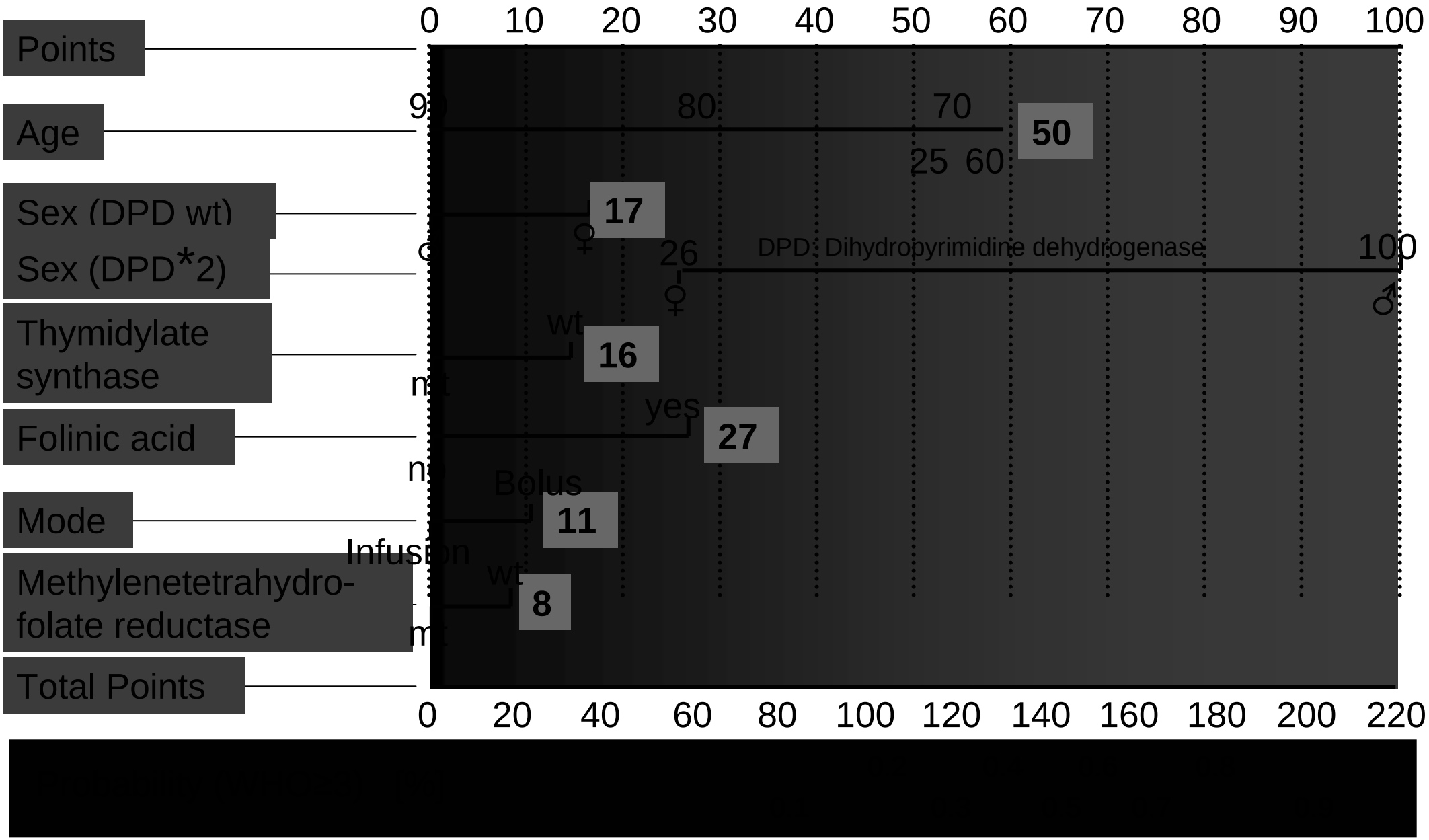
# Nomogram for the Prediction of 5-FU Toxicity

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# Predictive model for 5-FU WHO4-toxicity

| Factor | female | male |
|---|---|---|
| sex (DPD wt) | 17 | 0 |
| sex (DPD*2) | 9 (26) | 100 |
| TS (wt) | 16 | 16 |
| MTFR (wt) | 8 | 8 |
| Folinic acid | 27 | 27 |
| Bolus | 11 | 11 |
| age | 50 | 50 |
| total points | 138 | 212 |
| Probability | 0.48 | 0.95 |

Contribution of DPD*2 is negligible (4 %) in female but substantial (45 %) in male patients.

# What is the information content of a genetic test ?

| | |
|---|---|
| **Sensitivity** | likelihood that a patient with a given phenotype (ADR) will test positive |
| **Specifity** | likelihood that a patient without the phenotype (ADR) will test negative (i.e. not test false positive) |
| **Positive predictive value** | likelihood that a patient with a positive test will have the phenotype (ADR) |
| **Negative predictive value** | likelihood that a patient with a negative test will not have the phenotype (ADR) |

# Genotype and Phenotype

Limitations of present approach: Usually focus on one gene

Like most disease phenotypes, drug phenotypes (response, nonresponse, toxicity) are complex polygenic traits with nongenetic factors contributing to the manifestation of phenotypes.

The extent to which genetic factors contribute to phenotype will depend whether the candidate gene is a gene of major, moderate or minor effect.

Table 1. Partial list of candidate genes for pharmacogenomic studies of HMG-CoA reductase inhibitors.

| | Gene symbol | Gene name | OMIM No.* |
|---|---|---|---|
| ***Drug metabolism*** | *CYP3A4* | Cytochrome P450, subfamily IIIA, polypeptide 4 | 124010 |
| | *CYP3A5* | Cytochrome P450, subfamily IIIA, polypeptide 5 | 605325 |
| | *CYP2C8* | Cytochrome P450, subfamily IIC, polypeptide 8 | 601129 |
| | *CYP2C9* | Cytochrome P450, subfamily IIC, polypeptide 9 | 601130 |
| | *CYP2D* | Cytochrome P450, subfamily IID (CYP2D6) | 124030 |
| ***Drug transport*** | *ABCB1* | ATP-binding cassette, subfamily B, member 1 (P-glycoprotein) | 171050 |
| | *ABCC2* | ATP-binding cassette, subfamily C, member 2 (MRP2) | 601107 |
| | *SLCO1B1*† | Solute carrier family 21, member 6 (OATP1B1, aka OATP-C) | 604843 |
| | *SLCO2B1*† | Solute carrier family 21, member 9 (OATP2B1, aka OATP-B) | 604988 |
| | *SLC22A8* | Solute carrier family 22, member 8 (OAT3) | 607581 |
| ***Cholesterol biosynthesis/ drug targets*** | *FDFT1* | Farnesyl-diphosphate farnesyl-transferase (Squalene synthase) | 184420 |
| | *HMGCR* | 3-α-hydroxy-3-methylglutaryl coenzyme A reductase | 142910 |
| | *LDLR* | Low density lipoprotein receptor | 606945 |
| ***Lipoprotein metabolism*** | *ABCA1* | ATP-binding cassette, subfamily A, member 1 | 600046 |
| | *ABCG5* | ATP-binding cassette, subfamily G, member 5 | 605459 |
| | *ABCG8* | ATP-binding cassette, subfamily G, member 8 | 605460 |
| | *APOA1* | Apolipoprotein A-I | 107680 |
| | *APOB* | Apolipoprotein B | 107730 |
| | *APOC2* | Apolipoprotein C-II | 608083 |
| | *APOE* | Apolipoprotein E | 107741 |
| | *CETP* | Cholesteryl ester transfer protein | 118470 |
| | *LCAT* | Lecithin:cholesterol acyltransferase | 606967 |
| | *LEPR* | Leptin receptor | 601007 |
| | *LIPC* | Hepatic lipase | 151670 |
| | *MTP* | Microsomal triglyceride transfer protein | 157147 |
| | *PPARA* | Peroxisome proliferator-activated receptor-α | 170998 |
| | *PPARD* | Peroxisome proliferator-activated receptor-δ | 600409 |
| | *PPARG* | Peroxisome proliferator-activated receptor-γ | 601487 |
| | *SOAT1* | Sterol *O*-acyltransferase (ACAT) | 102642 |
| | *SCAP* | SREBP cleavage-activating protein | 601510 |
| | *SCARA3* | Scavenger receptor class A, member 3 | 602728 |
| | *SCARB1* | Scavenger receptor class B, member 1 (SRB1) | 601040 |
| | *SREBF1* | Sterol regulatory element-binding transcription factor 1 (SREBP1) | 184756 |
| | *SREBF2* | Sterol regulatory element-binding transcription factor 2 (SREBP2) | 600481 |
| | *TLR4* | Toll-like receptor 4 | 603030 |
| ***Related enzyme targets*** | *ACE* | Angiotensin-I converting enzyme | 106180 |
| | *CHIT1* | Chitinase 1 | 600031 |
| | *MMP3* | Matrix metalloproteinase 3 (Stromelysin-1) | 185250 |
| | *NOS3* | Nitric oxide synthase 3 | 163729 |
| | *PON1* | Paraoxonase 1 | 168820 |
| | *PON2* | Paraoxonase 2 | 602447 |
| | *PON3* | Paraoxonase 3 | 602720 |

# 41 Candidate genes with potential relevance for statin response

*(Zineh 2005)*

*Contribution of polymorphism of most genes in lowering cholesterol will be moderate.*

# Limitations to current statin pharmaco-genetics studies (*Zineh et al. 2005*)

- Generally not multi-gene studies (or studies considering combinations of several genes)
- Statistically significant results are not necessarily clinically meaningful
- Many studies - few results replicated
- Gene-environment, gene-disease and dietary factors not contolled
- Candidate polymorphisms often associated with baseline cholesterol

# Pharmacogenomics of Statins: Outlook

- Genotype groups with diminished lipid response may still show clinically useful effects!
- How predictive is lipid lowering efficacy for clinical endpoints?
- Will genetic testing to predict response and toxicity be feasible and cost-effective?

  - Maybe, but expectations are probably too high
- Large studies with many genes are needed

# Can Genotype-based Dose Selecetion Reduce Toxicity and Increase Response ?

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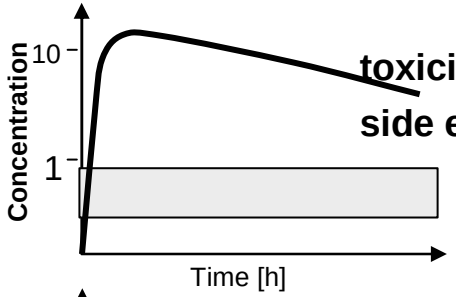
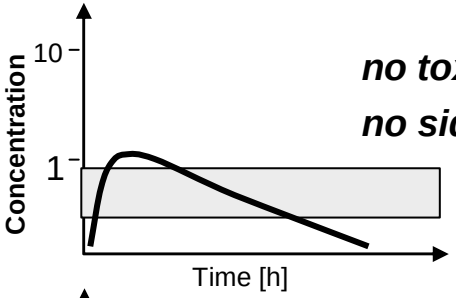
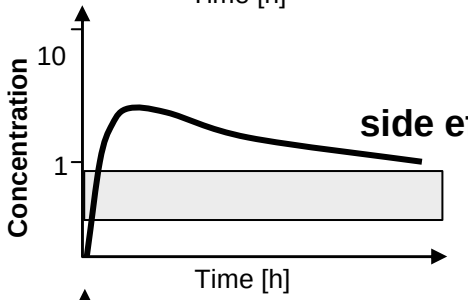
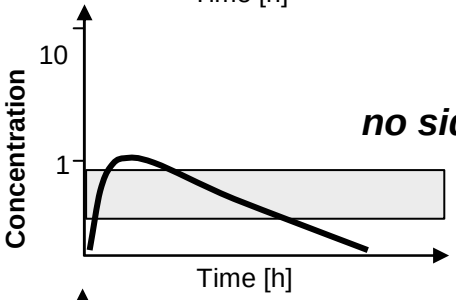
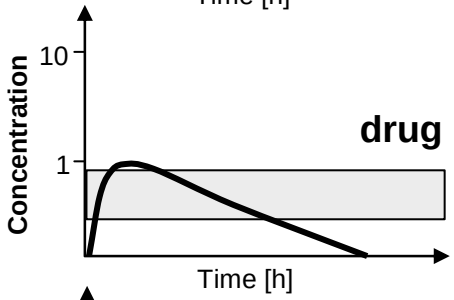
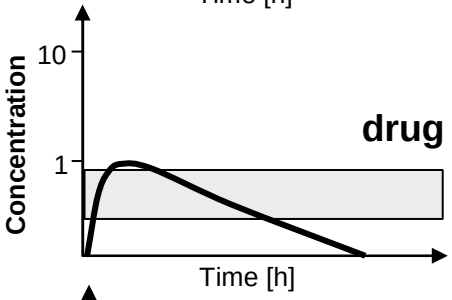
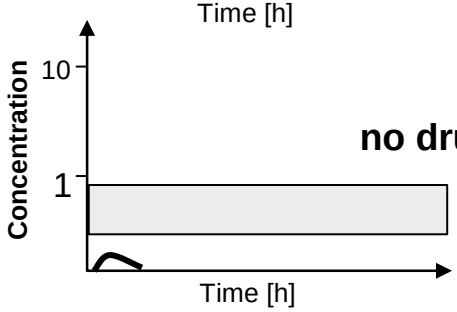
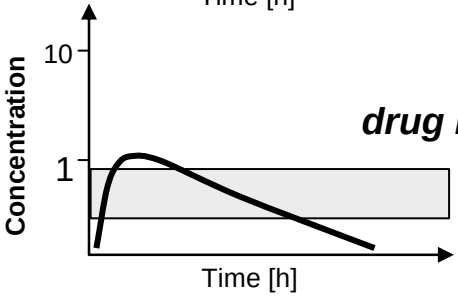
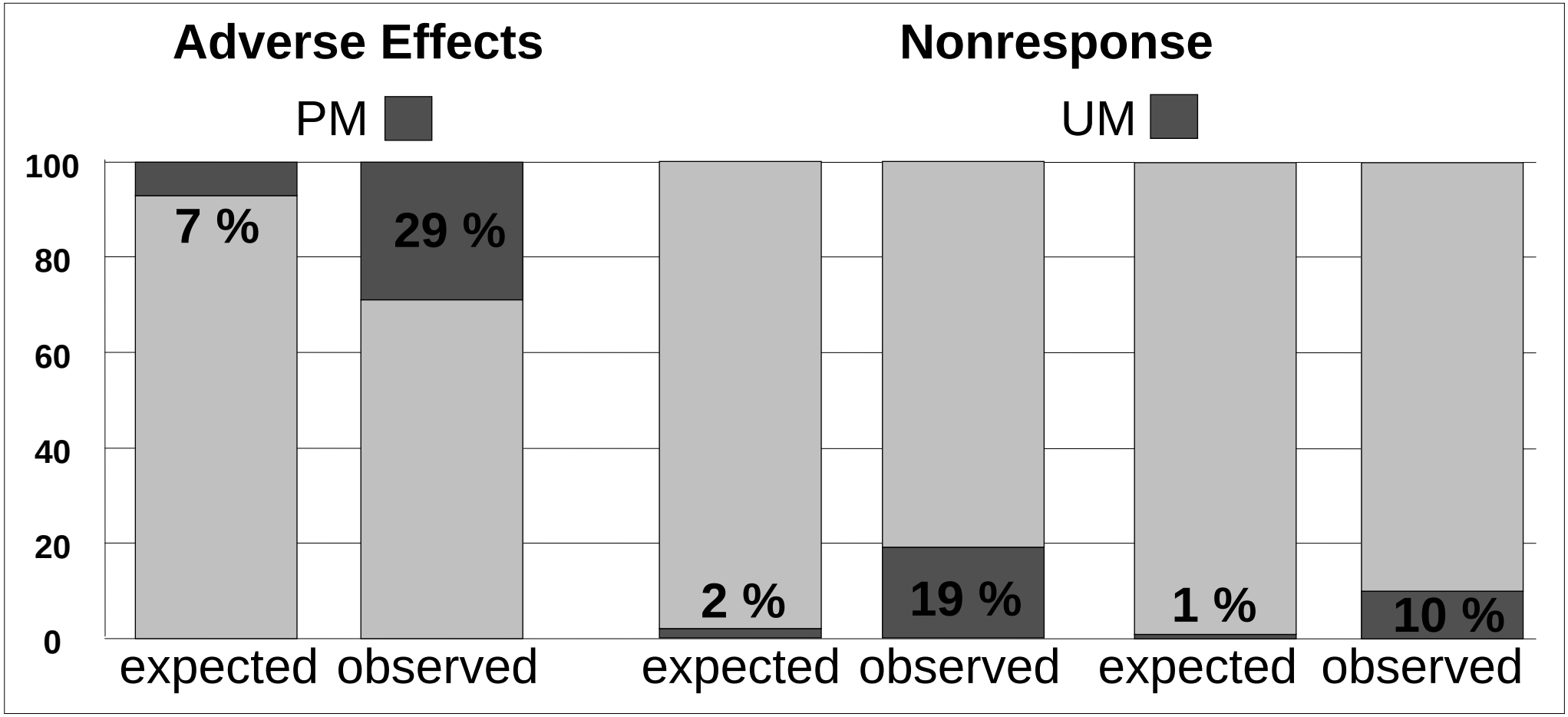
| Genotype | Dose | Concentration | Response | Dose adjusted | Concentration | Response |
|---|---|---|---|---|---|---|
| PM | 1 |  | **toxicity, side effects** | 0.1 |  | ***no toxicity, no side effects*** |
| IM | 1 |  | **side effects** | 0.2 |  | ***no side effects*** |
| EM | 1 |  | **drug response** | 1 |  | **drug response** |
| UM | 1 |  | **no drug response** | 4 |  | ***drug response*** |

# The Impact of CYP2D6 Genotype on Adverse Drug Reaction and Nonresponse During Treatment with Antidepressants




*Rau et al., 2004*

*Kawanishi et al., 2004*

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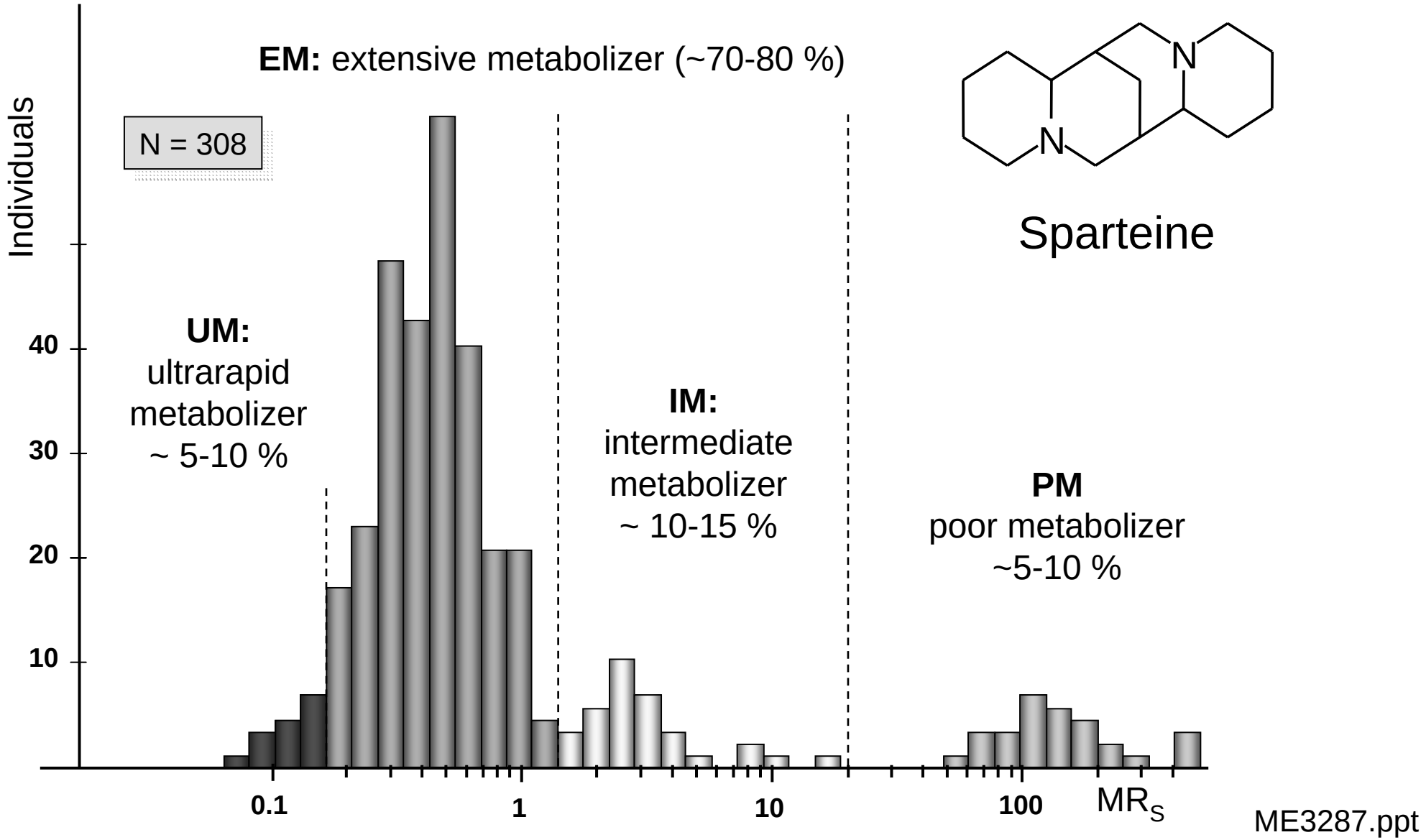
# CYP2D6 Oxidation Phenotypes in Caucasians

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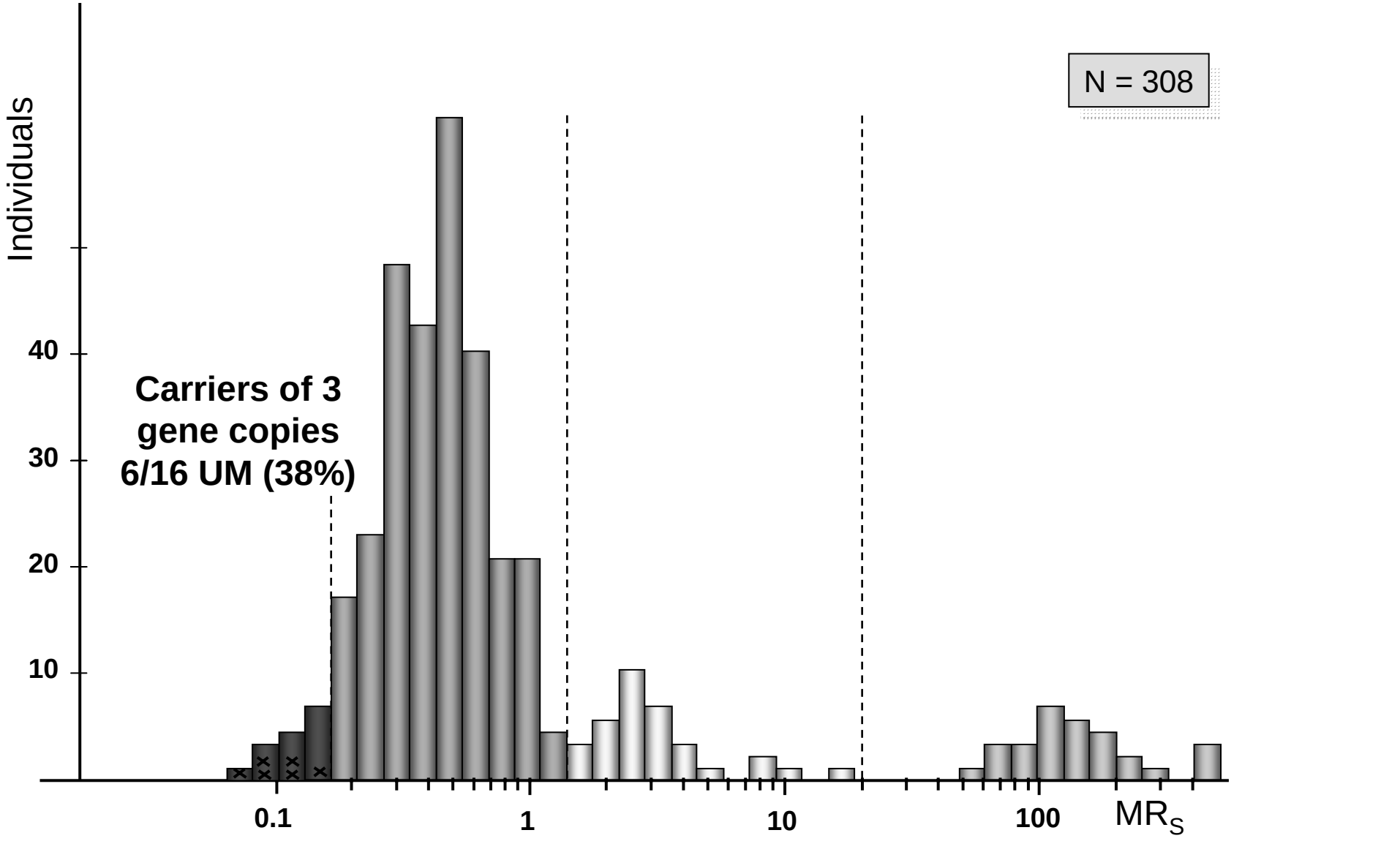
# Limited Predictivity of CYP2D6 Gene Duplication for UM Phenotype

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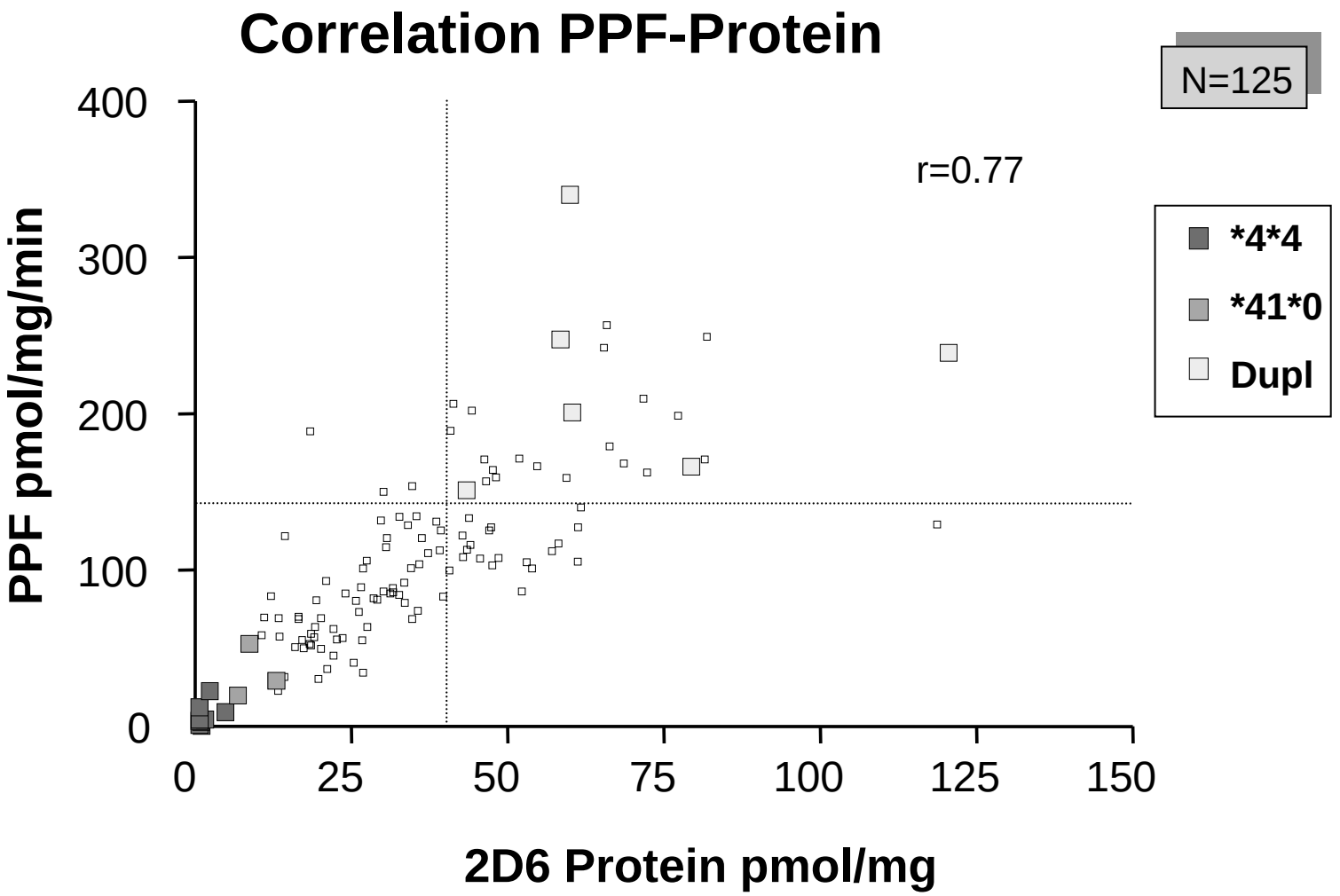
# CYP2D6 Protein and Propafenone Enzyme Activity in Human Liver

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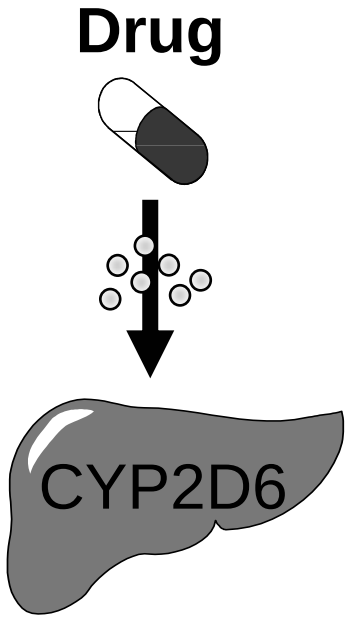
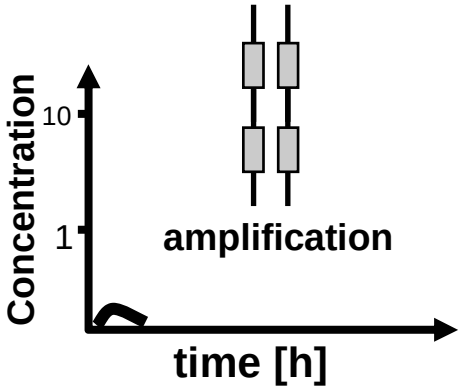
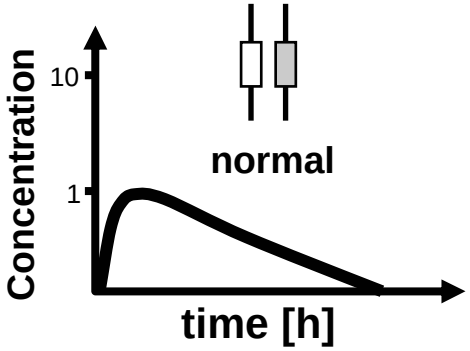
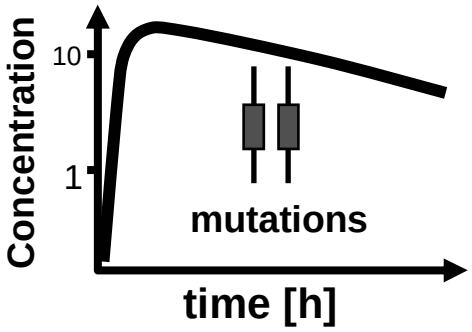
# Polygenic Nature of Drug Response: Antidepressants



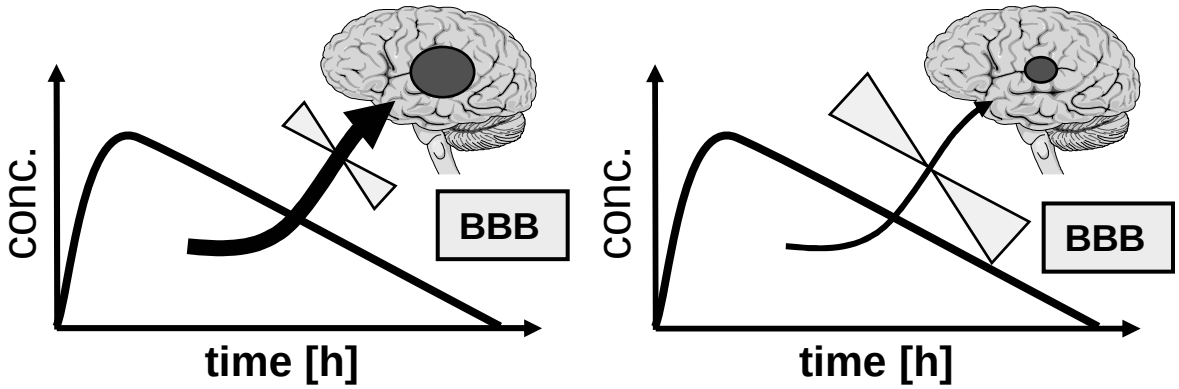

## Drug Metabolism

Poor predictive value of CYP2D6 and CYP2C19 genotype for severe adverse drug reactions and non-response leading to discontinuation of treatment

- Comparable doses used; compliance
- Measurement of drug levels
- Coadministration of drugs: Phenocopying
- Coexisting diseases; age; gender
- Predictive value of genotype for phenotype: UM genotype predicts only 20 – 30 % of UM phenotype

# Polygenic Nature of Drug Response: Antidepressants

## Drug Transport




MDR1 at blood brain barrier
Comparable plasma concentration,
yet different concentration at site
of action:

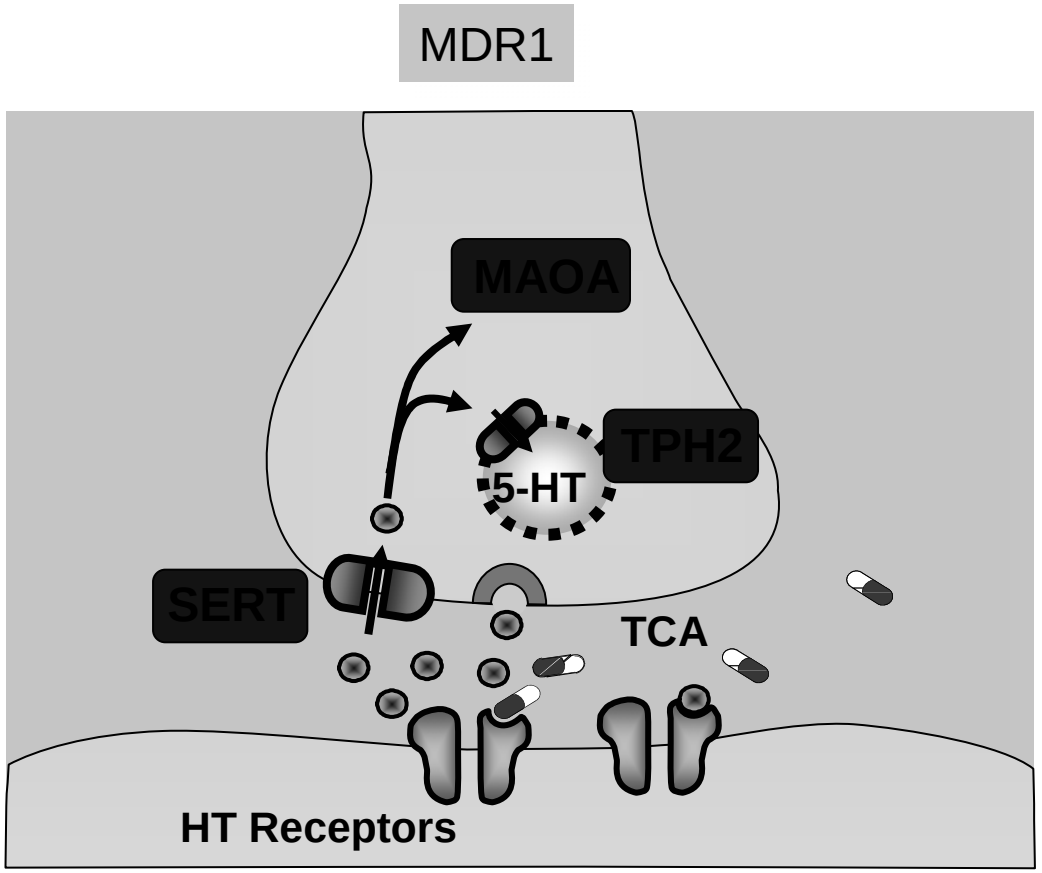
- Poor predictive value of drug concentration
- net concentration = influx (diffusion) – efflux (transport)
- Contribution of MDR1 polymorphism to

  response only at comparable concentrations

# Polygenic Nature of Drug Response: Antidepressants

## Drug Target

1. Concentration of serotonin in synaptic cleft is influenced by biosynthesis (TPH2), re-uptake (SERT) and catabolism (MAOA)
2. Inhibition of serotonin re-uptake depends on drug concentration in synaptic cleft
3. Mutations of receptors and signalling pathways affect neurotransmitter and drug effects



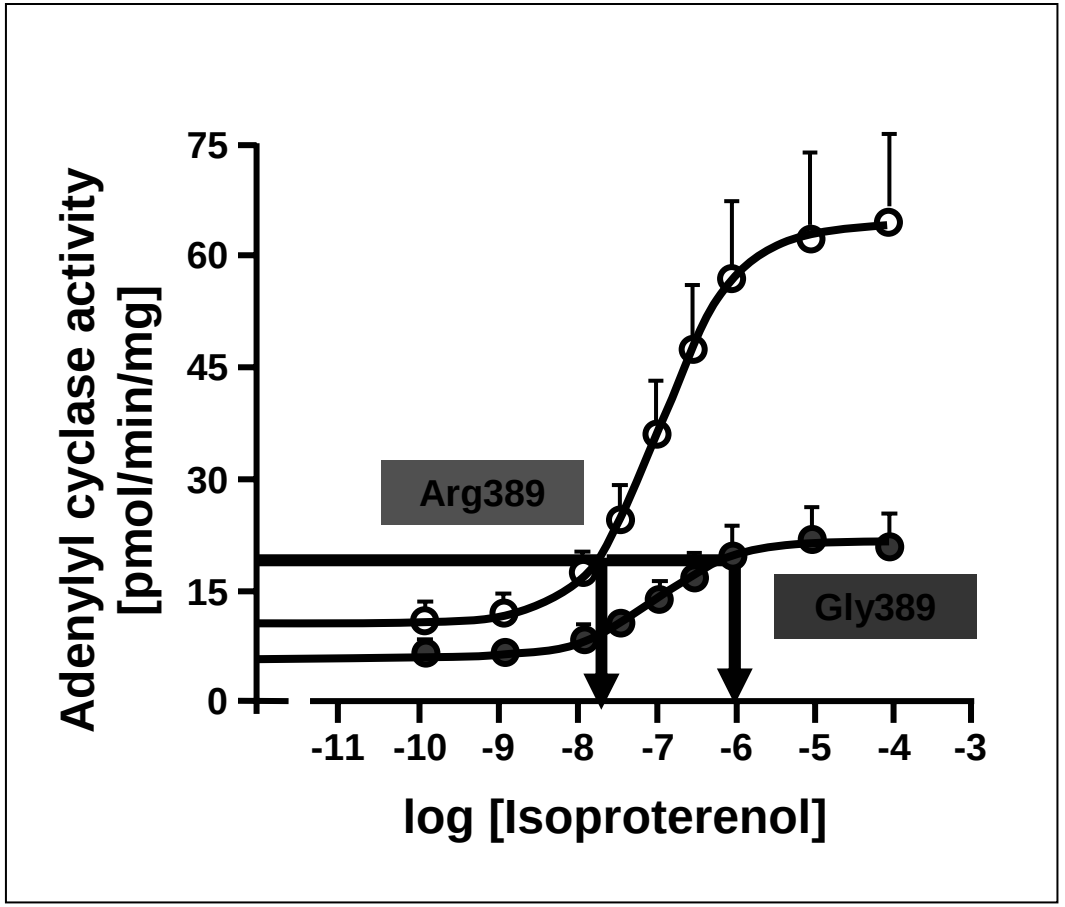
# Polygenic Nature of Drug Response: Antidepressants

## Drug Target

Contribution of receptor / signalling pathway to drug response should be assessed at comparable plasma levels

BBB: Stratification of patient groups for transporter polymorphisms

Comparable effect at receptor, but 10 fold difference in concentration required between genotypes




*(Mason et al. 1999)*

# Limitations to current statin pharmaco-genetics studies (*Zineh et al. 2005*)

- Generally not multi-gene studies (or studies considering combinations of several genes)
- Statistically significant results are not necessarily clinically meaningful
- Many studies - few results replicated
- Gene-environment, gene-disease and dietary factors not contolled
- Candidate polymorphisms often associated with baseline cholesterol

# Pharmacogenomics of Statins: Outlook

- Genotype groups with diminished lipid response may still show clinically useful effects!
- How predictive is lipid lowering efficacy for clinical endpoints?
- Will genetic testing to predict response and toxicity be feasible and cost-effective?

  - Maybe, but expectations are probably too high
- Large studies with many genes are needed