PROPAGATION OF PHARMACOGENETIC DIFFERENCES IN CYTOCHROME P450 INTO PHARMACOKINETIC AND PHARMACODYNAMIC MEASURES.

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

By

GEMMA DICKINSON BSc (hons)

Academic Unit of Clinical Pharmacology; Pharmacokinetics &

Pharmacogenetics Group.

The University of Sheffield

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SUMMARY OF WORK IN THIS THESIS

Literature reports of studies that investigate the impact of CYP polymorphisms on drug pharmacokinetics and response are often conflicting and the importance of genetic variation in drug metabolism for drug response remains unclear. Johnsson & Sheiner (2002) have advocated the need for 'smarter clinical trial design' and showed that simulation techniques can help in this process by integrating all the available information. However, current examples of clinical trial simulation rely heavily on data already available from *in vivo* studies and there is a need for utilising pharmacokinetic information gathered earlier on during drug development.

The aim of the current work was to integrate early preclinical data on drug metabolism into a clinical trial simulation paradigm in order to investigate (A) the impact of genetic polymorphisms in the cytochromes P450 on the pharmacokinetics and pharmacodynamics of 5 model drugs: dextromethorphan, *(S)*-warfarin, midazolam, omeprazole and tolbutamide, and (B) the predicted power of studies to detect the effects of such polymorphisms.

Simcyp® algorithms incorporate information on *in vitro* metabolism and *in vivo* kinetics with interindividual variability in the genetics of drug metabolising enzymes and other physiological and demographic features. In the current study these algorithms were linked to pharmacokinetic-pharmacodynamic models to describe the

time course of concentration and effect of the model drugs in virtual populations of subjects. The probability of detecting a statistically significant difference in the pharmacokinetics or response between CYP phenotypes/genotypes was assessed and the power of studies to detect such differences was calculated. Various aspects of study design (study size and enrichment) and drug characteristics (active metabolites, PD variability etc) were investigated in each case.

The study powers calculated from the simulations where largely consistent with the observed *in vivo* outcomes and helped to explain the aforementioned literature discrepancies. In conclusion, the simulations described have demonstrated the usefulness of clinical trial simulations, incorporating preclinical information on the genetics of drug metabolism for the prediction of drug pharmacokinetics and dynamics in virtual populations of individuals of varying drug metabolizing capability. In the future, clinical trial simulation may increasingly use prior *in vitro* data.

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Cheers!

Gemma Dickinson

PUBLICATIONS ARISING FROM THIS THESIS

Papers in Refereed Journals

Dickinson GL, Rezaee S, Proctor NJ, Lennard MS, Tucker GT, Rostami-Hodjegan A. (2007) Incorporating In Vitro Information on Drug Metabolism Into Clinical Trial Simulations to Assess the Effect of CYP2D6 Polymorphism on Pharmacokinetics and Pharmacodynamics: Dextromethorphan as a Model Application. *Journal of Clinical Pharmacology,* In Press.

Dickinson GL, Lennard MS, Tucker GT, Rostami-Hodjegan A. (2007) The impact of genetic variability in CYP2C9 on warfarin kinetics and dynamics: assessment of study power using a mechanistic model. *British Journal of Clinical Pharmacology.* In Press.

Book Chapters

Dickinson GL & Rostami-Hodjegan A (2007) Building virtual human populations: Assessing the propagation of genetic variability in drug metabolism to pharmacokinetics and pharmacodynamics. In Bertau M, Mosekilde E, Westerhoff H (Eds.), *Biosimulation in Drug Development,* (Chapter 16) Wiley-VCH, In Press.

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Poster Presentations

Dickinson GL, Lennard MS, Tucker GT, Rostami-Hodjegan A. (2006) The Propagation of Genetic Polymorphism in CYP2C9 into Tolbutamide Pharmacokinetics: An integrated model (ISSX; Manchester, UK). *Drug Metabolism Reviews,* 38(S1):63.

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Non-Refereed Abstracts, Reports and other Publication Posters

Oral Presentations

Dickinson GL, Lennard MS, Tucker GT, Rostami-Hodjegan A. (2005) Simulations as a tool to assess the propagation of genetic polymorphisms in drug metabolising enzymes into PK and PD outcomes: Warfarin and dextromethorphan as examples (PKUK; Brighton, UK).

Dickinson GL, Lennard MS, Tucker GT, Rostami-Hodjegan A. (2005) Simulations to assess the propagation of the impact of genetic polymorphism in CYP enzymes into PK and PD outcomes: Warfarin and dextromethorphan as examples (DMDG open meeting; Warwick, UK).

Dickinson GL, Rostami-Hodjegan A. (2005) The propagation of genetic polymorphism in CYP2C9 into warfarin pharmacokinetics (Graduate Research Presentation day; University of Sheffield, UK).

Poster Presentations

Dickinson GL, Lennard MS, Tucker GT, Rostami-Hodjegan A. (2005) Simulations to assess the propagation of the impact of genetic polymorphism in CYP enzymes into PK and PD outcomes: Warfarin and dextromethorphan as examples (AAPS; Nashville, USA).

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Dickinson GL, Rostami-Hodjegan A, (2004) Propagation of pharmacogenetic differences in cytochrome P450 into pharmacokinetic and pharmacodynamic measures (Graduate Research Presentation day; University of Sheffield, UK).

Dickinson GL, Rostami-Hodjegan A, Pratt P, Lennard.MS. (2003) methadone dose, methadone plasma concentration, and patient response to methadone maintenance therapy: Is there a link? (PKUK; Liverpool, UK).

ABBREVIATIONS

3-MM - 3-Methoxymorphinian

ADR - Adverse drug reaction

AUC - Area under the concentration time curve

AUEC - Area under the effect time curve

BSA – Body surface area

b.p. - blood pressure

B:P – Blood to plasma partition coefficient

BW – Body weight

CI – Cardiac index

CL – total clearance

CL_H – Hepatic clearance

CL_{int} – Intrinsic clearance

$CL_{i.v.}$ – intravenous (systemic) clearance

$CL_{p.o.}$ – oral clearance

CL_R - Renal clearance

$CLu_{H,int}$ – Unbound intrinsic clearance of drug in the liver

$CLu_{G,int}$ – Unbound intrinsic clearance of drug in the gut

C_{max} – Maximum blood/plasma concentration achieved over a particular time period

CO – Cardiac output

CrCL – Creatinine clearance

C_{ss} – steady state concentration

CTS - Clinical trial simulation

Cu_{50} - unbound concentration of drug producing 50% inhibition of PCA synthesis

CV - coefficient of variation

CYP - Cytochrome P450

D – Dose

DEX - Dextromethorphan

DOR - Dextrorphan

EC_{50} - Concentration that elicits 50% of the maximum stimulatory effect

E_H - The fraction of drug reaching the liver that escapes metabolism on first pass

EM – Extensive metaboliser

E_{max} – The maximum possible effect produced by the drug

f_a – Fraction of drug absorbed from the gut lumen

f_e – Fraction of drug excreted in the urine

F_G – Fraction of drug that is metabolised in the gut wall

F_H – The fraction of drug reaching the liver that is metabolised during first pass

fm – the fraction of parent drug converted to metabolite after an i.v. bolus dose

fu_B - fraction of drug unbound in blood

fu_{mic} - fraction of drug unbound in microsomes

fu_P - fraction of drug unbound in plasma

γ - Hill coefficient

GFR – Glomerular filtration rate

GM – Geometric mean

GMNPT – Geometric mean normal PT

GSA – Surface area of small intestine

GSD – Geometric standard deviation

HLM – Human liver microsome

IC_{50} - Concentration that elicits 50% of the maximum inhibitory effect

INR – International normalised ratio

IVIVE – *in vitro – in vivo* extrapolation

ISEF – Inter-system extrapolation factor

ISI – International sensitivity index

k_{10} – Elimination rate constant

k_{12} – Rate constant for transfer of drug from the central to peripheral compartments

k_{21} - Rate constant for transfer of drug from the peripheral to central compartments

k_a – Absorption rate constant

k_d – Degredation rate constant of prothrombin complex

k_{e0} – Rate constant for the elimination of drug from the effect compartment

K_m – the concentration at which half the V_{max} is achieved

k_{ofDOR} – Rate constant for the elimination of DOR

LV – Liver volume

LW – Liver weight

MDZ - Midazolam

MPPGL – Milligrams of Microsomal protein per gram of liver

OMZ - omeprazole

P_{app} – Permeability data obtained using Caco-2 cells

PCA – Prothrombin complex activity

PD - Pharmacodynamics

PK - Pharmacokinetics

PM – Poor metaboliser

$Potency_{(DOR)}$ – The pharmacological activity of DOR relative to DEX

PT – Prothrombin time

Q_{Art} – Blood flow through the hepatic artery

Q_{gut} – A flow term representing nominal blood flow in the gut

Q_H – Total liver blood flow

Q_{port} – Blood flow through the portal vein

rCYP – Recombinantly expressed CYP enzyme

SD - standard deviation

s.e.m - saccadic eye movement

τ - The dosing interval

tlag – Lag time between drug administration and appearance of drug in the plasma

t_{max} – The time at which the C_{max} is observed

TLB - Tolbutamide

UM – Ultra-rapid metaboliser

V – Initial volume of distribution

VKORC1 - Vitamin K epoxide reductase complex subunit 1

V_{max} – maximum velocity of enzymic reaction

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CHAPTER 1

Introduction

1 INTRODUCTION

1.1 Background

The work described in this thesis is concerned with the simulation and prediction of the pharmacokinetics and pharmacodynamics of selected drugs in virtual populations of individuals. The clearance of these drugs, as well as the fraction of drug escaping first pass hepatic and gut metabolism, was estimated for each individual by utilising *in vitro* data on drug metabolism. The simulations have incorporated variability from a number of demographic and physiological sources, in addition to genetic differences in drug metabolism. The pharmacokinetic data were used in conjunction with established pharmacokinetic-pharmacodynamic models, to allow the investigation of the impact of genetic polymorphisms of drug metabolism, on the pharmacokinetics and pharmacodynamics of the drugs studied. The findings were then used as the basis for clinical trial simulations (CTS), to investigate the importance of size, and other aspects of design, in determining the power of studies to observe such genotypic and phenotypic differences.

The aim of this introduction is to give an overview of drug development and how the practices of modelling and simulation can be used to support it. Clinical trial simulation is then discussed and its advantages highlighted. Finally, since this thesis is concerned with the influence of cytochrome P450 polymorphisms on drug pharmacokinetics and pharmacodynamics, the major elements of drug metabolism are discussed.

1.2 Drug Development

1.2.1 Stages of Drug Development

The traditional view of drug development represents the process as four stages designated as (i) discovery, (ii) pre-clinical research and development, (iii) clinical research and development and (iv) 'post-marketing' pharmacovigilance (Lipsky & Sharp, 2001) (Figure 1.1).

During drug discovery, compounds are screened against biochemical pathways or receptors that are thought to be implicated in the causation of a particular disorder or group of disorders. Once lead drug candidates have been recognized, they proceed to pre-clinical testing. During this stage, *in vitro* and *in vivo* (in animals) tests are carried out to elucidate the pharmacokinetic, pharmacodynamic and toxicological properties of the drug. Preclinical testing takes between 2 and 6.5 years (Lipsky & Sharp, 2001). If the characteristics of the drug, namely its efficacy and safety, are satisfactory at this stage, clinical testing may begin.

The clinical research and development stage is the longest, lasting up to 10 years (Lipsky & Sharp, 2001). It is further divided into three phases, I, II and III (Figure 1.1).

Phase I testing or 'first in man' studies are carried out in small groups of young, healthy subjects. The main aim of these studies is to characterise the pharmacokinetic properties of the drug and assess its safety for further testing (Lipsky & Sharp, 2001).

Phase II studies are carried out in patients who suffer from the disease or disorder that the drug is intended to treat. In this stage the safety, tolerability, effectiveness and appropriate dosage of the drug are studied in greater depth than in phase I (Lipsky & Sharp, 2001). A 'go/no go' decision will be made, based on the data gathered in phase II, as to whether to proceed to phase III (Lipsky & Sharp, 2001).

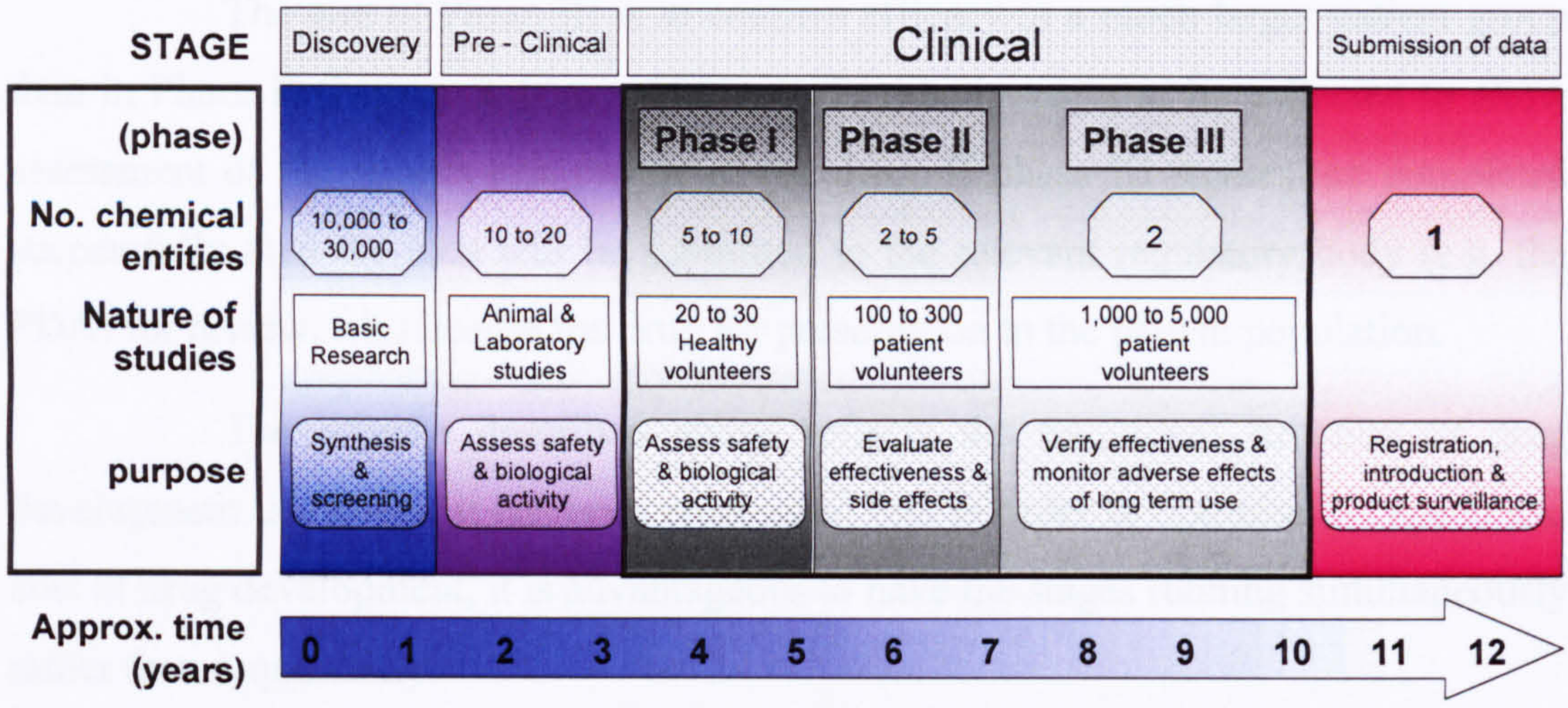

Figure 1.1 Schematic representation of the drug discovery and development process.

The aim of Phase III is to confirm efficacy in a much larger patient group than in Phase II (Lipsky & Sharp, 2001). Safety data must also be collected to allow assessment of the benefit-risk profile of the drug. If phase III studies are completed successfully then the data will be submitted to the relevant regulatory body (e.g. the FDA) for review, who license the drug for prescription to the patient population.

The scheme described above reflects the traditional structure of drug development. However, it has been recognised that in order to optimise the speed and cost of drug development, it is advantageous to have the stages running simultaneously rather than sequentially.

1.2.2 Pharmacokinetics & Pharmacodynamics: Essential Elements of Drug Development

Pharmacokinetics is defined as the quantitative description of the processes that determine the plasma concentration-time course of a drug, namely absorption, distribution, metabolism and excretion (ADME).

'Dosage' is a term describing the dose and frequency at which a drug is administered. The dosage of a drug is determined by a number of factors but most importantly, it relies on the assumption that there is a functional relationship between the concentration of a drug at a site of action and response to the drug (Rowland & Tozer, 1995). The more potent and efficacious the drug, the smaller the dose required. However, there are complexities to this relationship and the pharmacokinetic parameters of the drug also influence dosage. For example, a drug may have to be administered more or less frequently depending on how much of it reaches circulation and how long it remains in the body.

Pharmacokinetic data can be analysed using mathematical equations, which provide a representation of the relationship between the dose of a drug and the manner in which its plasma concentration changes with time. It relates the independent variables of time and dose to the dependent variable, concentration. Pharmacokinetic parameters such as clearance and volume of distribution are utilised in such models (Rowland & Tozer, 1995). Commonly used pharmacokinetic models can be divided into those that are empirical, compartmental or physiologically based. The compartmental type is the most frequently used, probably because it provides a continuous concentration-time profile in a body fluid that can be related to a continuous effect-time profile (Rowland & Tozer, 1995). Pharmacodynamic models

(Section 1.2.4) are used to describe the relationship between drug concentration and its effect at the site of action.

1.2.3 The Role of Pharmacokinetics in Drug Development

The exposure of an individual to a given drug can be obtained by measuring the area under the concentration time curve (AUC). The AUC after oral administration is dependent on the proportion of the dose that reaches the systemic circulation after passage from the gut and through the liver (F), the clearance (CL) and the dose of the drug (Rowland & Tozer, 1995) (Equation 1.1).

$$AUC = \frac{F \cdot D}{CL} \qquad \text{Equation 1.1}$$

Total CL is defined as the volume of blood that is completely cleared of drug per unit time, and encompasses clearance by the liver, the kidneys, the biliary system, the lungs and blood.

Exposure is one of the most important factors in determining the pharmacodynamic response of an individual to a particular drug. Therefore, it is important for drug companies to determine the pharmacokinetic characteristics of a drug as early as possible in drug development. However, the usefulness of pharmacokinetic data is dependent on the pharmacokinetic-pharmacodynamic relationship (1.2.4). Today, pharmacokinetics is considered at each stage of the drug development process, from discovery to post-marketing. The pharmacokinetic basis of the ADME processes, and their importance in drug development will be discussed further in Section 1.3.1.1.

1.2.4 Defining the Pharmacokinetic-Pharmacodynamic Relationship

Several mathematical models have been developed to describe the relationship between drug concentration and effect. Factors that will influence the choice of model include the nature of the drug, the response being measured, the effect seen after drug or placebo administration, the degree of linearity in the concentration-effect curve, and the potential for achieving the maximal response. Under steady state conditions, the most commonly used models are (1) the linear effect-concentration model, (2) the log-linear effect-concentration model, and (3) the simple and sigmoidal

E_{max} models (Gabrielsson & Weiner, 2000; Meibohm & Derendorf, 1997). All these are 'direct response' models represented by Figure 1.2.

The two types of model utilised in the work described in this thesis are:

The E_{max} model, where the effect-concentration relationship is described by Equation 1.2:

$$E = E_0 \pm \frac{E_{max} \cdot C}{EC_{50} + C} \qquad \text{Equation 1.2}$$

where, 'E' is the effect at a given concentration, C, E_{max} is the maximal effect, C is the concentration of active moiety in the plasma or a remote effect compartment (see below), and the EC_{50} is the concentration required to elicit 50% of the E_{max}. EC_{50} is a measure of the potency of the drug (i.e. its affinity for a receptor), whereas E_{max} reflects its activity.

The E_{max} model describes the concentration-effect relationship over a wide range of concentrations from E_0 (baseline effect) in the absence of drug, to the maximal effect at concentrations twice as high as the EC_{50}.

Sigmoidal E_{max} model: The sigmoidal E_{max} model is a variation of the simple E_{max} model. The relationship is sometimes known as the Hill equation:

$$E = E_0 \pm \frac{E_{max} \cdot C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}} \qquad \text{Equation 1.3}$$

Where γ is the shape factor describing the steepness of the curve (Rowland & Tozer, 1995) (Figure 1.3). The larger the value of γ, the greater the change in response with concentration around the EC_{50} value (Rowland & Tozer, 1995). When γ is equal to 1, the sigmoidal E_{max} model collapses to the simple E_{max} model. Patients may differ widely in their values of EC_{50} and γ for a given drug (Chan *et al.*, 1994; Moghadamnia *et al.*, 2003; Rostami-Hodjegan *et al.*, 1998) (Section 1.3.2).

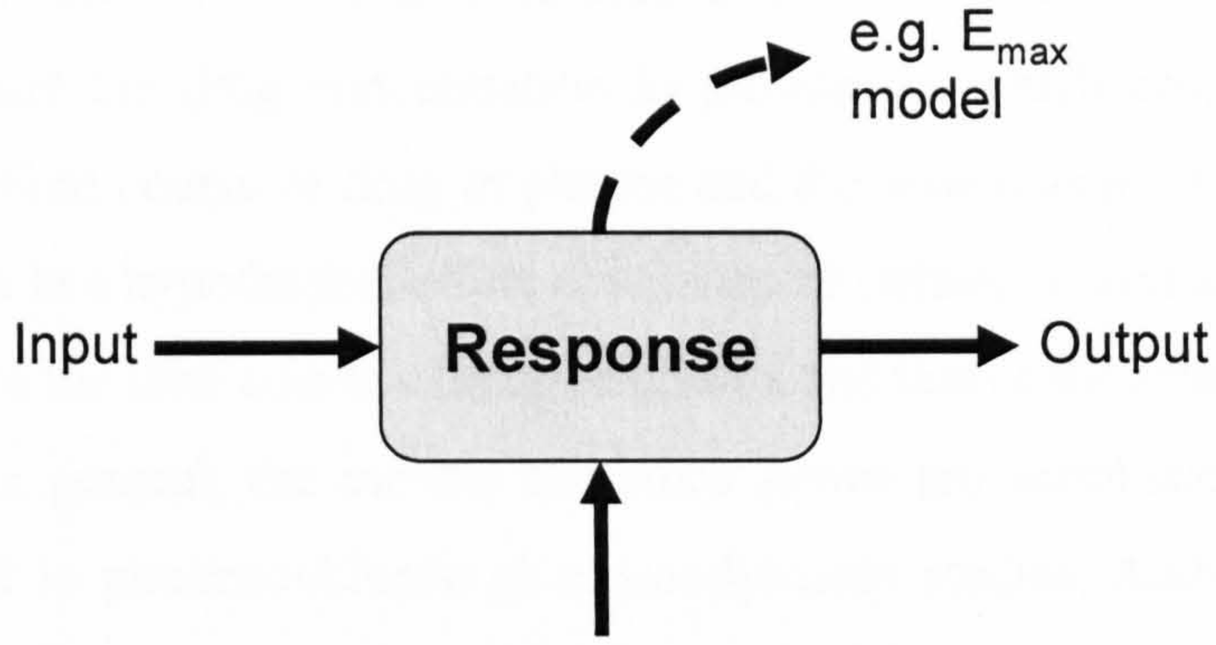

Figure 1.2 A schematic representation of a direct response model (see Figure 1.4 for comparison with an indirect model) (Gabrielsson & Weiner, 2000).

In the two models described above, the concentration term, C, may represent either the drug concentration in plasma (in which case there is no delay between the time course of drug in plasma and the time course of effect), or the drug concentration in a hypothetical effect compartment (which is used to account for a time delay between the time course of drug in plasma and that of the effect).

In general, the models described above are simplistic representations of those utilised in pharmacokinetic-pharmacodynamic studies. Additional complexities may involve the incorporation of a placebo response, the modelling of inhibitory response, where the effect may not exceed the baseline effect ($E_{max} \leq E_0$), or the case where two drug molecules compete for the same receptor site (e.g. the combined action of parent drug and its active metabolite). These topics are described in the appropriate sections of this thesis, as and when they arise.

Alternatively, response may be modeled using more mechanistic models, such as indirect response (turnover) models. For some drugs, the lag between the plasma concentration and pharmacological effect is not only the result of a delay in the drug in plasma reaching the effect compartment (see above). It can also represent the accumulation or depletion of some biological entity resulting in a build up or gradual loss of effect within the body. In this case, an indirect model is used when prior information on the mechanism of drug action is available (Gabrielsson & Weiner, 2000). In contrast to direct response models, indirect response models are used to describe the action of drugs that influence the build up (or loss) of response, rather than acting directly on the magnitude of response (Figure 1.4).

In such models, the measured response to a drug (E) may be due to factors controlling the turnover rate, k_{syn} (input or production), or the fractional turnover rate, k_{deg} (loss) of the biological factor mediating response (Gabrielsson & Weiner, 2000). The rate of change of the response over time with no drug present can be described by Equation 1.4:

$$\frac{dR}{dt} = k_{syn} - k_{deg} \times E \qquad \text{Equation 1.4}$$

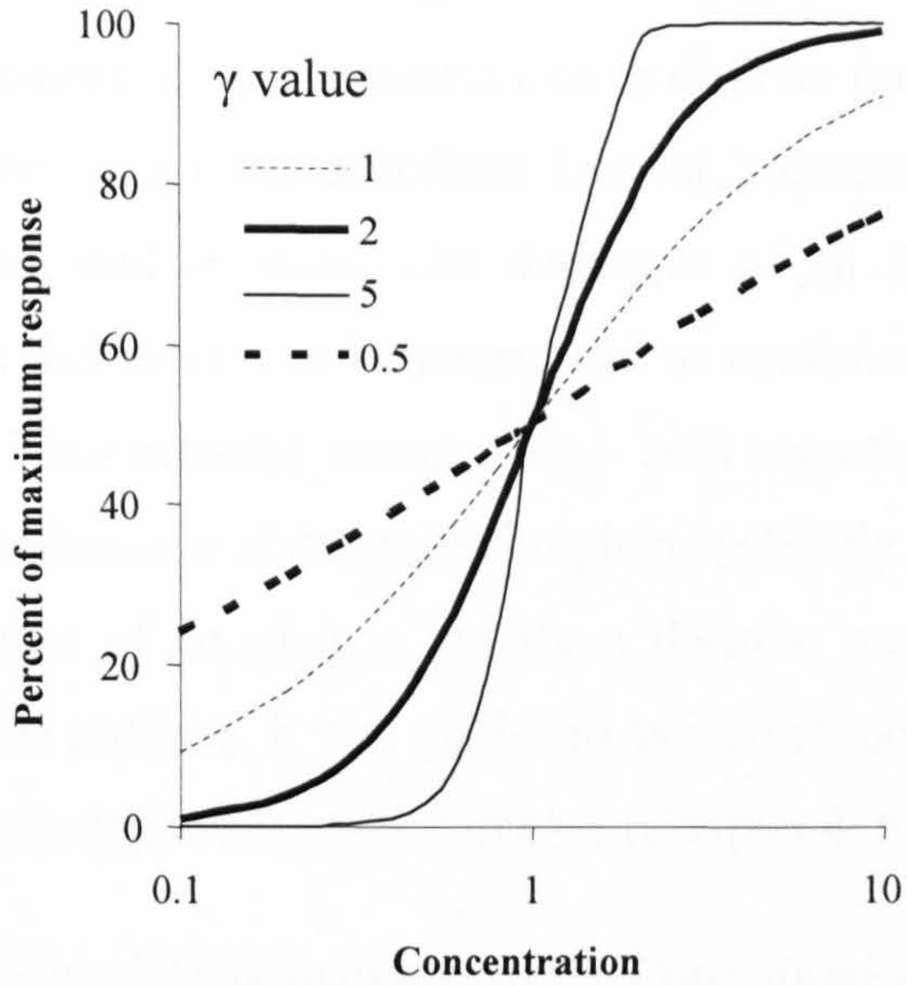

Figure 1.3 A concentration – response plot according to equation 1.3 for four hypothetical drugs that have the same EC_{50} value but different values of the shape factor, γ (Rowland & Tozer, 1995).

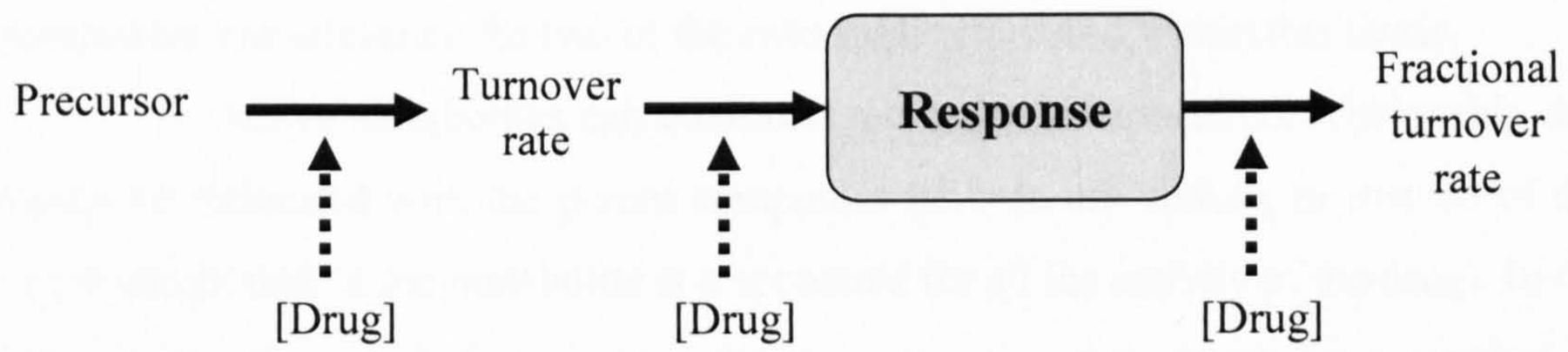

Figure 1.4 A schematic representation of an indirect response model (see Figure 1.2 for comparison with a direct model). [Drug] indicates the sites of potential interaction between drug and target (Gabrielsson & Weiner, 2000).

There are a wide range of techniques to measure response *in vivo*. As a result, measurements may take continuous or discrete forms. Continuous data may take on any value within a finite or infinite interval, whereas discrete data takes on values that are distinct and separate. The outcomes of all the pharmacodynamic models utilised within this thesis can be considered as continuous variables (warfarin – INR; midazolam – EEG activity; omeprazole – acid secretion rate; tolbutamide – insulin secretion rate). The case of dextromethorphan is slightly more complex as the response variable (number of coughs) is strictly a discrete measurement. However, for the purposes of data analysis, it was modelled as a continuous variable, although the final results were recorded to the nearest integer (Chapter 4; Section 4.2).

1.2.4.1 Additional Complexities of the Concentration-Effect Relationship

In many cases plasma concentration has been known to correlate poorly with response. In addition to the factors discussed above (delay in drug reaching effect compartment or delay in drug eliciting its effect *via* a biological entity), a number of other reasons may be responsible for this poor correlation. These include the presence of active metabolites (as receptor agonists or antagonists (Danhof & Mandema, 1995)) or chirality in the drug molecule (Lee & Williams, 1990). The presence of active metabolites has relevance for two of the case studies outlined within this thesis.

Active metabolites can confound the concentration-effect relationship and should be measured with the parent compound (if both are active), or instead of the parent compound (if the metabolite is responsible for all the activity of the drug). In the former case, the interaction between the two moieties may occur by a number of different mechanisms, namely, (i) competitively, where both compounds compete for the same receptor site, (ii) additively, where they act at different receptor sites, or (iii) synergistically, where the moieties act at different receptor sites as in (ii), but the resulting effect is greater than the sum of the effects at the two sites (Greco *et al.*, 1995).

For some drugs the maximal effect may be observed after the drug concentration reaches its peak in the plasma (Figure 1.5). Possible causes of such a delay are a poorly perfused tissue, a drug that has poor membrane permeability, or an indirect mechanism of action.

1.2.5 Biomarkers

The use of biological markers, or 'biomarkers', to predict the relationship between exposure to and the safety or efficacy of a drug product offers the potential of accelerating and cutting the costs of drug development. A biomarker is defined as a "physical sign or laboratory measurement that may be detected in association with a pathological process and that have putative diagnostic and/or prognostic utility" (Rolan *et al.*, 2003). They facilitate the prediction of clinical responses that may be more difficult, costly or time-consuming to measure directly (Rolan *et al.*, 2003). In early drug development, biomarkers may be used as a guide to dose selection and escalation, a process which would otherwise involve more trial and error and guess-work (Rolan *et al.*, 2003). Whether many biomarkers are sufficiently representative of the clinical response to a drug is a subject of much debate.

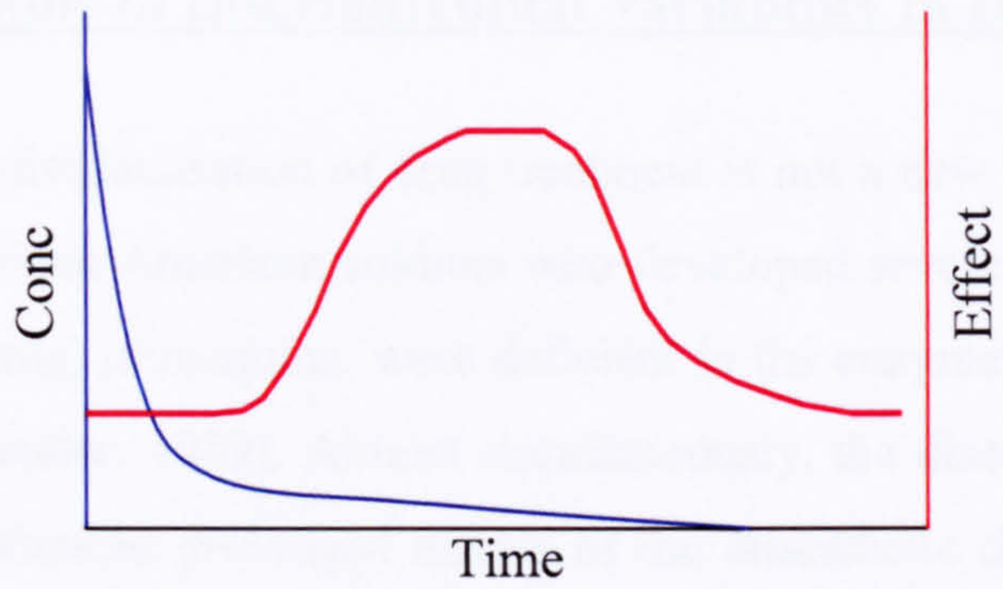

Figure 1.5 Representation of a time delay between drug in plasma and drug effect.

1.3 The Role of Interindividual Variability in Drug Response

The individualisation of drug treatment is not a new idea. In the 1950's it was found that African-American soldiers who developed severe anaemia after taking the anti-malarial drug, primaquine, were deficient in the enzyme glucose-6-phosphate dehydrogenase (Beutler, 1959). Almost simultaneously, the discovery was made that patients who experienced prolonged effects of the anaesthetic drug, succinylcholine, possessed an atypical from of a cholinesterase enzyme (Kalow & Staron, 1957). Since this time, a large amount of research has been conducted to elicit the physiological, demographic, ethnic and genetic basis of individual variation in drug response. It is now a widely held view that the personalisation of medicine to the individual is inevitable to the future of therapeutic medicine. However, it is also apparent that solid examples of the use and success of individualised medicine are few and far between. There are a number of possible reasons for this:

i) Lack of awareness on the part of clinicians.

ii) The difficulty, complexity, and often high cost associated with performing the assays, necessary to determine many of the genetic factors leading to interindividual variability in drug response.

iii) The rarity of many of the factors leading to the extreme or 'outlier' responses.

iv) The possibility (or probability) that several, if not many, genetic, demographic or environmental factors may contribute to variation and the difficulty in characterising all of them.

Historically, the philosophy in the clinic with regard to drug therapy has been the 'one size fits all' approach. It is now generally accepted that at least for some drugs, this attitude is not likely to lead to the best therapy. Indeed, when the average value of a population parameter is considered in isolation, without regard for the variability of the individual values around the mean, a great deal of information is lost. We hence have little idea of what is likely to occur in 'outlier' individuals. Given that these individuals are likely to be those most at 'risk' from adverse events or ineffective therapy, the situation is not ideal.

There are a small number of examples of situations where genetic testing has been used prior to treatment to inform the clinician on how best to proceed with

drug therapy for a particular individual. Pharmacogenetic information is contained in about ten percent of labels for drugs approved by the FDA and a significant increase of labels containing such information has been observed over the last decade (http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm). For example, in November 2004, the labelling of the drug Camptosar (irinotecan) was updated to include information on its metabolism by UGT1A. Camptosar is a drug used to treat colon/rectal cancer. Nearly 70% of patients require a dose reduction in order to prevent neutropenia. Since exposure to the drug is dependent on its metabolism by UGT1A1, patients with certain enzyme mutations are expected to require a lower dose of the drug.

1.3.1 Pharmacokinetics as a Source of Variability

Interindividual variability arises from a number of sources (Figure 1.6). These could be environmental factors (e.g. food, pollutants, time of day and season, location etc.), genetics, disease, age, concomitant medication and compliance (Rowland & Tozer, 1995). With the exception of compliance, each of these factors affects variability by either altering the pharmacokinetics or the pharmacodynamics of a particular drug (Figure 1.6).

A major source of variability in response can be attributed to interindividual differences in the plasma concentration – time profiles of drugs. Therefore, factors affecting the ADME of drugs are of importance in understanding overall variability in pharmacokinetics.

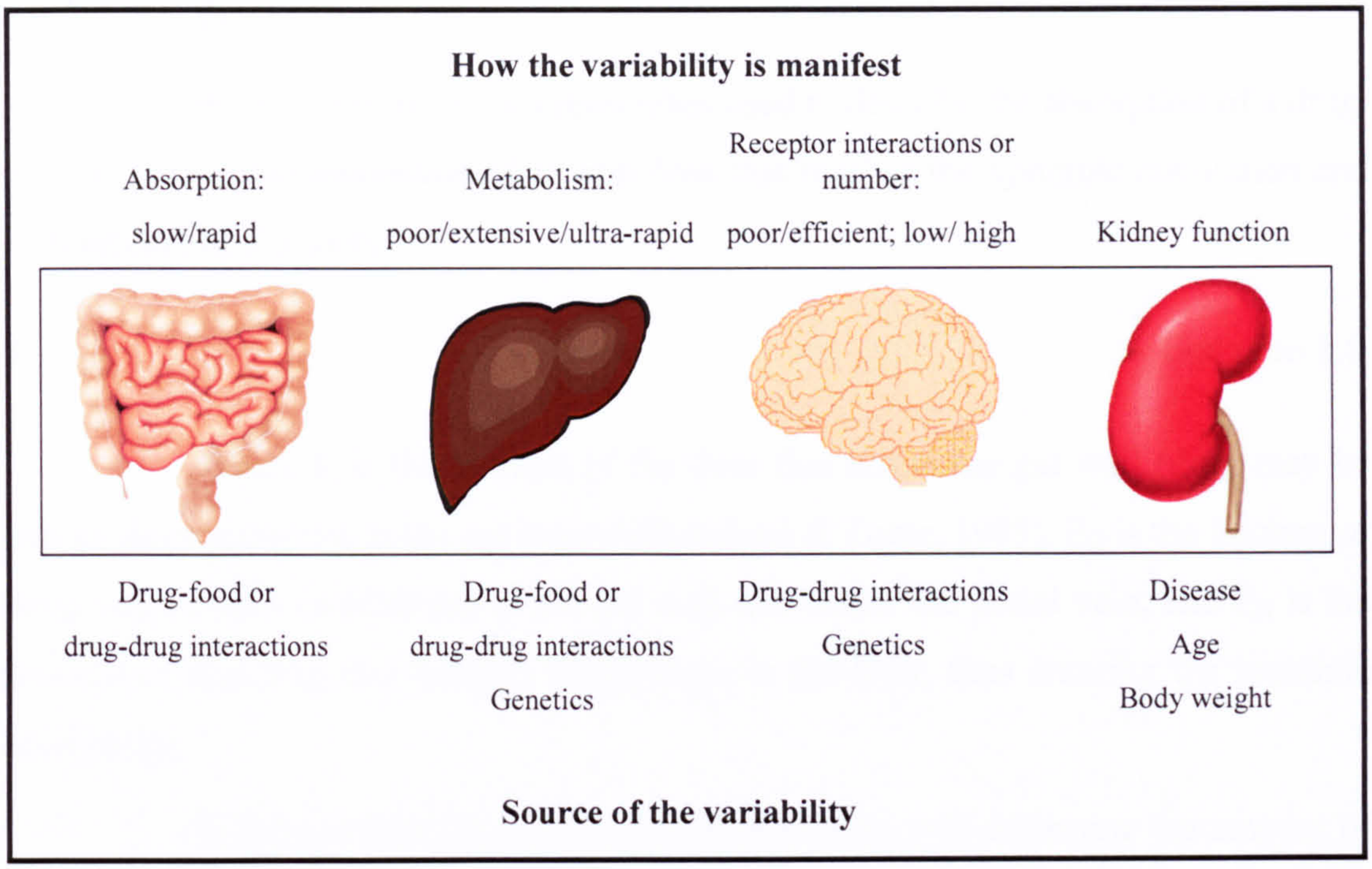

Figure 1.6 Sources of variability in pharmacokinetics and pharmacodynamics and how these variabilities are manifest (Ingelman-Sundberg, 2001).

1.3.1.1 Drug Absorption

Bioavailability (F) is a term often used to describe the absorption of a drug. It is defined as the proportion of an oral dose that reaches the systemic circulation and is described by Equation 1.5:

$$F = f_a \times F_G \times F_H \qquad \text{Equation 1.5}$$

Where f_a is the fraction of the dose that enters the gut wall (drug may be lost by decomposition in the gut lumen) (Rowland & Tozer, 1995), F_G is the fraction of drug that escapes metabolism in the gut wall and enters the portal vein, and F_H is the fraction of the drug that escapes metabolism in the liver, thus entering the systemic circulation.

It follows that the extent of drug absorption will determine the amount of drug that reaches the systemic circulation from the gut lumen and hence, the dose requirement. The rate of absorption is also important in determining the time span of absorption, and therefore the frequency of dosing, particularly in the case of sustained release formulations.

Each of the parameters, f_a, F_G, and F_H is sensitive to a number of factors that differ between individuals. For example, f_a is in many cases affected by gastrointestinal motility (particularly for drugs with low permeability). F_G is sensitive to variation in drug metabolising enzyme activity, diet, and active secretion of the drug back into the gut by the multidrug efflux pump, P-glycoprotein (P-gp), which may be susceptible to interindividual fluctuations in both abundance and activity. Variability in the first pass metabolism of the drug by the liver (F_H) is a result of genetic, environmental and physiological (e.g. liver blood flow and size) differences in drug metabolism.

1.3.1.2 Distribution

Distribution refers to the reversible transfer of drug from one location to another within the body (Rowland & Tozer, 1995). Distribution of drugs to and from blood and other tissues occurs at different rates and to varying extents (Rowland & Tozer, 1995). Several factors are responsible for the distribution pattern of a drug over time. These include the perfusion of different tissues by blood, the ability of the drug to cross membranes (e.g. the blood brain barrier), drug binding in the blood and tissues, and partitioning into fat (Rowland & Tozer, 1995).

The fraction of drug that is unbound in blood (fu_B) often varies between individuals. For very highly bound drugs such variability can be large (Lin & Lu, 1997), but is normally less than that of other pharmacokinetic parameters (Yacobi *et al.*, 1977). The fu_B of a drug is a function of its affinity for plasma proteins (Rowland & Tozer, 1995), each of which has a finite number of binding sites. Because of this, binding is also dependent on the concentrations of both drug and protein in the blood (Rowland & Tozer, 1995). The concentration of protein in the plasma can vary due to factors such as age, liver cirrhosis, pregnancy, trauma and stress.

Interindividual variability in the distribution of drugs is also a result of variations in the size and composition of different organs. For example, differences in body fat content between individuals, and in particular between males and females, may lead to differences in the volume of distribution. A highly lipid soluble drug may distribute more into the adipose tissue of a female with high body fat and become less available to the eliminating organs. Consequently, the half life of the drug may be higher in females compared to males.

1.3.1.3 Drug Metabolism

For the majority of drugs, metabolism is a major route of elimination from the body (Williams *et al.*, 2004). Some of the important questions that need to be answered during the development of a new drug are: (i) what are the major (and minor) routes of metabolism? (ii) are any active and/or toxic metabolites produced?, (iii) what enzymes are involved?, and (iv) are there any potentially important drug-drug interactions?

It is advantageous to identify the pathways involved in the metabolism of a drug, the structure of the metabolites generated, and the enzymes involved, as early in the drug development process as possible. Such information can be used to predict the effect of genetic and environmental factors on pharmacokinetics, to predict the likelihood of clinically important drug-drug interactions, and to select appropriate animal species for toxicology and efficacy testing before the drug is given to humans (Williams & Ette, 2000). The use of *in vitro* systems to characterise drug metabolism and its extrapolation to *in vivo* pharmacokinetics is discussed further in Section 1.5.4.2.

During pre-clinical development, the quantitative prediction of *in vivo* metabolic clearance from *in vitro* data is arguably one of the most important objectives of *in vitro* metabolism studies. Clearance can be used to assess the likelihood of

individual drug interactions occurring, to determine appropriate dosage regimen for *in vivo* studies, and to provide information on concentration-time profiles and on the risk of drug toxicity. Several methods have been employed to determine clearance from *in vitro* data (Section 1.5.4.2).

Polypharmacy, the administration of several therapeutic drugs to a patient concurrently, is commonplace in the developed world, and inpatients often receive an average of five different drugs at any one time during their hospitalisation (Rowland & Tozer, 1995). Adverse drug reactions including drug interactions have been estimated to be one of the leading cause of death in the western world (Lazarou *et al.*, 1998), although some concerns have been raised with respect to the methods of analysis of these data (Kvasz *et al.*, 2000). Moreover, drug interactions are a leading cause of the removal of drugs from the market (Table 1.1) (www.fda.gov).

Interindividual differences in drug metabolism are caused by a wide range of factors, the most important probably being induction or inhibition of enzyme activity by drugs and other xenobiotics, and the genetic makeup of the individual. Variants of genes coding for drug metabolising enzymes can result in higher, lower or no activity, or they may lead to a complete absence of the enzyme (Lin & Lu, 1997). Such genetic variation is termed a 'polymorphism' when the monogenic trait occurs at a single gene locus with a frequency of more than (arbitrarily) 1% (Meyer, 1991). Specific examples of polymorphisms of drug metabolising enzymes will be discussed in Section 1.4.

Some of the potential consequences of genetic polymorphisms of drug metabolising enzymes are: (i) unwanted responses (toxic effects) as a result of elevated plasma concentrations through impaired clearance (ii) a lack of pro-drug activation, or (iii) a dependence on alternative routes of elimination. If these alternative routes are also compromised (e.g. by renal impairment, drug–drug or drug–food interactions), the outcome may be more severe (Dickins & Tucker, 2001).

The extent of the consequences of such polymorphisms for the pharmacokinetics of a drug depends on a number of factors (Dickins & Tucker, 2001): (i) whether the polymorphic enzyme metabolises the parent drug or the metabolite(s) or both, (ii) the contribution of the polymorphic enzyme to overall elimination of the drug, (iii) the potency of any active metabolites, and (iv) the viability of the other, competing pathways of elimination. Such pharmacogenetic variability in drug metabolism is discussed further in Section 1.4, and its effects on pharmacokinetics and pharmacodynamics in Chapter 2.

Table 1.1 Reasons for recent withdrawals of drugs from the US market (www.fda.gov). DDI = Drug-drug interaction

		Reason for Withdrawal			
Year	Drug	DDI	Torsades de Points	Hepato-toxicity	Other
1998	Terfenadine	✓	✓		
	Mibefradil	✓	✓		
	Bromfenac			✓	
1999	Astemizole	✓	✓		
	Grepafloxacin		✓		
2000	Troglitazone			✓	
	Cisapride	✓	✓		
	Alosetron				✓
2001	Cerivastatin	✓			✓
	Rapacuronium				✓

1.3.1.4 Excretion

Many drugs are eliminated from the body partly or entirely by renal (or in some cases biliary) excretion. In the present work, the renal route of elimination is not a major focus of attention, since it plays a relatively minor role in the clearance of the drugs selected for study. The only covariates considered with respect to this route are those known to affect creatinine clearance (e.g. age or body weight).

As well as being influenced by plasma drug concentration, protein binding and renal function, the renal clearance (excretion) of a drug is affected by urine flow and urine pH. These two factors vary widely between individuals, which leads to considerable interindividual differences in the excretion of drugs from the body (Rowland & Tozer, 1995). The urine flow of an individual is sensitive to fluid intake and to the administration of diuretic drugs. Interindividual differences in urine pH are brought about by differences in diet, drug intake, and clinical status, as well as being due to natural diurnal variation (Rowland & Tozer, 1995).

Biliary excretion of drugs is sensitive to the ingestion of environmental substances or toxins, pathophysiological conditions that cause cholestasis (Shargel & Yu, 1999), and to variation in the activity of certain transporters (e.g. OATP; Yamashiro *et al.*, 2006).

1.3.2 Pharmacodynamics as a Source of Variability

Drug response is likely to be the result of a complex function of the influence of many genes interacting with environmental and behavioural factors. Whether pharmacokinetic–pharmacodynamic variability translates into clinically relevant differences in drug response depends on further issues including compliance, the availability of alternate drugs and doctor/patient perception of side-effects (Dickins & Tucker, 2001).

Sources of pharmacodynamic variability include genetics (e.g. of transporters or receptors) and demographics (e.g. developmental differences in the abundance of receptors or hormonal influence on the regulation of receptors).

The consequence of polymorphisms in receptors that mediate drug response depends on a number of contributing factors (Dickins & Tucker, 2001): (i) the point on which the concentration-effect relationship typical concentrations arising from 'normal' doses fall, (ii) the therapeutic index and utility of the drug, and (iii)

whether pharmacokinetic variability is outweighed by pharmacodynamic variability in receptor sensitivity or density, or in the turnover of a particular endogenous receptor ligand.

There is substantial evidence that genetic variation in drug receptor and effector proteins is associated with variable drug response (Johnson, 2001; Evans & McLeod, 2003). Examples of genes for which genetic polymorphisms have been reported include those encoding β-adrenergic receptors (Liggett, 2000), μ-opioid receptors (Hollt, 2002; Lotsch *et al.*, 2002) and G proteins (Johnson & Lima, 2003). All of these polymorphisms have the effect of altering response to drug therapy, independent of dose and exposure levels, and of increasing interindividual variability in response.

1.3.3 Other Sources of Variability in Drug Response

There are a number of sources of variability in human drug response that do not fit into either of the above two categories. These include disease status, compliance with the prescribed drug regimen, and variability in prescribing. These factors will not be discussed, since they are not considered as sources of variability in the pharmacokinetic-pharmacodynamic models used in this thesis. Pharmacokinetic-pharmacodynamic models are usually carried out either under controlled conditions in healthy individuals where such variables do not apply, or in hospitalised patients where compliance should not be an issue.

1.4 Variability in Drug Metabolising Enzymes

A major focus of the present work is the propagation of pharmacogenetic differences in cytochrome P450 enzymes into pharmacokinetic and pharmacodynamic measures. Therefore, a background to drug metabolism and its determinants is given below.

1.4.1 Background to Drug Metabolism

The liver is the main organ responsible for drug metabolism; other organs that contribute to a lesser extent include the gastrointestinal tract (either via enzymes in the gut wall or flora that inhabit the gut), kidneys, lungs, blood, brain, skin and placenta. The metabolism of drugs and xenobiotics is divided into two phases, termed phase one and phase two.

1.4.2 Phase One Drug Metabolism

Phase one reactions involve the oxidation, reduction, hydrolysis and hydration of drug compounds. Their primary aim is to render the drug molecule more hydrophilic (polar), to facilitate its excretion in the urine. Phase one reactions may also introduce a relatively reactive group into the molecule, which then serves as a point of attachment for a conjugate group by the enzymes in phase two metabolism (Section 1.4.3). The cytochrome P450 (CYP) enzymes are primarily responsible for the majority of phase one drug metabolism, with other hepatic enzymes such as xanthine oxidase, monoamine oxidase, flavin containing monooxygenase and epoxide hydrolase playing a lesser role. All CYP enzymes possess a porphyrin-haem complex as the catalytic centre, but they differ in their amino acid sequence. They are subject to induction, inhibition, and genetic polymorphism. Hence, a main focus of screening strategies within pharmaceutical drug development is on how metabolism by CYP enzymes may affect the pharmacokinetics of drugs.

1.4.2.1 The Cytochromes P450 (CYPs)

The nomenclature for the CYP's is based on amino acid sequence (Figure 1.7). Those sequences with more than 40% homology are grouped into the same family (Guengerich, 1995). Within families, those sequences with 40% or more homology are grouped into a subfamily (Guengerich, 1995). Different genes in the

same subfamily are distinguished by the second Arabic numeral (e.g. CYP3A4, CYP3A5) and have at least 60% homology with one another.

To date, 76 *CYP* gene families and 233 subfamilies have been described in animals (http://drnelson.utmem.edu/CytochromeP450.html). However, a relatively small number of these enzymes (expressed by the *CYP1, 2* and *3* gene families) are responsible for the majority of drug oxidations in man.

1.4.2.2 The CYP Subfamilies Relevant to this Thesis

The CYP2C Subfamily: CYP2C is the second most abundant protein in human liver after CYP3A (Rowland-Yeo *et al.*, 2004). It consists of four members: CYP2C8, CYP2C18, CYP2C9, and CYP2C19, each of which exhibits polymorphism (Daly, 2003).

CYP2C9 plays a major role in the metabolism of a large number of clinically important drugs such as *(S)*-warfarin (Rettie *et al.*, 1992), tolbutamide (Sullivan-Klose *et al.*, 1996) and phenytoin (Veronese *et al.*, 1993).

Two variant alleles of CYP2C9 (*CYP2C9*2 and CYP2C9*3*) are common in Caucasians, but no null alleles (alleles that result in absent or non-functional protein) have been identified. Each of the two variant alleles confers a different degree of decreased activity as compared to the wild type (*CYP2C9*1*). Hence, there are six common genotypes of *CYP2C9* in Caucasians, each associated with a different level of activity; the frequency of these genotypes in the Caucasian population is shown in Figure 1.8. Figure 1.9 shows the difference in the metabolic ratio of losartan (a CYP2C9 substrate) between CYP2C9 extensive (EM), intermediate (IM) and poor metabolisers (PM).

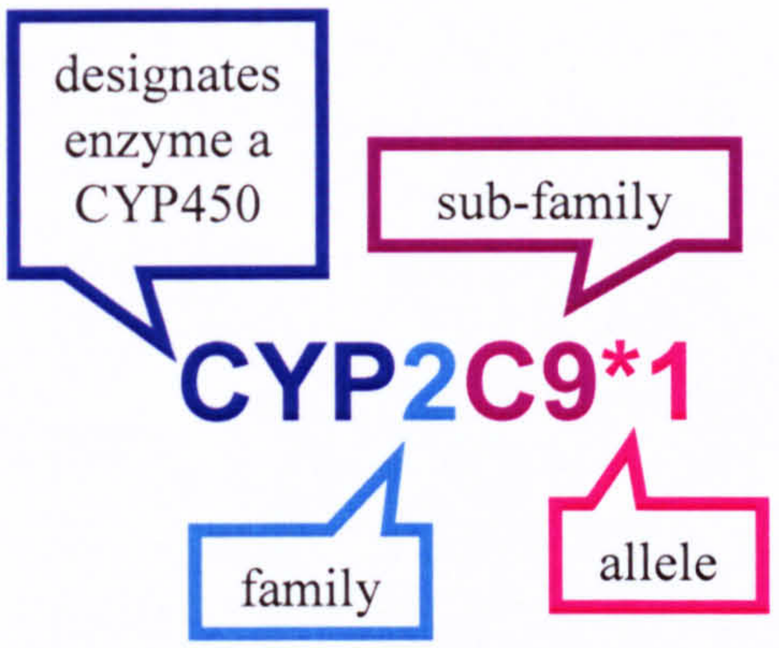

Figure 1.7 The internationally accepted method for naming CYP enzymes (http://www.cypalleles.ki.se).

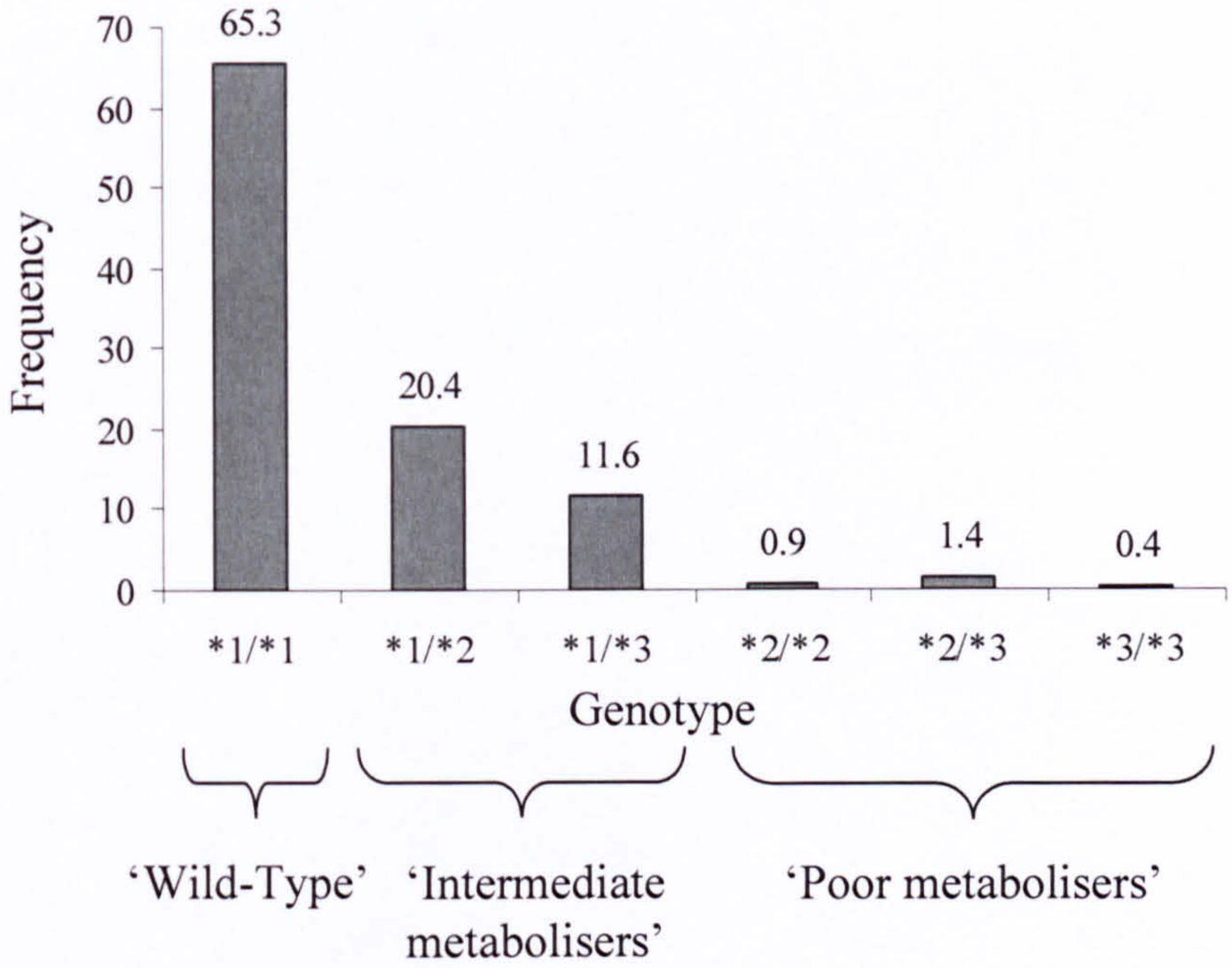

Figure 1.8 Frequencies of each of the common *CYP2C9* genotypes (Lee *et al.*, 2002).

Eight alleles have been identified for the *CYP2C19* gene (Wedlund, 2000) Individuals with two defective alleles are classed as PM's, with a frequency of around 2-4% of Caucasians (Kalow, 1986). Most (*CYP2C19*3, *4, *5, *6, *7* and *8) are rare in Caucasian populations (Wedlund, 2000). *CYP2C19*2* is the most common allele associated with an inactive gene product in Caucasians, accounting for up to 85% of those responsible for impaired metabolism (Desta *et al.*, 2002). Most of the remaining PM's are the result of the *3 allele (Wedlund & Wilkinson, 1996).

Figure 1.10 shows the differences in the metabolic ratio (MR) of omeprazole between *CYP2C19* genotypes.

The CYP2D Subfamily: CYP2D6 is the only member of the CYP2D subfamily to be expressed in human liver. Despite it only making up around 3% of total human liver CYP content (Figure 1.12), CYP2D6 is probably the best understood of the polymorphic CYP's due to both its earlier discovery (Eichelbaum *et al.*, 1979; Idle *et al.*, 1978; Lennard *et al.*, 1983), and its contribution to the metabolism of an appreciable percentage of marketed drugs (at least 25% of the drugs on the market; Rendic, 2002).

There are a large number of variant alleles of CYP2D6. The fully functional alleles are CYP2D6*1 and CYP2D6*2, with CYP2D6*9, CYP2D6*10 and CYP2D6*41 conferring reduced activity. There are also a number of null alleles (e.g. CYP2D6*3, *4, *5, *6, *7 and *8), giving rise to many possible genotypes. Generally, those made up of the *1 and *2 alleles are grouped as the EM genotypes. PM genotypes are those which result from a combination of two null alleles, and some refer to all the other combinations as IM's, although this is the subject of debate. The occurrence of ultra-rapid metabolisers of CYP2D6 substrates has been established, and results from an individual having multiple copies of the active *CYP2D6* genes. PMs have a prevalence of about 8% in Caucasians (Cascorbi, 2003), and ultra rapid metabolisers a prevalence of 1 -3% in middle Europeans (Cascorbi, 2003).

The activity of CYP2D6 is highly variable between individuals. A greater than 100-fold difference in activity was reported by Ingleman-Sundberg *et al.* (1999), which was mainly the result of both genetic variability and inhibition of CYP2D6 by other compounds (Ingelman-Sundberg *et al.*, 1999).

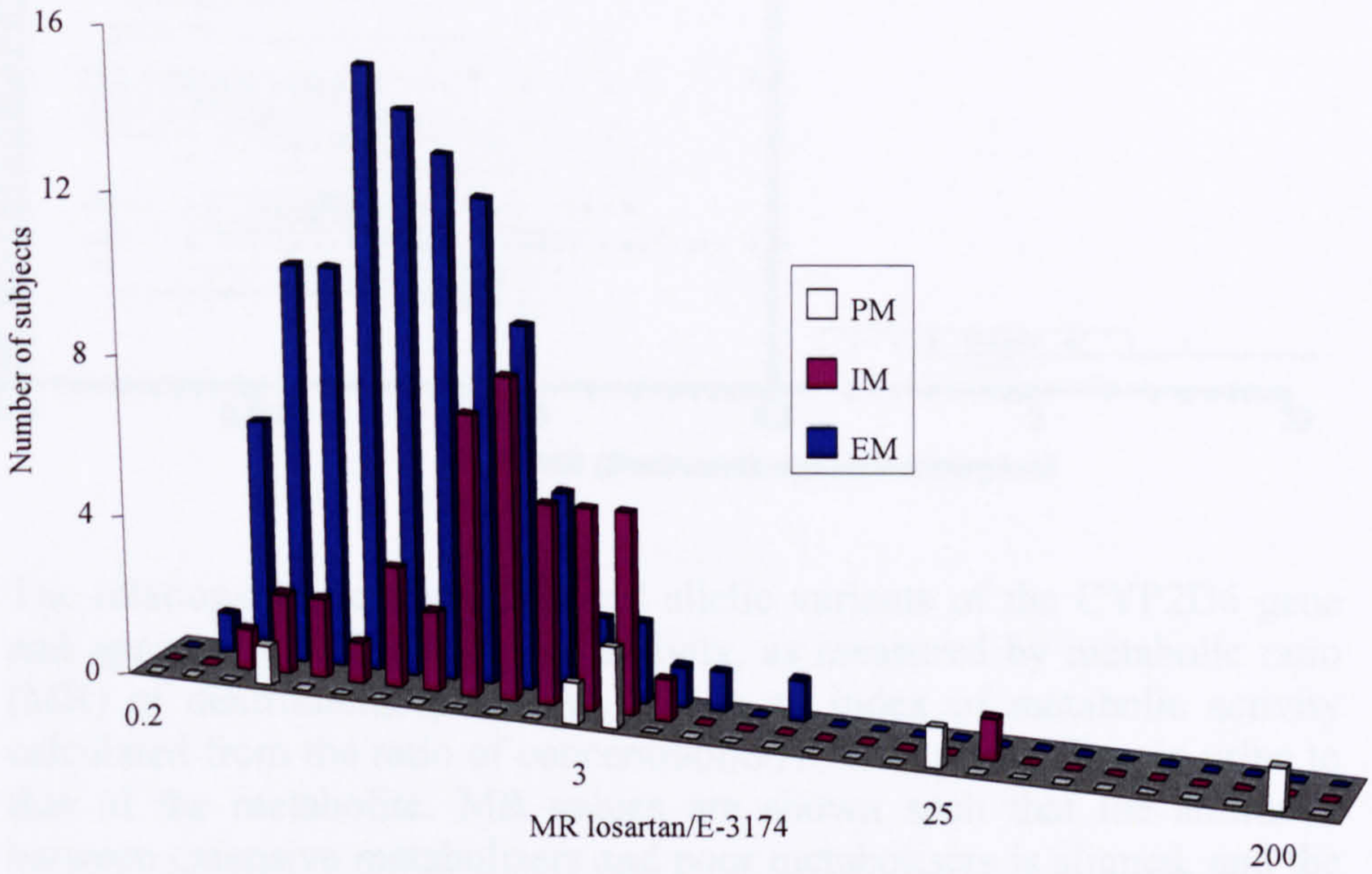

Figure 1.9 Frequency distribution of the metabolic ratio (MR) of losartan in *CYP2C9* genotyped subjects (PM = *2/*2, *2/*3 and *3/*3; IM = *1/*2 and *1/*3; EM = *1/*1) (Sandberg *et al*., 2004).

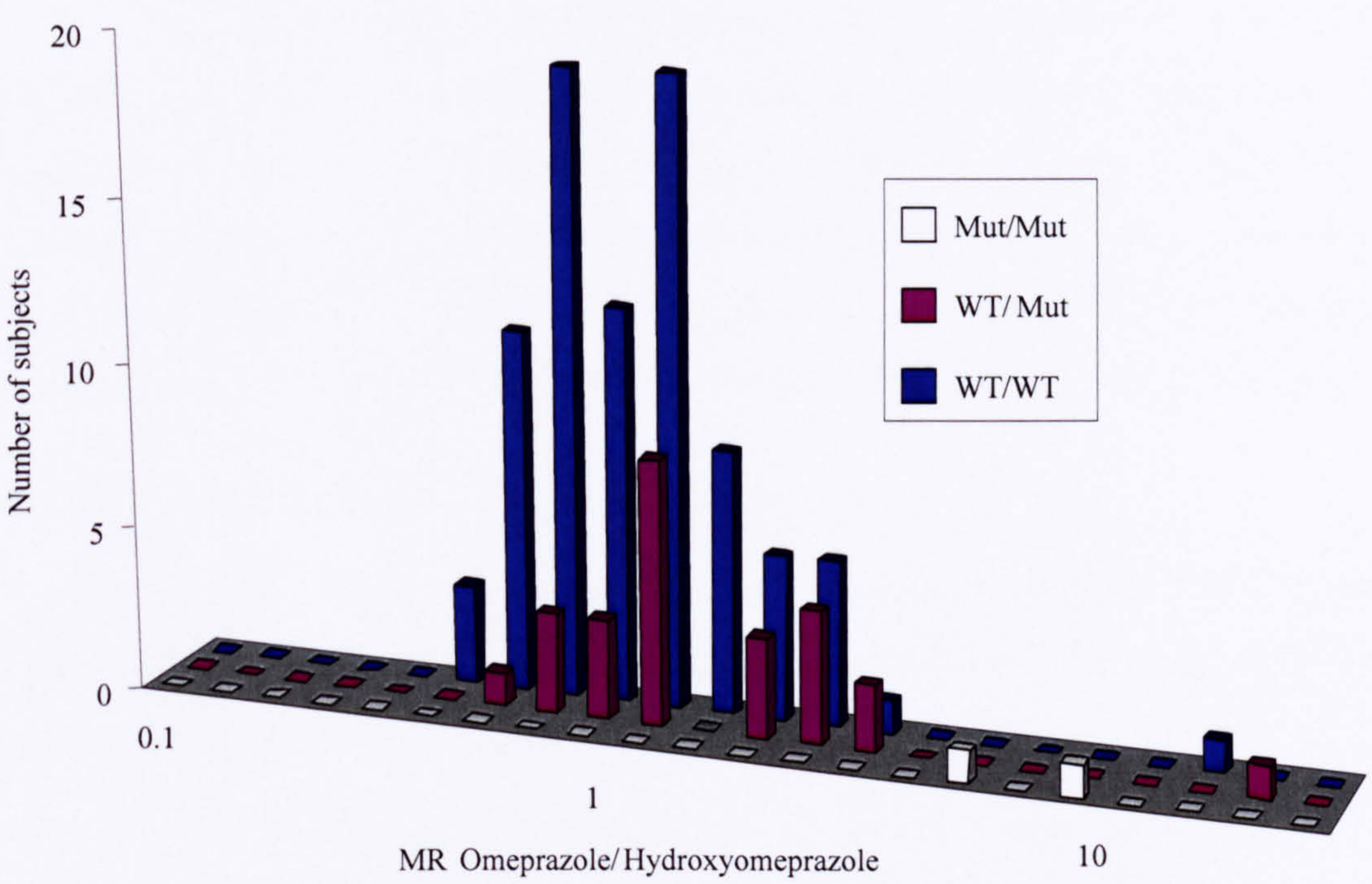

Figure 1.10 Frequency distribution of the metabolic ratio (MR) of omeprazole in *CYP2C19* genotyped subjects (WT = wild type; Mut = mutant allele either *2 or *3) (Sagar *et al*., 1998).

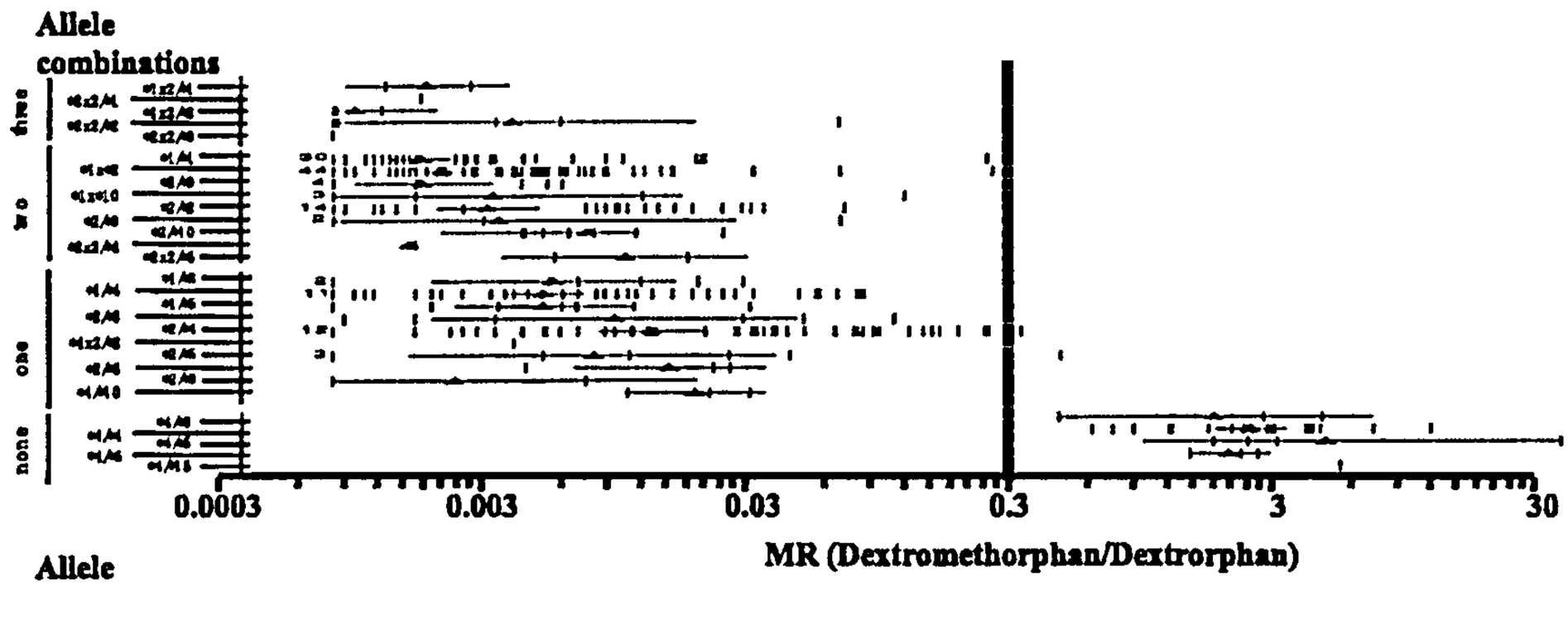

Figure 1.11 The relationship between different allelic variants of the CYP2D6 gene and apparent CYP2D6 enzyme activity, as measured by metabolic ratio (MR) of dextromethorphan. An MR is an index of metabolic activity calculated from the ratio of concentration/AUC of parent drug in urine to that of the metabolite. MR values are shown such that the antimode between extensive metabolisers and poor metabolisers is aligned, and the scales are normalized on the basis of fold variation from the antimode. [data from Sachse *et al.* (1997), represented as in Ozdemir *et al.* (2004)].

The CYP3A Subfamily: The CYP3A subfamily has three main members, CYP3A4, CYP3A5 and CYP3A7 (which is only expressed in foetal tissue). CYP3A is the most abundant isoform present in human liver (Figure 1.12; Rowland-Yeo *et al.*, 2004), and is also the predominantly expressed enzyme in the gut wall (Paine *et al.*, 2006). There is marked (5 to 82 fold) interindividual variation in the hepatic activity of CYP3A (Gibbs & Hosea, 2003), and up to a 30 fold variation in the gut (Paine *et al.*, 1997).

CYP3A4 is the most abundant CYP enzyme in human liver (Figure 1.12). It also has a very broad substrate specificity, and capable of metabolising at least 60% of current therapeutic drugs (Rendic, 2002), including midazolam, dextromethorphan and omeprazole, three of the drugs studied in this thesis. Due to the presence of CYP3A4 in the gut, the isoform is especially susceptible to dietary effects. Although CYP3A4 is polymorphic (Ingelman-Sundberg, 1999), genetic variants are rare, and due to the wide interindividual variability in CYP3A4 activity regardless of genotype, the effect of such variants is difficult to discern.

CYP3A5 is polymorphic, and only individuals with at least one *CYP3A5*1* allele express a significant level of protein (Kuehl *et al.*, 2001). This is equivalent to around 10 – 30% of the Caucasian population (Wrighton *et al.*, 1990). When expressed, the amount of CYP3A5 in the liver constitutes a significant proportion of total CYP3A content (46 – 85%) (Lin *et al.*, 2002). Thus, CYP3A5 may contribute to the wide interindividual variability in CYP3A-metabolised drugs (Kuehl *et al.*, 2001).

1.4.2.3 Abundances of CYP Enzymes in Human Liver

Figure 1.12 shows the estimated relative abundance of each of the most common CYP isoforms based on a meta-analysis by Rowland-Yeo *et al.* (2004) of data from between 42 and 241 human livers.

CYP3A accounts for up to 35% of the total content of the common CYP enzymes in the human liver (Rowland-Yeo *et al.*, 2004).

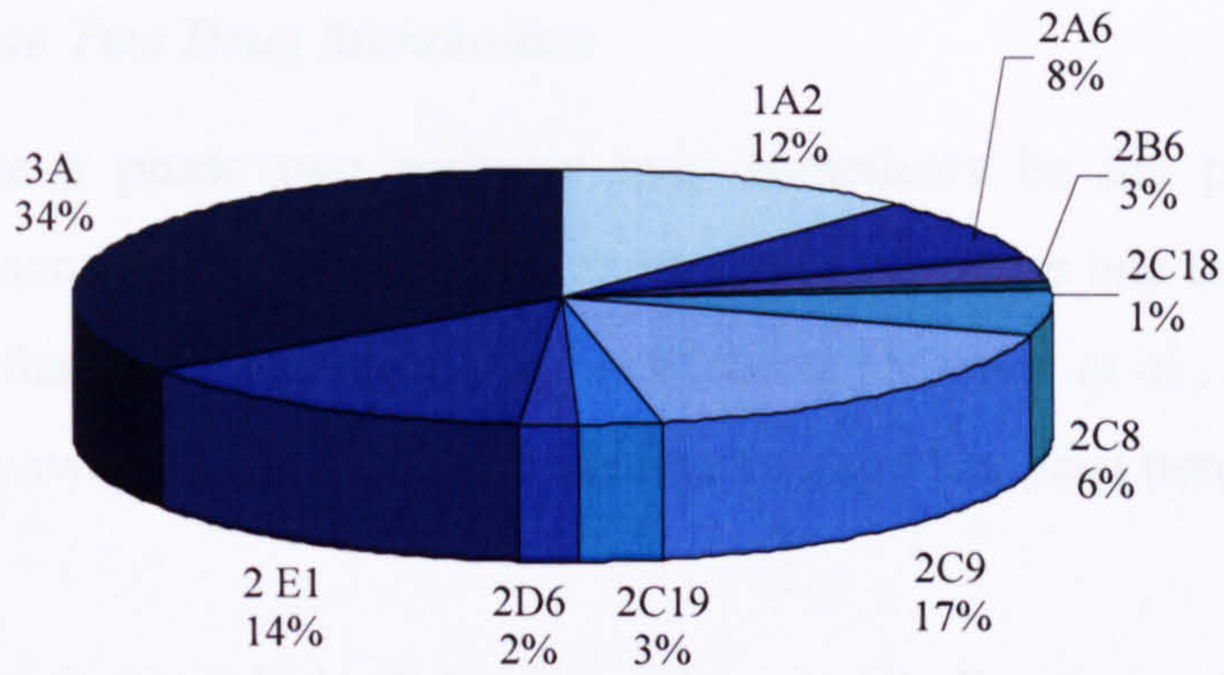

Figure 1.12 Estimated relative abundance of cytochrome P450 isoforms determined from a meta-analysis of all reported data and those cited by Rowland-Yeo *et al*. (2004). Note that very rare CYP isoforms were not included in the analysis.

1.4.3 Phase Two Drug Metabolism

Since a phase two pathway may sometimes be the primary route of metabolism for some drugs without any requirement for phase one metabolism, it has been suggested that the term 'phase two' is obsolete (Josephy *et al.*, 2005). Although this view is acknowledged, the traditionally terminology has been used throughout this thesis.

The primary 'aim' of phase two drug metabolism is to attach a conjugate to the drug molecule, to render it inactive and less lipid soluble than its precursor, so that it can be excreted in the urine or bile. The groups most commonly conjugated to drug molecules are glucuronyl, sulphate, methyl, acetyl, glycyl and glutathione.

Many phase two enzymes have been shown to be polymorphic, such as thiopurine methyltransferase, *N*-acetyltransferase, glucuronosyltransferase. Phase two enzyme polymorphisms are of importance only when they are responsible for the primary metabolism of the compound or of a metabolite, if the latter is responsible for some or all of the effects of the drug.

1.5 Modelling & Simulation

The advent of computer technology has radically changed drug development. However, many hold the view that we are far from utilising such techniques to their full potential and *'in silico'* techniques are in their infancy (Smith, 2002). A well known analogy to the use of simulation in the pharmaceutical industry comes from Beresford *et al.* (2002). The paper describes how the Boeing 777 aircraft was designed, manufactured and tested using only simulation, and nothing was physically produced until the manufacturers were confident that they had the final product in their electronic files (Beresford *et al.*, 2002). The authors argue that if such a feat is possible for aeronautical engineering, why not for pharmaceuticals (Beresford *et al.*, 2002)? Some argue that in the future, it will be possible to model in detail the complete physiology of the body, allowing us to administer drugs '*in silico*'. This will allow us to evaluate whether they are effective and/or toxic and to determine the ideal dose, before they are administered to animals or humans. Just as Boeing would be unlikely to manufacture an aircraft, only to push it off a cliff to see if it flies, it is an inefficient, and unfortunate feature of the drug development process, that the advancement of many drugs is terminated during the later stages because of undesirable yet unforeseen characteristics.

The focus of modelling and simulation in drug development within this thesis, will be the use of clinical trial simulation (CTS), whose main aim is to maximise the information content obtained during the drug development process in order to ensure the greatest chance of 'success' in a clinical trial (Bonate, 2000). The concept was first utilised by statisticians working within the field of drug development (Bonate, 2000). Indeed, the term clinical trial simulation is still often used, in its narrower sense, to describe the use of pharmacostatistical techniques within drug development. However, for the purposes of this thesis, a broader interpretation of the term will be utilised.

1.5.1 The Need for More Efficient Clinical Trials

Declining productivity has been a concern of the pharmaceutical industry for some time. Figure 1.13 demonstrates the apparently decreasing number of new chemical entities (NCEs) produced by drug companies in the United States over a ten-

year period (FDA, 2004). However, the amount of money spent on drug development appears to be increasing at an almost exponential rate (Figure 1.14).

Figures 1.13 and 1.14 are taken from a recent whitepaper, published by the FDA, stating that "During the last several years, the number of new drug and biologic applications submitted to FDA has declined significantly; and the number of innovative medical device applications has also decreased. In contrast, the costs of product development have soared over the last decade".

Nonetheless, Schmid and Smith (2005) have put forward a counter-argument to the above evidence, supporting the belief that declining innovation in the pharmaceutical industry may be a myth (Schmid & Smith, 2005). The authors argue that, whereas the total number of NCE's brought to the market does indeed appear to have been in decline over the 10 year period from 1993 to 2003 (Figure 1.13), a more representative view can be gained by looking at a longer period of time, from 1945 to 2004 (59 years). They observe an overall increase in the number of drugs launched in the USA when the extended time period is analysed (Figure 1.15).

However, despite their convincing counter argument, Schmidt and Smith (2005) dispute only the claim of declining innovation in the pharmaceutical industry. They make no attempt to dispute the problem of increasing cost. Therefore, even if their argument were true, it is undisputable that the cost of producing a single drug product is increasing at an unsustainable rate. Change is needed to improve the efficiency of drug development (Rooney *et al.*, 2001). The process could be made faster, and more efficient, hence reducing costs and increasing the number of new drug launches. Recently, Williams *et al.* (2006) commented on the large number of inadequately powered studies, carried out during drug development, to determine the effect of a particular drug metabolising enzyme polymorphism on the pharmacokinetics of drugs. They observe that studies that claim to either prove or disprove the functional relevance of the polymorphism may be misleading, because the studies are underpowered. The authors went on to suggest that clinical trial simulation is a valuable tool for ensuring the use of adequately sized samples for clinical studies.

In 2002 Johnsson and Sheiner advocated the need for 'smarter clinical trial design', and showed that simulation can help in this process by integrating all the information, in order to extract more evidence against the null hypothesis in clinical trials. They argued that, although greater reliance on simulations may involve a larger number of assumptions, and hence, give rise to less confidence in the results, this approach can still be effective (Jonsson & Sheiner, 2002).

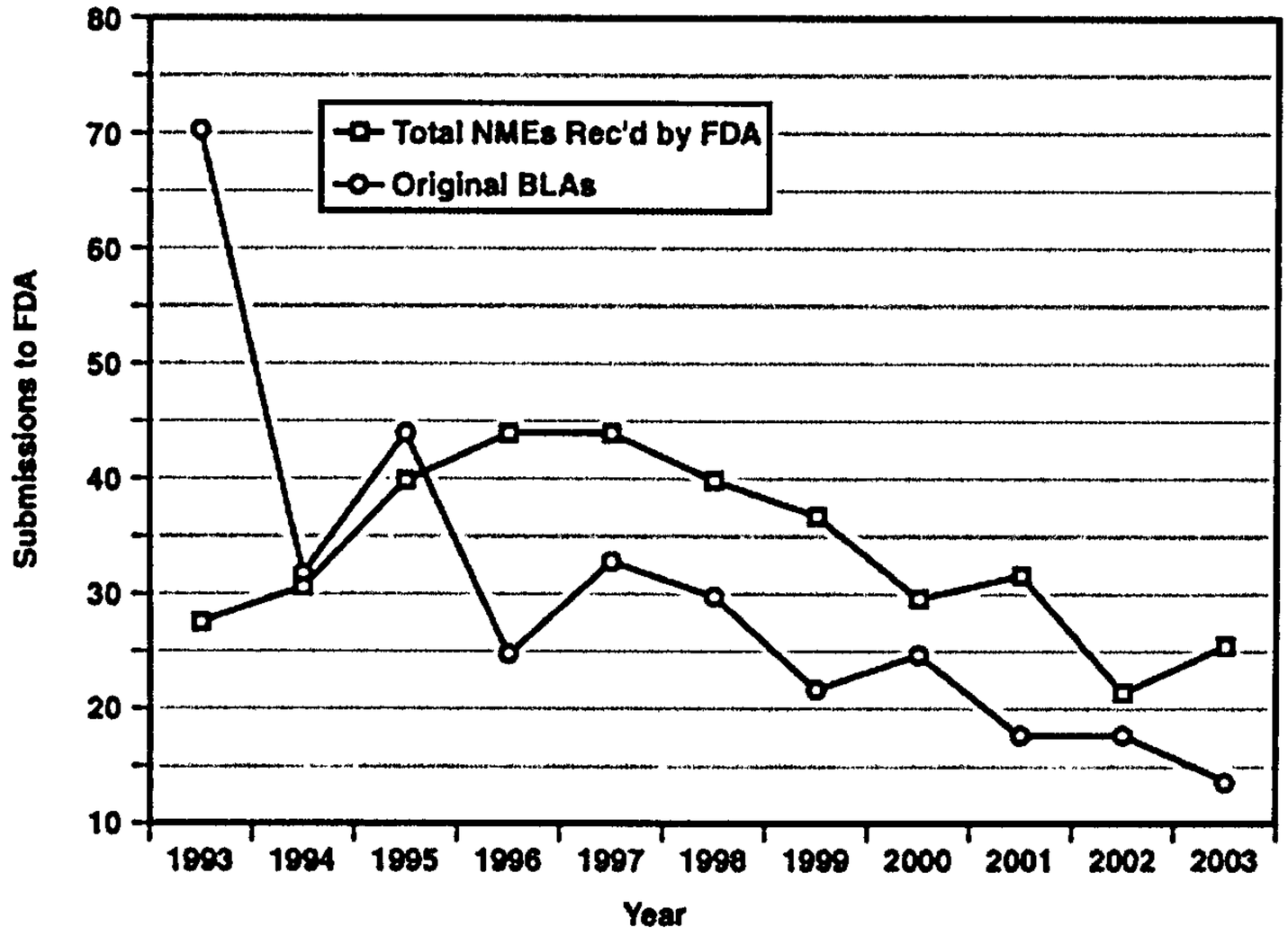

Figure 1.13 The number of new medical entities (NMEs) with a novel chemical structure and the number of biologics license applications (BLAs) submitted to the FDA over a 10-year period (FDA, 2004).

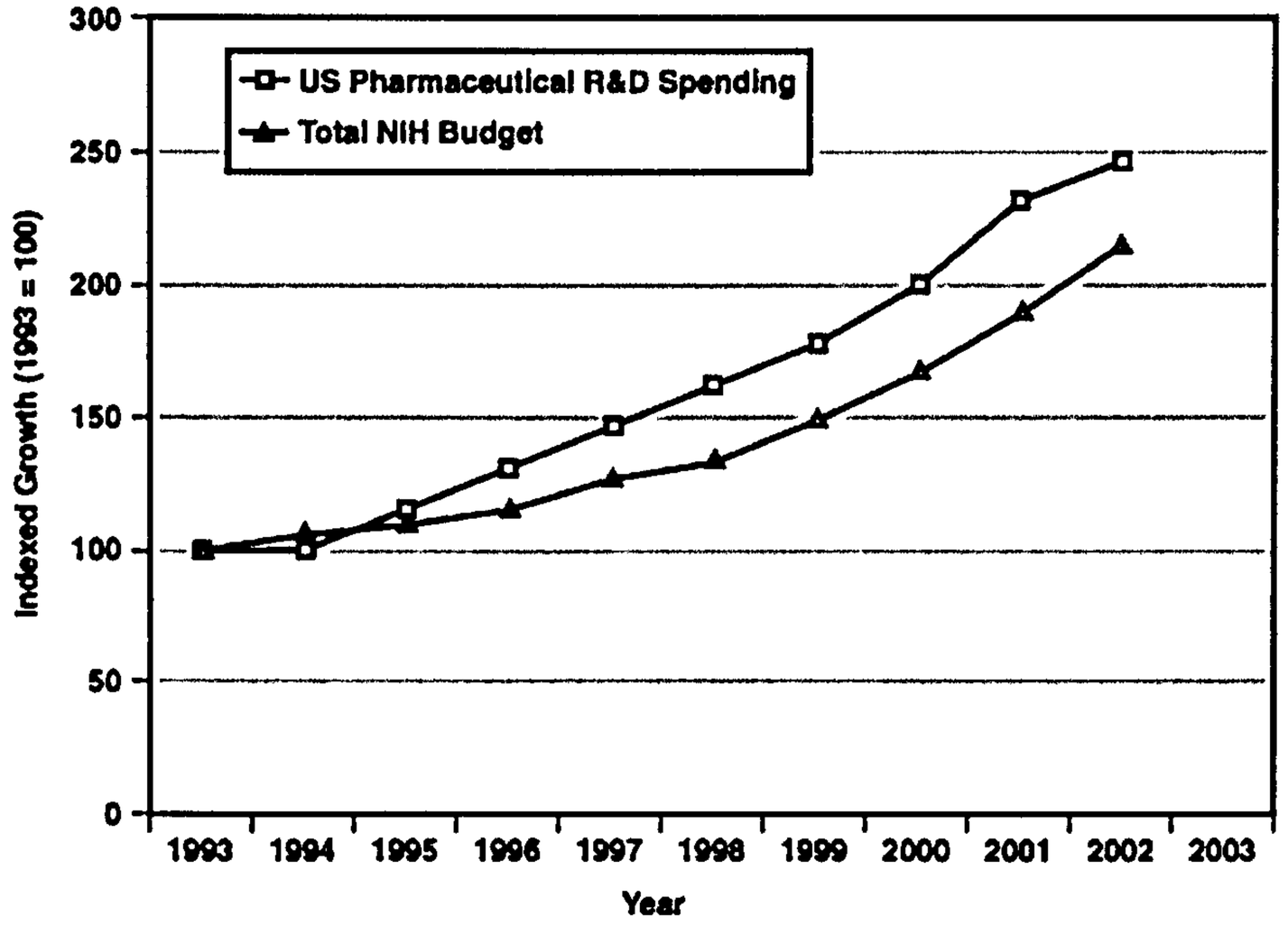

Figure 1.14 Amount spent on biomedical research at the National Institute of Health in the USA over a 10-year period. (FDA, 2004).

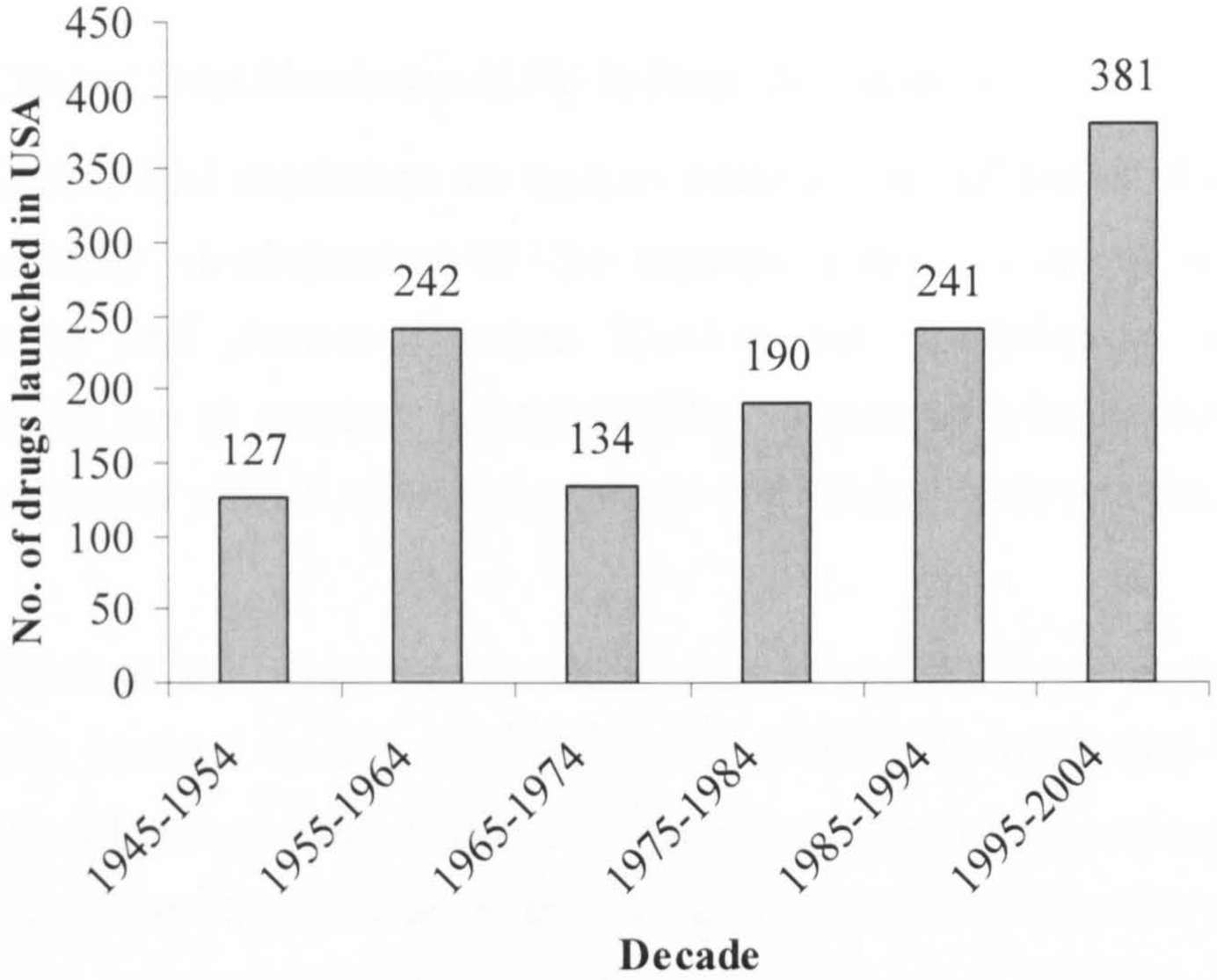

Figure 1.15 Number of drugs launched in the USA between 1945 and 2004 (Schmid & Smith, 2005).

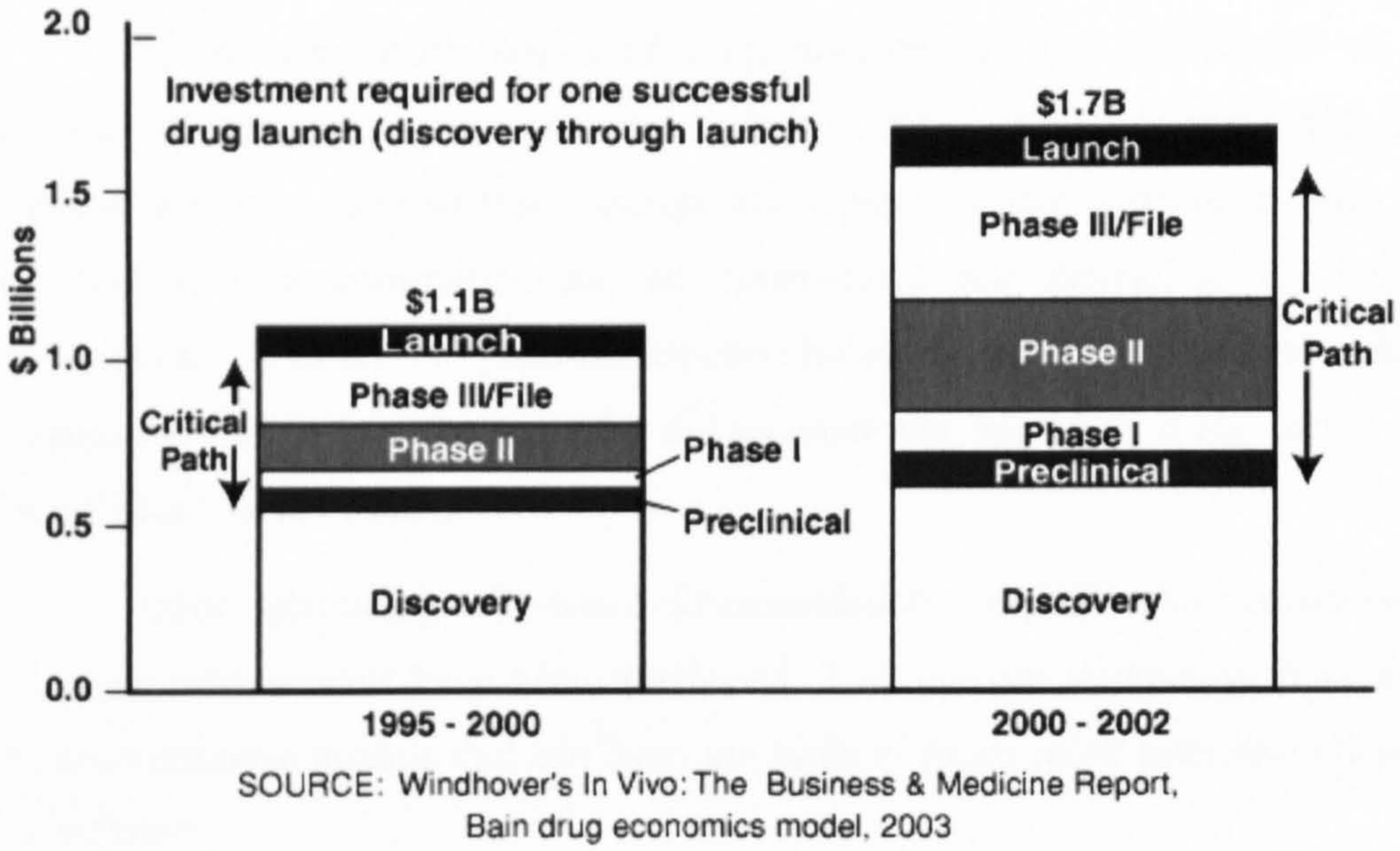

Figure 1.16 A breakdown of the costs involved in bringing one new drug to market from discovery to launch. The recent increase in costs is also demonstrated (FDA, 2004).

1.5.2 Clinical Trial Simulation (CTS) in Drug Development

Clinical trial simulations are built on mathematical and statistical models that are essentially simplifications of the complex systems involved in drug pharmacokinetics and pharmacodynamics. Clinical trial simulation is a term encompassing the use of premises and assumptions, to generate a large number of replications of virtual clinical trials that represent real clinical trials (Blesch *et al.*, 2003).

Much of the basis for modelling and simulation during early drug development is provided by the use of computer methods for predicting human pharmacokinetics from pre-clinical data. Pharmacokinetic-pharmacodynamic models developed from information gathered from preclinical studies also come into play at this stage. Data collected using these methods can be used to optimize dosage regimens and to design subsequent phase I and even phase II studies. As discussed in Section 1.5.4, it is possible to predict the parameters describing the absorption, distribution, metabolism and elimination of a drug. from *in vitro* data., and this information can be used to predict the time course of a drug in plasma.

In the very early stages of drug development the information on a compound can be scarce and somewhat 'patchy'. Thus, it is important that the available data is integrated so that coherent and comprehensive conclusions may be drawn from it. The information can be incorporated into physiologically based pharmacokinetic (PBPK) or pharmacokinetic-pharmacodynamic models to allow investigators to ask 'what if' questions, and evaluate the impact of study design on outcome (Blesch *et al.*, 2003).

Once physiologically-based-pharmacokinetic and/or pharmacokinetic-pharmacodynamic models have been developed, it is possible to develop them into more comprehensive models that can form the basis of much more extensive clinical trial simulation.

Just as the data from preclinical studies can be used in the design of phase I or patient studies, data from the former should be utilised to design safe and efficient phase II and III studies. Clinical trial simulations are often based on models derived from early data in humans (Aarons *et al.*, 2001). If physiological models are made available in the early stages of drug development, the differences in the parameters that

determine variability can be determined. Therefore, it is possible to define the pharmacokinetics of a drug *a priori* in patients who are to be recruited for phase II and III studies. However, this approach is rarely utilised, because much of the data in drug development comes from compartmental fitting of pharmacokinetic parameters.

In the future, virtual clinical trials may replace many of those carried out in humans. Furthermore clinical trial simulation may considerably cut the cost of drug development However, cost benefits aside, clinical trial simulation has the potential to change the face of drug development by making it safer (both by reducing the requirement for human volunteers and by ensuring that trials in human are safer and more efficient).

1.5.3 Examples of Successful Applications of CTS

1.5.3.1 Estimating Dosage

The estimation of dosage appears to have been one of the most popular uses for clinical trial simulation, several examples are available in the literature outlining successes in this area, and some of these are outlined below.

A new anticancer agent: Gieschke and colleagues (1997) successfully used clinical trial simulation to devise a dosing regimen to be used in phase III trials for an anticancer agent developed at Hoffman-La Roche. The authors also described how the model was utilised to examine the impact of reducing the dose by 50% on efficacy and adverse event profiles. It was found that the lower dose was equally as efficacious as the higher one (Gieschke *et al.*, 1997).

Docetaxel: Veyrat-Follet and colleagues (2000) used pharmacokinetic-pharmacodynamic models, developed from data gathered from phase II studies, to assess the advantages of a 125 mg/m^2 dose over a 100 mg/m^2 dose. The authors investigated the effect of the docetaxel dose on disease progression and survival time of small cell lung cancer sufferers. The simulation showed no clinical advantage of the higher dose over the lower one (Veyrat-Follet *et al.*, 2000).

T cell dose: Gooley *et al.*, (1994) used clinical trial simulation to calculate the optimal dose of T-cells, to be administered to bone marrow transplant patients with HLA-mismatched unrelated donors. The authors predicted the appropriate dose and also showed that the standard dose-escalation regimens that had been utilised previously were likely to give inaccurate predictions (Gooley *et al.*, 1994).

1.5.3.2 Estimating Adequate Sample Size

Perhaps one of the most well-known examples of the successful use of clinical trial simulation comes from Hale *et al.* (1998). This group used simulation techniques to estimate the sample size associated with a particular power and thus decide on an appropriate size for their clinical trial of mycophenolate mofetil, an immunosuppressive agent. The simulations were based on phase II clinical trial data (Hale *et al.*, 1998).

1.5.4 Incorporating Early Preclinical Data into CTS

It is clear from the above examples that most of the clinical trial simulation currently carried out during drug development rely heavily on data from *in vivo* studies. However, if modelling and simulation techniques are to benefit the drug development process to the full, clinical trial simulation must be carried out much earlier during drug development. Clearly, there is a need for utilising pharmacokinetic information gathered earlier in drug development.

Without data from clinical studies, there are currently two ways to extract the relevant information about a drug: (a) quantitative structure-property relationship analysis (QSPR) and (b) through the use of *in vitro* systems. QSPR operates on the basis that all of the physical and chemical and therefore, pharmacological and toxicological properties of a drug are related to its chemical structure, and are consequently, predictable. Since the present work concerns the use of *in vitro* data within clinical trial simulation to investigate the effects of genetic variability in metabolism on the pharmacokinetics and pharmacodynamics of drugs, the remainder of this section will focus on *in vitro* techniques. F_G and F_H will be discussed in the greatest detail, since they are the only parameters affected by genetic variability in drug metabolism, and hence, by polymorphisms of the CYP enzymes.

1.5.4.1 Prediction of Absorption

As discussed in Section 1.3.1.1, the oral absorption of a drug is dependent on f_a, F_G and F_H. Numerous *in vitro* systems (e.g. animal tissue, Caco-2 cell lines etc.) have been utilised in assessing the intestinal absorption (represented by f_a) potential of new drug candidates. However, a major disadvantage of all these techniques, is that they do not incorporate the effect of physiological factors such as gastrointestinal transit time, gastric emptying rate, or gastric pH (Balimane *et al.*, 2000).

The prediction of F_G can be achieved by assuming that the affinity of a drug for the metabolising enzymes in the gut wall, is equivalent to that in the liver. Thus, F_G and F_H can be extrapolated from *in vitro* data using the techniques described in Section 1.5.4.2.

1.5.4.2 Prediction of Metabolism

QSPR models may be used to predict how a molecule will interact with the active site of a drug-metabolising enzyme. In their review, Bugrim *et al.* (2004) state that as many as 39 molecular descriptors have been identified as correlating with metabolism by CYP enzymes. However, such methods are notoriously unreliable and are not used systematically within drug development.

In vitro systems can provide an inexpensive, high throughput method for the prediction of drug clearance and drug-drug interactions during early development. Various systems are available including human liver microsomes, recombinantly expressed CYP enzymes, purified and reconstituted CYP enzymes, and isolated hepatocytes. Each has its own advantages and disadvantages, which are discussed below. The extrapolation of *in vitro* information to the *in vivo* setting is discussed further below.

(i) *Human liver microsomes:* Hepatic microsomes are widely available and can be easily stored at -80°C. However, a major disadvantage associated with the use of human liver microsomes in these studies, is the artefactual variability in CYP protein expression and function. This may be caused by (a) the time delay between tissue harvesting and freezing, (b) differences in the cause of death and medications taken by the donor, and (c) differences in environment, diet and lifestyle between the donors. Variability in the levels of CYP expression and function also arises from genetic differences due to polymorphisms of the CYP enzymes (Section 1.4.2.1). Unless genotyping of the microsomes is undertaken, investigators cannot be sure of the reasons underlying variability between samples. A further disadvantage of the use of human liver microsomes results from the presence of lipids and proteins, which can bind to drug molecules and cause a decrease in the free concentration of the drug in the medium (Venkatakrishnan *et al.*, 2003).

(ii) *Recombinantly expressed enzymes* (rCYPs)*:* Recent advances in molecular biology have led to the stable expression of CYP enzymes in a wide range of systems including yeast, bacteria, insect and mammalian cells. The wide availability

of such systems offers investigators a viable alternative to the use of human tissue preparations as a source of hepatic enzymes. An important advantage of rCYPs is the reproducibility of information gained from them. However, it has become apparent that although rCYPs are identical to their human counterparts in terms of amino acid sequence, their intrinsic catalytic activity may differ substantially from human liver CYPs. However, this problem can be rectified through the use of inter-system extrapolation factors (ISEFs; Proctor *et al.*, 2004). An important advantage of rCYPs is the availability of different genetic variants of the same CYP isoform.

(iii) *Hepatocytes:* Human hepatocytes are available commercially and are a popular and well established tissue for drug metabolism studies. They are whole, living cells, containing the full complement of drug metabolising enzymes and transport systems, and any concentration gradients mediated by transporters that may affect exposure of substrate/inhibitor to enzymes, will still be present. However, hepatocyte systems are expensive, and some transporters rapidly lose their functional activity after isolation of hepatocytes. Furthermore, maintenance of hepatocyte cultures can be problematic (Tucker *et al.*, 2001; Venkatakrishnan *et al.*, 2003). An important disadvantage of the use of human hepatocytes, is that experimental results are dependent on the source of the hepatocytes. Thus, the activity of any enzymes and transporters are dependent on the genotype and environment of the human donor.

To make use of data gathered from *in vitro* systems, there must be a means of 'scaling up' to the *in vivo* setting. This process is called *in vitro-in vivo* extrapolation (IVIVE), and involves the use of scaling factor*s*. Methods for IVIVE were first described 30 years ago by Rane *et al.* (1977). The researchers used *in vitro* intrinsic clearance data from isolated perfused rat liver experiements to predict the hepatic extraction ratio of seven drugs in humans (Rane *et al.*, 1977). More recently, with the increased availability of human liver samples, and the advent of new technology for producing recombinant drug metabolising enzymes, methods for the prediction of drug clearance and metabolic drug-drug interactions have been refined and widely implemented (Iwatsubo *et al.*, 1996; Rostami-Hodjegan & Tucker, 2004).

1.5.4.3 Prediction of Efficacy/Toxicity

The extrapolation of *in vitro* data on drug response to human drug efficacy and toxicity is a relatively new area of research. However, a number of successful studies have demonstrated its potential usefulness for drug development (Cleton *et al.*,

2000; Cox *et al.*, 1998; Visser *et al.*, 2003). For example, Cox *et al.* (1998) have predicted *in vivo* opioid EEG response from *in vitro* mu-receptor binding data. In 2003, Visser and colleagues utilised data on the *in vitro* binding affinity of benzodiazepines to $GABA_A$ receptors to successfully describe the *in vivo* properties of these drugs (Visser *et al.*, 2003).

Methods such as these could allow the efficacy and toxicity of a drug molecule to be predicted in man before the drug leaves the laboratory. Mechanism based pharmacokinetic-pharmacodynamic models will also lead to a better understanding of interindividual variability in human drug response (Visser *et al.*, 2003). A further advantage of incorporating *in vitro* pharmacodynamic information into clinical trial simulation is the potential for simulating the influence of receptor polymorphisms such as those in the β_2-adrenoceptors (Liu *et al.*, 2003) or the herg channel protein (Bezzina *et al.*, 2003), on drug response. Currently, information on receptor binding is not routinely available in the early stages of drug development, where it has the potential to be most useful.

1.6 Aims and Objectives

This thesis is intended to gauge the feasibility of incorporating a mechanistic approach to the extrapolation of *in vitro* data on drug metabolism to *in vivo* drug clearance, within the framework of pharmacokinetic-pharmacodynamic simulation and clinical trial simulation. The general aims of the work are to (a) expand the framework of clinical trial simulation, such that early information on drug metabolism gathered during pre-clinical drug development can be incorporated and used to design effective clinical studies; (b) assess whether it is possible to recover *in vivo* variability in drug response caused by genetics; and (c) provide a paradigm for future clinical trial simulation in drug development. The specific aims of the thesis are as follows:

- To gather *in vitro* metabolism data for selected drugs, and integrate these data with other pharmacokinetic and pharmacodynamic information to simulate and predict both pharmacokinetic and pharmacodynamic profiles in virtual populations of individuals.
- To enter genetic or phenotypic information on the occurrence and impact on drug metabolism of CYP polymorphisms into the model, for the purpose of investigating these genetic effects on the pharmacokinetic and pharmacodynamic profiles of the drugs.
- To investigate the impact of CYP polymorphisms on the pharmacokinetics and pharmacodynamics of the drugs, using a range of sample sizes, for the prediction of the outcome of published studies.
- To estimate the size and optimise the design of clinical studies that will define accurately the effect of the CYP polymorphism on the pharmacokinetics or pharmacodynamics of the selected drugs.
- To use these simulations and predictions to suggest possible reasons for the success/failure of published studies to observe an effect of CYP polymorphisms on pharmacokinetics and/or pharmacodynamics.

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CHAPTER 2

The Propagation of Pharmacogenetic Differences in Cytochrome P450 into the Pharmacokinetics and Pharmacodynamics of Therapeutic Drugs: A Review of the Literature

2 LITERATURE REVIEW

2.1 Background

The history of pharmacogenetics stretches as far back as 510 B.C. when Pythagoras noted that ingestion of fava beans resulted in a potentially fatal reaction in some, but not all, individuals (Nebert, 1999). In the many centuries that have followed, there have been numerous landmarks that have shaped this field of research, and have led to the current wave of interest. The latter began in the late 1970s with the discovery of the debrisoquine/sparteine hydroxylation (CYP2D6) polymorphism (Eichelbaum *et al.*, 1979; Mahgoub *et al.*, 1977; Tucker *et al.*, 1977). This discovery sparked a surge of studies which would attempt to define the pharmacokinetic characteristics of a drug based on interindividual differences in drug-metabolising capacity. Pharmacodynamic studies of the same type were close to follow when it was hypothesised that polymorphic drug metabolism would be a source of interindividual variation in drug response. Recently, the interest in this area has once again been catalysed (albeit under the new name of 'pharmacogenomics') with the completion of the Human Genome Project.

A current area of debate has focused on the usefulness of pharmacogenetic testing in the individualisation of drug therapy ('personalised medicine') (Chapter 1; Section 1.3). Many authors strongly support such an approach, for example Ingelmann-Sundberg suggested in 2001 that "Because of the rapid development in the field it is believed that in a couple of years from now, pharmacogenetics will be a standard tool in drug development and clinical practice" and recently, Eichelbaum and colleagues stated that "With the complete sequence of the human genome available, individualized medicine may soon become reality" (Eichelbaum *et al.*, 2006; Ingelman-Sundberg, 2001).

However, such opinions are not supported by substantive evidence and others take a more circumspect view. For example, Holtzman (2001) has stated that "there are few cases where testing patients for certain enzymes involved in drug metabolism may help but … other factors, such as diet and smoking, are as important", and Tucker (2004) has suggested that "the promise of pharmacogenetics has largely remained unfulfilled" (Holtzman, 2001; Tucker, 2004).

Many studies have been published on the influence of CYP polymorphisms on drug pharmacokinetics and pharmacodynamics. Recently, Williams *et al.* (2006) have emphasized the importance of adequate sample size in such studies. Ensuring adequate statistical power becomes an even greater issue with respect to defining the influence of pharmacodynamic outcomes because variability in pharmacodynamics is generally greater than that in pharmacokinetics (Levy et al., 1994) (Table 2.1).

A result of underpowering of studies may be the generation of false negative results or, where more than one study has been performed, conflicting data.

The main aims of this chapter were to summarise and evaluate critically the published literature on the influence of CYP polymorphisms on drug pharmacokinetics and pharmacodynamics to help clarify the available evidence in support of such effects. Because they are the subject of the work described in this thesis, the examples of DEX, *(S)*-warfarin, MDZ, OMZ and TLB will be discussed in some detail.

Using the results of the simulations described later in this thesis, it may be possible to explain why some studies have failed to detect an influence of CYP polymorphisms on drug pharmacokinetics or pharmacodynamics, where others investigating the same drug have shown differences between phenotypes/genotypes (Chapter 1; Section 1,6).

The purpose of the research was not to provide an exhaustive list of all the reported studies that detail investigations into the influence of CYP polymorphisms on drug pharmacokinetics and/or pharmacodynamics, but to gather a large enough sample of such studies to be representative of the real situation and to allow inferences to be made.

Table 2.1 Pharmacokinetic and pharmacodynamic variability in humans (adapted from Levy *et al.* (1994).

Drug	n	Coefficient of Variation (%)		Reference
		Pharmacokinetics (total CL or AUC)	Pharmacodynamics (EC_{50}, IC_{50} or slope)	
Prednisolone	7	17.4 (low dose) 9.8 (high dose)	37.2 (cortisol suppr.) 48.4 (basophil suppr.) 81.6 (T-helper suppr.)	Wald *et al.,* 1992
Deacetylmetipranolol	6 6	40.1 31.6	63.9 112	Janku *et al.,* 1992
Ibuprofen	38	34	137	Kauffman & Nelson, 1992
Hydroxymidazolam	8	8.0	45.2 (EEG) 57.1 (s.e.m)	Mandema *et al.,* 1992
Diltiazem	32	29.5 (low dose) 27.2 (high dose)	76.6	Dias *et al.,* 1992
Labetalol	7	34.3	46.8 (systolic b.p) 41.9 (diastolic b.p)	Saotome *et al.,* 1993

s.e.m = saccadic eye movement; b.p = blood pressure

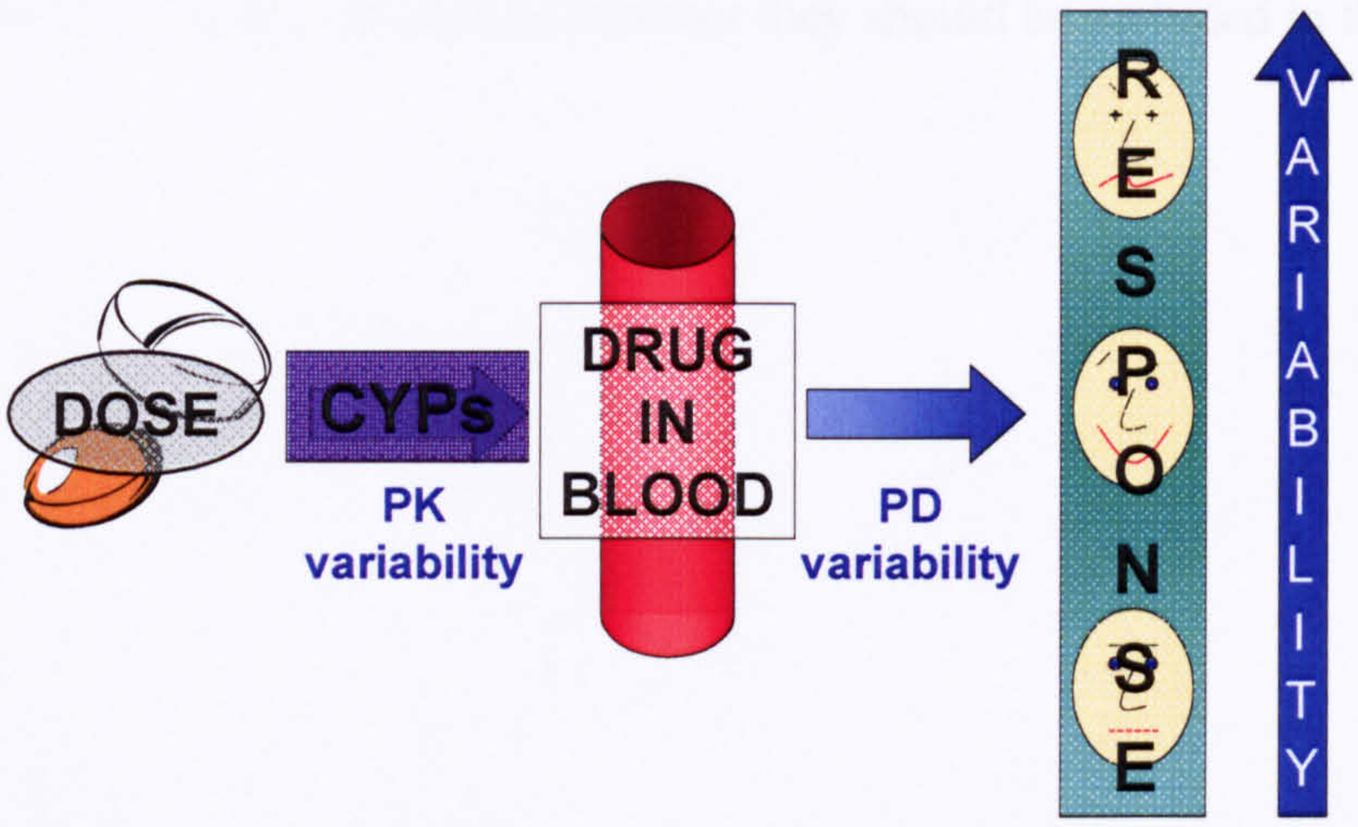

Figure 2.1 A diagram to illustrate that population variability in drug response is often greater than variation in plasma drug concentrations.

2.2 Methods

A literature search was carried out in the electronic databases OVID MEDLINE (1966–2006) and WEB OF SCIENCE (1981–2006) using the following combination of keywords "(cytochome P450 or CYP*), (pharmacokinetic*, pharmacodynamic* or response), and polymorphism" (Figure 2.1).

The drug name and CYP enzyme identified in each of the initial papers were included as key-words in a second stage search in place of the words 'pharmacokinetic*, pharmacodynamic* or response' along with the words 'polymorphism' and 'cytochrome P450 or CYP'.

Key reviews were also scrutinised for further reports of studies on the influence of CYP polymorphisms on the pharmacokinetics and/or pharmacodynamics of drugs. In total, 2283 reports were identified, and their titles and, if necessary, their abstracts were reviewed to determine whether they should be included in the analysis.

1st Stage: OVIDE MEDLINE and WEB OF SCIENCE searched:

Keywords:

polymorphism + CYP cytochrome P450 + Pharmacokinetic * Pharmacodynamic* Response

n = 265 reports selected from 1735 reports identified

2nd Stage: Using the drug name and specific CYP identified in 1 st stage reports:

Keywords:

polymorphism + CYP cytochrome P450 + [name of CYP] + [Drug name]

n = 78 extra reports selected from 536 reports identified

3rd Stage: Bibliographies of key reviews searched for further references

n = 12 more reports identified

Total = 355 reports

Figure 2.2 A schematic representation of the three stages of the literature search.

2.3 Results

Two hundred and thirty pharmacokinetic studies encompassing 115 drugs, and 124 pharmacodynamic studies of 54 drugs were identified. Details of these studies are shown in the appendix (Table 2.10 and Table 2.11).

Out of 230 pharmacokinetic studies, 70 (encompassing 70 drugs) were reports of a drug/CYP relationship that had not been investigated by others. These are subsequently referred to as 'unique' studies. The remaining 160 pharmacokinetic studies (involving 44 drugs) described drug/CYP relationships that had been investigated previously, and reported in at least one of the other 159 studies. These reports are subsequently referred to as 'repeated' studies.

The results from 78 (52%, involving 44 drugs) 'repeated' studies were 'consistent' with one another, in that they reported similar outcomes (Figure 2.1). The results from the 82 (48% involving 21 drugs) 'repeated' studies were 'inconsistent' with one another, in that the outcomes were opposing (Table 2.2, Figure 2.3).

Out of the 78 'consistent', 'repeated' pharmacokinetic studies, 7 (9%, involving 3 drugs) report negative results (i.e. no significant relationship between CYP pheno/genotype and drug pharmacokinetics), and 71 (91%, involving 20 drugs) report consistently positive results.

Table 2.2 A list of 'repeated' studies into the effect of CYP polymorphisms on drug PK.

CYP	Drug	Studies			
		+	-	Number of studies	Outcome
1A2	Clozapine	1	1	2	Inconsistent
2B6	Bupropion	1	1	2	Inconsistent
	Ibuprofen	2		2	Consistently positive
2C9	Repaglinide	1	1	2	Inconsistent
	Celecoxib	1	1	2	Inconsistent
	Diclofenac	2	4	6	Inconsistent
	Glimepiride	3		3	Consistently positive
	Glyburide	3		3	Consistently positive
	Ibuprofen	1	1	2	Inconsistent
	Losartan	3	1	4	Inconsistent
	Phenprocoumon	2		2	Consistently positive
	Phenytoin	5		5	Consistently positive
	Tolbutamide	6		6	Consistently positive
	Warfarin	4	1	5	Inconsistent
2C19	Amitriptyline	3		3	Consistently positive
	Diazepam	4	1	5	Inconsistent
	Fluvoxamine		2	2	Consistently negative
	Hexobarbital	2		2	Consistently positive
	Imipramine	2		2	Consistently positive
	Lansoprazole	8		8	Consistently positive
	Mephobarbital	2		2	Consistently positive
	Omeprazole	14	1	15	Inconsistent
	Phenobarbital	1	1	2	Inconsistent
	Phenytoin	3	4	7	Inconsistent
	Proguanil	4		4	Consistently positive
	Rabeprazole	5		5	Consistently positive
	Tolbutamide		2	2	Consistently negative
2D6	Amitriptyline	1	1	2	Inconsistent
	Carvedilol	1	1	2	Inconsistent
	Dextromethorphan	5		5	Consistently positive
	Fluvoxamine	1	1	2	Inconsistent
	Haloperidol	2		2	Consistently positive
	Metoprolol	5		5	Consistently positive
	Mianserin	2		2	Consistently positive
	Propranolol	1	1	2	Inconsistent
	Risperidone	2	1	3	Inconsistent
	Tamoxifen	2		2	Consistently positive
	Midazolam		3	3	Consistently negative
	Paclitaxel	1	2	3	Inconsistent
3A5	Cyclosporine	3	2	5	Inconsistent
	Midazolam	3	4	7	Inconsistent
	Saquinavir	1	1	2	Inconsistent
	Sirolimus	2		2	Consistently positive
	Tacrolimus	6		6	Consistently positive
	Total	**121**	**39**	**160**	

'+' & 'positive' indicate studies that detected a significant difference in the pharmacokinetics of the drug between CYP phenotypes/genotypes; '-' and 'negative' indicate studies that did not detect a significant difference in the pharmacokinetics of the drug between CYP phenotypes/genotypes.

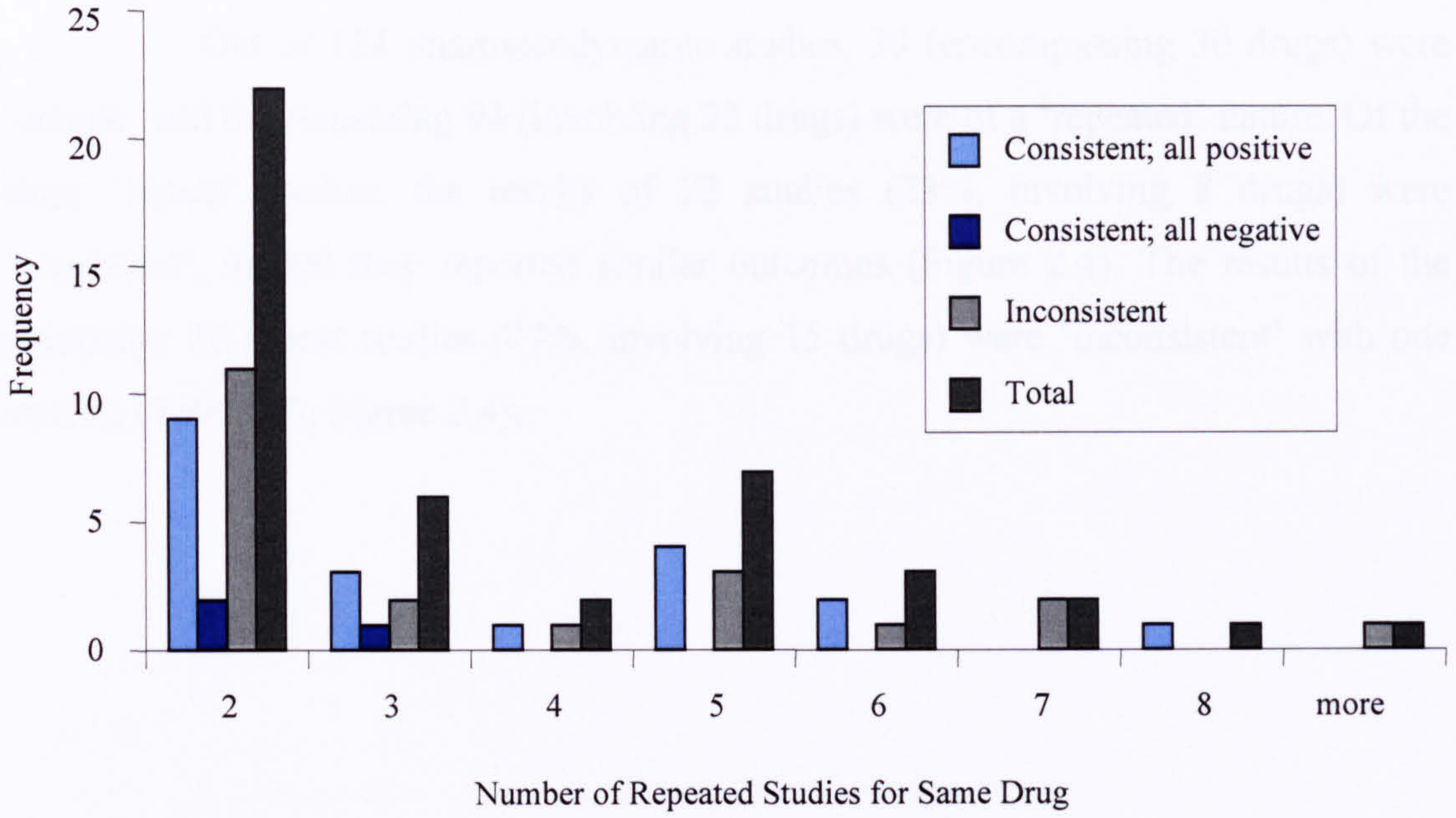

Figure 2.3 Frequency of 'repeated' pharmacokinetic reports that were 'consistent' and 'inconsistent' with each other. 'Consistent' studies have been divided into two groups – 'all negative' and 'all positive' to indicate whether they consistently observed a significant difference in drug pharmacokinetic between CYP phenptypes/genotypes or they consistently did not detect a difference.

Out of 124 pharmacodynamic studies, 30 (encompassing 30 drugs) were 'unique' and the remaining 94 (involving 23 drugs) were of a 'repeated' nature. Of the latter 'repeat' studies, the results of 22 studies (23%, involving 8 drugs) were 'consistent', in that they reported similar outcomes (Figure 2.4). The results of the remaining 72 repeat studies (77%, involving 15 drugs) were 'inconsistent' with one another (Table 2.3; Figure 2.4).

Table 2.3 A list of 'repeated' studies into the effect of CYP polymorphisms on drug pharmacodynamics.

CYP	Drug	Studies			
		+	-	Number of studies	Outcome
1A2	Clozapine	1	1	2	Inconsistent
	Acenocoumarol	6		6	Consistently positive
	Glyburide	2		2	Consistently positive
2C9	Glimepiride	1	1	2	Inconsistent
	Irbesartan	1	1	2	Inconsistent
	Phenprocoumon		3	3	Consistently negative
	Phenytoin	2	1	3	Inconsistent
	Tolbutamide	1	2	3	Inconsistent
	Warfarin	17	1	18	Inconsistent
	Cyclophosphamide	2		2	Consistently positive
2C19	Lansoprazole	4	1	5	Inconsistent
	Omeprazole	9	2	11	Inconsistent
	Proguanil	1	2	3	Inconsistent
	Rabeprazole	3	5	8	Inconsistent
	Tolbutamide		2	2	Consistently negative
	Codeine	2		2	Consistently positive
	Dextromethorphan		2	2	Consistently negative
2D6	Haloperidol	1	1	2	Inconsistent
	Metoprolol	2	2	4	Inconsistent
	Phenformin	1	1	2	Inconsistent
	Propafenone	3		3	Consistently positive
	Risperidone	1	2	3	Inconsistent
3A5	Simvastatin	1	1	2	Inconsistent
	Total	**62**	**32**	**94**	

'+' & 'positive' indicate studies that detected a significant difference in the pharmacodynamics of the drug between CYP phenotypes/genotypes; '-' and 'negative' indicate studies that did not detect a significant difference in the pharmacodynamics of the drug between CYP phenotypes/genotypes.

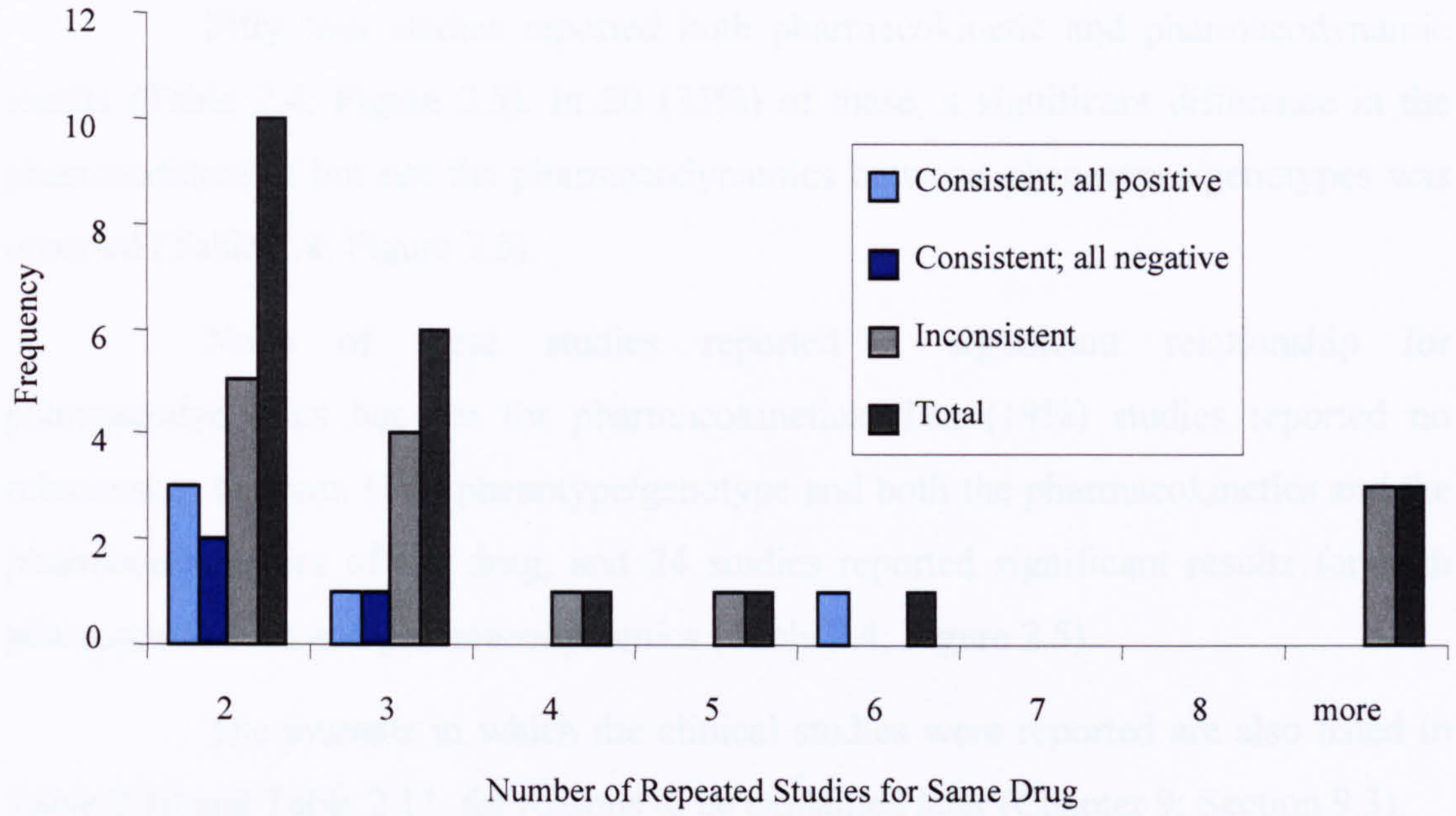

Figure 2.4 Frequency of 'repeated' pharmacodynamic reports that were 'consistent' and 'inconsistent' with each other. 'Consistent' studies have been divided into two groups – 'all negative' and 'all positive' to indicate whether they consistently observed a significant difference in drug pharmacodynamics between CYP phenotypes/genotypes or they consistently did not detect a difference.

Fifty four studies reported both pharmacokinetic and pharmacodynamic results (Table 2.4; Figure 2.5). In 20 (35%) of these, a significant difference in the pharmacokinetics but not the pharmacodynamics between phenotypes/genotypes was reported (Table 2.4; Figure 2.5).

None of these studies reported a significant relationship for pharmacodynamics but not for pharmacokinetics. Ten (19%) studies reported no relationship between CYP phenotype/genotype and both the pharmacokinetics and the pharmacodynamics of the drug, and 24 studies reported significant results for both pharmacokinetics and pharmacodynamics (Table 2.4; Figure 2.5).

The journals in which the clinical studies were reported are also listed in Table 2.10 and Table 2.11, for reasons to be explained later (Chapter 9; Section 9.3).

Table 2.4 List of studies for which both pharmacokinetic and pharmacodynamic data were reported. If the study showed a significant difference in pharmacokinetics or pharmacodynamics between phenotypes/ genotypes, a '✓' was recorded. If there was no significant difference, a '✗' was recorded. PK – pharmacokinetics; PD - pharmacodynamics

CYP	Drug	Reference	PK	PD
1A2	Clozapine	Van der Weide *et al.*, 2003	✗	✗
2B6	Methadone	Crettol *et al.*, 2005	✓	✗
2C8	Repaglinide	Niemi *et al.*, 2003	✓	✗
2C9	Diclofenac	Kirchheiner *et al.*, 2003	✓	✗
	Fluvastatin	Kirchheiner *et al.*,2003	✓	✗
	Glimepiride	Suzuki *et al.*, 2006	✓	✓
		Niemi *et al.*, 2002	✓	✗
	Glyburide	Kirchheiner *et al.*,2002	✓	✓
		Yin *et al.*, 2005	✓	✓
	Ibuprofen	Kirchheiner *et al.*,2002	✓	✓
	Irbesartan	Chen *et al.*, 2006	✓	✓
	Losartan	Sekino *et al.*, 2003	✓	✓
	Methadone	Crettol *et al.*, 2005	✗	✗
	Nateglinide	Kirchheiner *et al.*,2004	✓	✗
	Tolbutamide	Kirchheiner *et al.*,2002	✓	✗
		Shon *et al.*, 2002	✓	✓
		Lee *et al.*, 2002	✓	✗
	Warfarin	Sconce *et al.*, 2005	✓	✓
		Scordo *et al.*, 2002	✓	✓
		Kamali *et al.*, 2004	✓	✓
		Takahashi *et al.*, 2003	✗	✗
2C19	Lansoprazole	Ieiri *et al.*, 2001	✓	✓
	Methadone	Crettol *et al.*, 2005	✗	✗
	Omeprazole	Chang *et al.*, 2005	✓	✓
		Hu *et al.*, 2005	✓	✓
		Kita *et al.*, 2001	✓	✓
		Furuta *et al.*, 1999	✓	✓
		Shirai *et al.*, 2001	✓	✓
	Proguanil	Kaneko *et al.*, 1999	✓	✓
		Kaneko *et al.*, 1999	✓	✗
	Rabeprazole	Horai *et al.*, 2001	✓	✓
		Hu *et al.*, 2005	✓	✗
		Ieiri *et al.*, 2001	✓	✓
	Tolbutamide	Kirchheiner *et al.*,2002	✗	✗
		Shon *et al.*, 2002	✗	✗
2D6	Dextromethorphan	Abdul-Manap *et al.*, 1999	✓	✗
		Capon *et al.*, 1996	✓	✗
	Donepezil	Varsaldi *et al.*, 2006	✗	✗
	Flecainide	Tenneze *et al.*, 2002	✗	✗
	Haloperidol	Brockmoller *et al.*, 2002	✓	✓
		Pan *et al.*, 1999	✓	✗
	Metoprolol	Zineh *et al.*, 2004	✓	✗
		Kirchheiner *et al.*,2004	✓	✓
		Fux *et al.*, 2005	✓	✗
	Mianserin	Mihara *et al.*, 1997	✓	✓
	Nateglinide	Kirchheiner *et al.*,2004	✗	✗
	Phenformin	Oates *et al.*, 1983	✓	✓
	Propafenone	Cai *et al.*, 2002	✓	✓
	Propranolol	Huang *et al.*, 2003	✓	✗
	Risperidone	Reidel *et al.*, 2005	✓	✗
		Kakihara *et al.*, 2005	✗	✗
	Timolol	Nieminen *et al.*, 2005	✓	✗
	Tolterodine	Brynne *et al.*, 1998	✓	✗
3A5	Alprazolam	Park *et al.*, 2006	✓	✗

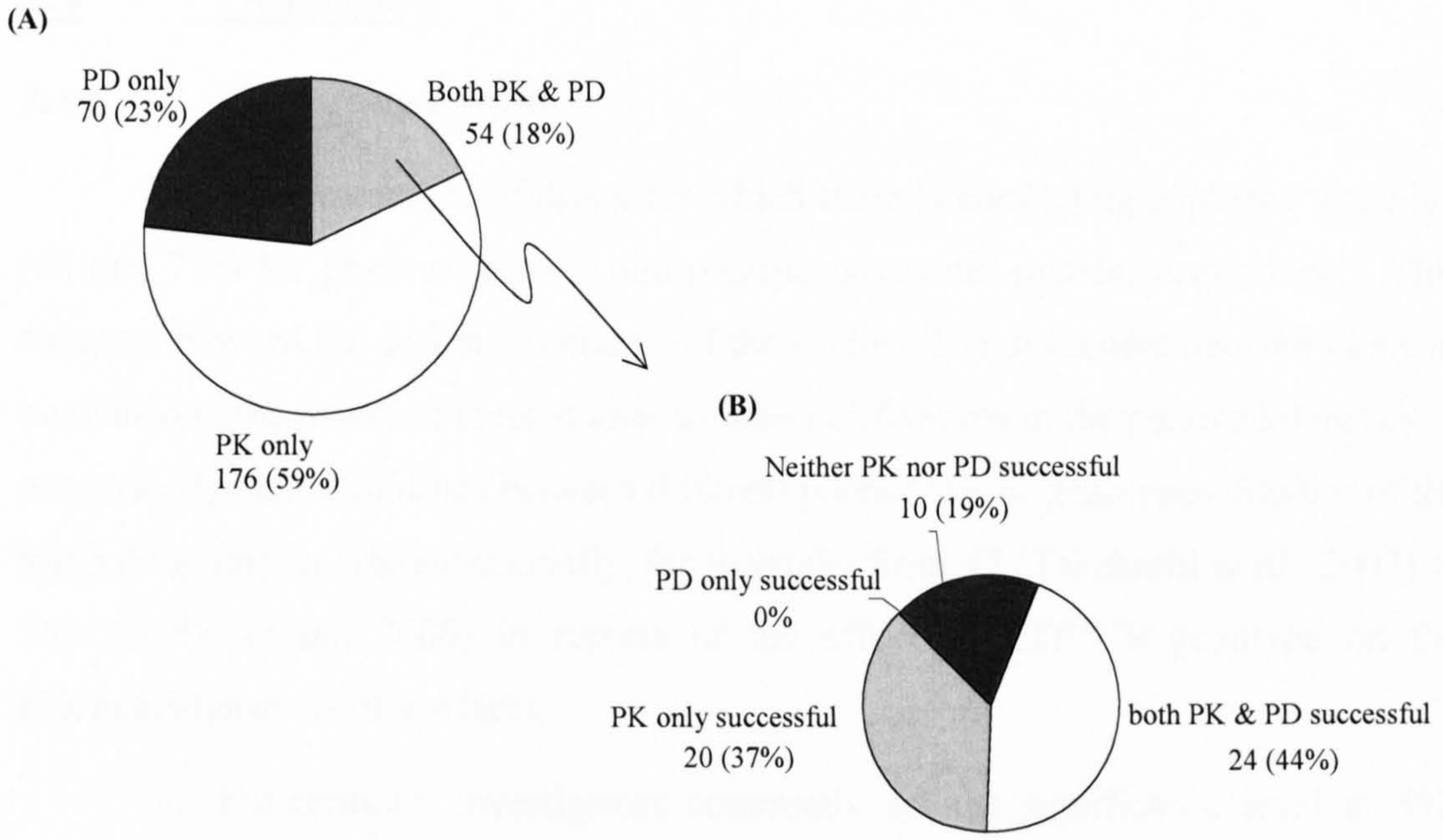

Figure 2.5 (A): The number of studies reported in the literature that provide only pharmacokinetics, only pharmacodynamics or both pharmacokinetic and pharmacodynamic data (B): The success (siginificant differences between phenotypes/genotypes observed) or not of studies that report both pharmacokinetics and pharmacodynamics.

2.4 Discussion

2.4.1 *Conflicting Results*

The percentage of drugs for which there is conflicting evidence was high (48 and 77% for pharmacokinetic and pharmacodynamic studies, respectively). This suggests flaws in the design or conduct of the studies. The discrepant data are likely to be related to the power of these studies to detect differences in the pharmacokinetics or pharmacodynamics of drugs between different phenotypes or genotypes. Studies of the same drug vary in size substantially, for example, from 47 (Takahashi *et al.*, 2003) to 561 (Taube *et al.*, 2000) in reports of the effect of *CYP2C9* genotype on the pharmacodynamics of warfarin.

Furthermore, investigators commonly set the significance level at 5%. Consequently, 5% of studies are likely to be subject to type I errors, giving false positive results. Thus, out of the 230 pharmacokinetic studies, and 124 pharmacodynamic studies identified, around 12 and 6, respectively, will result in false positive outcomes. When a number of different outcomes are used (e.g. AUC, C_{max}, CL for pharmacokinetic studies or several response measures for pharmacodynamics studies) the likelihood of false positive outcomes is even greater.

Many authors have been somewhat selective when reviewing reports of the impact of CYP polymorphisms on the pharmacokinetics and/or pharmacodynamics of drugs, concentrating on 'positive' and less on 'negative' outcomes. Conflicting data from two or more studies may indicate (i) a type 1 error in the studies showing a positive outcome, or (ii) the presence of only small difference between phenotypes/genotypes, which may be difficult to observe and clinically unimportant. In 63% (42/67) of the drugs investigated in the present work (including both pharmacokinetic and pharmacodynamic studies) for which there was more than one report, at least one study was in evidence that suggested a negative result.

The majority of the 115 drugs (61%; pharmacokinetic) and 54 drugs (56%; pharmacodynamic) had not been investigated independently by different groups. Considering the inconsistencies uncovered by the present analysis, good practice should ensure that 'repeat' studies are adequately powered to detect any hypothesised difference between phenotypes/ genotypes. Assessment of power is much easier to carry out if based on the results of a previous study.

'Repeated' pharmacokinetic reports involving only 20 drugs (45%) were consistent with each other, all independently showing a link between phenotype/genotype and pharmacokinetics. The corresponding number for pharmacodynamic reports was 5 (22%).

Because it is often overlooked that pharmacokinetic and pharmacodynamic variability are additive, investigators may be less likely to observe differences in pharmacodynamics between CYP phenotypes/ genotypes than in pharmacokinetics, when using similar study sizes. This is supported by the results from the 54 studies for which both pharmacokinetic and pharmacodynamic information was available (Table 2.4), in that 37% detected a significant link between CYP phenotype/genotype and pharmacokinetics but have been unable to demonstrate a similar link for pharmacodynamics.

In conclusion, it is apparent that review authors often make the erroneous assumption that a significant relationship between pharmacokinetics and genotype/phenotype implies a significant relationship between phenotype/genotype and pharmacodynamics. Furthermore, investigators should pay close attention to pharmacodynamic variability and power their studies accordingly.

2.4.2 *The Model Drugs*

In order to investigate the value of clinical trials simulation in the design and conduct of studies investigating differences in pharmacokinetics and pharmacodynamics between different CYP phenotypes/genotypes, specific drugs were chosen based on

(i) The availability of comprehensive *in vitro* enzyme kinetic data, preferably from studies carried out in recombinant systems.

(ii) The availability of a pharmacokinetic-pharmacodynamic model obtained by fitting *in vivo* data.

(iii) The presence of literature reports that have investigated the influence of CYP phenotype/genotype on the pharmacokinetics and/or pharmacodynamics of the drug.

Based on the criteria above a number of drugs were selected and prioritised so that those for which the most data were available and those that were most widely used clinically, were investigated first. Within the time constraints of this

thesis, 5 drugs were studied, and these were dextromethorphan (DEX), *(S)*-warfarin, tolbutaimde (TLB), midazolam (MDZ) and omeprazole (OMZ). Published pharmacodynamic data were available for all these drugs (Table 2.10 & Table 2.11), except for MDZ.

2.4.2.1 Dextromethorphan

All five studies investigating the effect of CYP2D6 phenotype on the pharmacokinetics of DEX have shown significant differences using 22, 6, 7, 65 and 50 subjects, respectively (Abdul Manap *et al.*, 1999; Capon *et al.*, 1996; Casner, 2005; Desmeules *et al.*, 1999; Pope *et al.*, 2004). Only two corresponding pharmacodynamic studies have been performed, both reporting no significant differences between CYP2D6 phenotypes, using 6 and 22 subjects (Abdul Manap *et al.*, 1999; Capon *et al.*, 1996).

2.4.2.2 *(S)*-Warfarin

Of the five drugs selected, most studies have been published on warfarin. Five have investigated the impact of *CYP2C9* genotype on *(S)*-warfarin pharmacokinetics and 18 on the pharmacodynamics of the drug. The results for both sets of studies generally show genotypic differences. However, since there are 6 common *CYP2C9* genotypes, for a study to be recorded as 'positive', only one of the comparisons between the wild-type genotype and any other single genotype needs to be significantly different. One pharmacokinetic study and one pharmacodynamic study produced negative findings. However, the data for *(S)*-warfarin are more complex than those for DEX. This is because, instead of simply comparing one phenotype with the other as is the case for CYP2D6 and DEX, all 6 common *CYP2C9* genotypes must be evaluated. Therefore, it becomes difficult to define 'success' in discerning the effect of *CYP2C9* genotype on *(S)*-warfarin pharmacokinetics and pharmacodynamics. For example, whereas a study may differentiate *(S)*-warfarin pharmacodynamics between subjects of *1*3 genotype and wild type subjects, the same study may not be adequately powered to detect differences between *2*2 genotype and the wild type. Owing to its rarity in the Caucasian population, it is likely that no subjects with the *CYP2C9* *2*2 genotype will be present in the relatively small number of subjects normally recruited for these types of studies (see Chapter 5).

Table 2.5 Summary of studies recovered that have investigated the effect of CYP2D6 phenotype on (A) the pharmacokinetics, and (B) the pharmacodynamics of DEX.

(A) CYP	Reference	Significant effect of genotype? Yes	No
2D6	Casner, 2005	✓	
	Desmeules *et al.*, 1999	✓	
	Abdul-Manap *et al.*, 1999	✓	
	Pope *et al.*, 2004	✓	
	Capon *et al.*, 1996	✓	

(B) CYP	Reference	Significant effect of genotype? Yes	No
2D6	Abdul-Manap *et al.*, 1999		×
	Capon *et al.*, 1996		×

Table 2.6 Summary of studies recovered that have investigated the effect of *CYP2C9* genotype on (A) the pharmacokinetics, and (B) the pharmacodynamics of *(S)*-warfarin.

(A) CYP	Reference	Significant effect of genotype? Yes	No
2C9	Herman *et al.*, 2005	✓	
	Sconce *et al.*, 2005	✓	
	Kamali *et al.*, 2004	✓	
	Scordo *et al.*, 2002	✓	
	Takahashi *et al.*, 2003		×

(B) CYP	Reference	Significant effect of genotype? Yes	No
2C9	Aqilante *et al.*, 2006	✓	
	Khan *et al.*, 2004	✓	
	Joffe *et al.*, 2004	✓/×	
	Aithal *et al.*, 1999	✓	
	Tabrizi *et al.*, 2002	✓	
	Loebstein *et al.*, 2001	✓/×	
	Peyvandi *et al.*, 2004	✓	
	Margaglioni *et al.*, 2002	✓	
	Higashi *et al.*, 2002	✓	
	Lindh *et al.*, 2005	✓	
	Hillman *et al.*, 2004	✓	
	Taube *et al.*, 2002	✓/×	
	Sconce *et al.*, 2005	✓	
	Kamali *et al.*, 2004	✓	
	Takahashi *et al.*, 2003		×
	Chern *et al.*, 2006	✓	
	King *et al.*, 2004	✓	
	Scordo *et al.*, 2002	✓	

2.4.2.3 Midazolam

Seven studies have investigated the influence of *CYP3A5* genotype on MDZ pharmacokinetics. Of these, only three found significant differences between genotypes. The 'successful' trials used 19, 31 and 64 subjects (Goh et al., 2002; Wong et al., 2004; Yu et al., 2004) whereas those that were 'unsuccessful' used 21, 39, 57 and 58 subjects (Eap *et al.*, 2004b; Floyd *et al.*, 2003; Lepper *et al.*, 2005; Shih & Huang, 2002). These findings suggest that the power of the studies may have been low, but not so underpowered as to render the effect of phenotype on the pharmacokinetics of MDZ impossible to detect. There are no published studies investigating the influence of CYP3A5 polymorphisms on the pharmacodynamics of MDZ.

The three studies that have investigated the influence of CYP3A4 genotype on MDZ pharmacokinetics (Eap *et al.*, 2004b; Floyd *et al.*, 2003; Lepper *et al.*, 2005) were all 'unsuccessful' in detecting differences between genotypes (Table 2.7). The wide interindividual variability in the activity of CYP3A4 (5 to 82 fold *in vitro*) (Gibbs & Hosea, 2003) may account for these negative findings.

2.4.2.4 Omeprazole

All except one of the 13 reports that have investigated the effect of CYP2C19 genotype/phenotype on the pharmacokinetics of OMZ showed significant differences, study sizes ranging from 6 (Kita *et al.*, 2001) to 160 (Chang *et al.*, 1995) subjects.

Of the 10 studies on the effect of CYP2C19 phenoype on the pharmacodynamics of OMZ, 8 reported 'positive' results, using between 6 and 160 subjects (Chang *et al.*, 1995; Egan *et al.*, 2003; Furuta *et al.*, 1999b; Hu *et al.*, 2005; Kita *et al.*, 2001; Shimatani *et al.*, 2003; Sim *et al.*, 2006; Tanigawara *et al.*, 1999).

The two 'unsuccessful' studies recruited 119 and 174 subjects (Miyoshi *et al.*, 2001; Ohkusa *et al.*, 2005). However, in contrast to the 'unsuccessful' studies, most of the pharmacodynamic studies (as well as many of the pharmacokinetic studies) that found significant differences between CYP2C19 genotypes/phenotypes, used 'enriched' populations, in that they had higher abundances of certain genotypes than are present in Caucasian populations. Thus, subjects were either pre-selected based on their genotypes, or studies were carried out in other ethnic groups.

Table 2.7 Summary of studies recovered that have investigated the effect of *CYP3A5* genotype on the pharmacokinetics of MDZ.

CYP	Reference	Significant effect of genotype? Yes	No
3A5	Lepper *et al.*, 2005		×
	Goh *et al.*, 2002	✓	
	Wong *et al.*, 2004	✓	
	Shih *et al.*, 2002		×
	Eap *et al.*, 2004		×
	Floyd *et al.*, 2003		×
	Yu *et al.*, 2004	✓	

Table 2.8 Summary of studies recovered that have investigated the effect of CYP2C19 phenotype on (A) the pharmacokinetics, and (B) the pharmacodynamics of OMZ.

(A) CYP	Reference	Significant effect of genotype? Yes	No
2C19	Qiao *et al.*, 2006	✓	
	Fu *et al.*, 2004		×
	Fu *et al.*, 2003	✓	
	Hu *et al.*, 2005	✓	
	Ohnishi *et al.*, 2005	✓	
	Zhou *et al.*, 1999	✓	
	Chang *et al.*, 1995	✓	
	Yasuda *et al.*, 1995	✓	
	Andersson *et al.*, 1998	✓	
	Furuta *et al.*, 1999	✓	
	Kita *et al.*, 2001	✓	
	Shirai *et al.*, 2001	✓	
	Yin *et al.*, 2004	✓	
	Tybring *et al.*, 1997	✓	
	Sakai *et al.*, 2001	✓	

(B) CYP	Reference	Significant effect of genotype? Yes	No
2C19	Shimatani *et al.*, 2003	✓	
	Chang *et al.*, 1995	✓	
	Hu *et al.*, 2005	✓	
	Egan *et al.*, 2003	✓/×	
	Ohkusa *et al.*, 2005		×
	Miyoshi *et al.*, 2001		×
	Kita *et al.*, 2001	✓	
	Furuta *et al.*, 1999	✓	
	Tanigawara *et al.*, 1999	✓	
	Sim *et al.*, 2006	✓	
	Kita *et al.*, 2001	✓	
	Shirai *et al.*, 2001	✓	

2.4.2.5 Tolbutamide

All of the 6 studies that investigated the effect of *CYP2C9* genotype on TLB pharmacokinetics were 'successful' and used study sizes of 15 (Lee *et al.*, 2002), 16 (Lee *et al.*, 2003), 18 (Shon *et al.*, 2002), 23 (Kirchheiner *et al.*, 2002a), 23 (Jetter *et al.*, 2004) and 63 (Wang *et al.*, 2005a). However, as is the case for *(S)*-warfarin, and since there are 6 common *CYP2C9* genotypes, only one of the comparisons between the wild-type genotype and any other single genotype needed to be significantly different for the study to be recorded as 'successful'.

The pharmacodynamic studies appear to have been somewhat underpowered. Only one out of the three studies investigating the influence of *CYP2C9* genotype on TLB pharmacodynamics (Shon *et al.*, 2002) was successful. All used similar numbers of subjects (15; Lee *et al.*, 2002), (18; Shon *et al.*, 2002) (23; Kirchheiner *et al.*, 2002a).

Table 2.9 Summary of studies recovered that have investigated the effect of *CYP2C9* genotype on (A) the pharmacokinetics, and (B) the pharmacodynamics of TLB.

(A) CYP	Reference	Significant effect of genotype? Yes	No
2C9	Shon *et al.*, 2002	✓	
	Jetter *et al.*, 2004	✓	
	Kirchheiner *et al.*, 2002	✓	
	Lee *et al.*, 2002	✓	
	Lee *et al.*, 2003	✓	
	Wang *et al.*, 2005	✓	

(B) CYP	Reference	Significant effect of genotype? Yes	No
2C9	Kirchheiner *et al.*, 2002		✗
	Shon *et al.*, 2002	✓	
	Lee *et al.*, 2002		✗

Table 2.10 Summary of studies recovered that have investigated the effect of CYP phenotype/genotype on the pharmacokinetics of drugs. The highlighted studies indicate drugs for which both positive and negative outcomes were reported in the literature.

			Significant effect of genotype?		
Enzyme	**Drug**	**Reference**	**Yes**	**No**	**Journal**
1A2	Clozapine	(van der Weide *et al.*, 2003)		×	Pharmacogenetics
		(Bertilsson *et al.*, 1994)	✓		British Journal of Clinical Pharmacology
2B6	Bupropion	(Kirchheiner *et al.*, 2003a)		×	Pharmacogenetics
		(Loboz *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
	Mephobarbital	(Kobayashi *et al.*, 2004)		×	Pharmacogenetics
	Methadone	(Crettol *et al.*, 2005)	✓		Clinical pharmacology & Therapeutics
2C8	Fluvastatin	(Kirchheiner *et al.*, 2003b)		×	Clinical pharmacology & Therapeutics
	Ibuprofen	(Martinez *et al.*, 2005)	✓		British Journal of Clinical Pharmacology
		(Garcia-Martin *et al.*, 2004)	✓		Clinical pharmacology & Therapeutics
	Paclitaxel	(Henningsson *et al.*, 2005)		×	Clinical Cancer Research
	Repaglinide	(Niemi *et al.*, 2003)	✓		Clinical pharmacology & Therapeutics
		(Bidstrup *et al.*, 2006)		×	British Journal of Clinical Pharmacology
2C9	Acenocoumarol	(Thijssen *et al.*, 2001)	✓		Clinical pharmacology & Therapeutics
	Celecoxib	(Kirchheiner *et al.*, 2003e)	✓		Pharmacogenetics
		(Brenner *et al.*, 2003)		×	Clinical Pharmacokinetics
	Diclofenac	(Brenner *et al.*, 2003)		×	Clinical Pharmacokinetics
		(Yasar *et al.*, 2001)		×	European Journal of Clinical Pharmacology
		(Dorado *et al.*, 2003)	✓		European Journal of Clinical Pharmacology
		(Kirchheiner *et al.*, 2003c)	✓		British Journal of Clinical Pharmacology
		(Shimamoto *et al.*, 2000)		×	European Journal of Clinical Pharmacology
		(Morin *et al.*, 2001)		×	European Journal of Clinical Pharmacology
	Doxepin	(Kirchheiner *et al.*, 2002d)	✓		Pharmacogenetics

Flurbiprofen	(Lee *et al.*, 2003)	✓		European Journal of Clinical Pharmacology
Fluvastatin	(Kirchheiner *et al.*, 2003b)	✓		Clinical pharmacology & Therapeutics
Glimepiride	(Suzuki *et al.*, 2006)	✓		Diabetes Research & Clinical Practice
	(Wang *et al.*, 2005b)	✓		Clinical pharmacology & Therapeutics
	(Niemi *et al.*, 2002)	✓		Clinical pharmacology & Therapeutics
Glyburide	(Kirchheiner *et al.*, 2002b)	✓		Clinical pharmacology & Therapeutics
	(Yin *et al.*, 2005b)	✓		Clinical pharmacology & Therapeutics
	(Niemi *et al.*, 2002)	✓		Clinical pharmacology & Therapeutics
Ibuprofen	(Kirchheiner *et al.*, 2002c)	✓		Clinical pharmacology & Therapeutics
	(Garcia-Martin *et al.*, 2004)		×	Clinical pharmacology & Therapeutics
Imatinib	(Gardner *et al.*, 2006)		×	Clinical pharmacology & Therapeutics
Irbesartan	(Chen *et al.*, 2006)	✓		Meth & Findings in Exp & Clin Pharmacol
Losartan	(Lee *et al.*, 2003)		×	Pharmacotherapy
	(Yasar *et al.*, 2002)	✓		Clinical pharmacology & Therapeutics
	(Allabi *et al.*, 2004)	✓		Clinical pharmacology & Therapeutics
	(Sekino *et al.*, 2003)	✓		European Journal of Clinical Pharmacology
Methadone	(Crettol *et al.*, 2005)		×	Clinical pharmacology & Therapeutics
Nateglinide	(Kirchheiner *et al.*, 2004b)	✓		Clinical Pharmacokinetics
Phenprocoumon	(Kirchheiner *et al.*, 2004c)	✓		Pharmacogenetics
	(Ufer *et al.*, 2004)	✓		Xenobiotica
Phenytoin	(Allabi *et al.*, 2005)	✓		Pharmacogenetics & Genomics
	(Mamiya *et al.*, 1998)	✓		Epilepsia
	(Rosemary *et al.*, 2006)	✓		Indian Journal of Medical Research
	(Odani *et al.*, 1997)	✓		Clinical pharmacology & Therapeutics
	(Aynacioglu *et al.*, 1999)	✓		British Journal of Clinical Pharmacology
Piroxicam	(Perini *et al.*, 2005)	✓		Clinical pharmacology & Therapeutics
Tamoxifen	(Jin *et al.*, 2005)		×	Journal of the National Cancer Institute
Tolbutamide	(Shon *et al.*, 2002)	✓		Pharmacogenetics

		(Jetter *et al.*, 2004)	✓		European Journal of Clinical Pharmacology
		(Kirchheiner *et al.*, 2002a)	✓		Pharmacogenetics
		(Lee *et al.*, 2002)	✓		Clinical pharmacology & Therapeutics
		(Lee *et al.*, 2003)	✓		Journal of Clinical Pharmacology
		(Wang *et al.*, 2005a)	✓		Chinese Journal of Clinical Pharmacology
	Torsemide	(Vormfelde *et al.*, 2004)	✓		Clinical pharmacology & Therapeutics
	Warfarin	(Herman *et al.*, 2005)	✓		Pharmacogenomics Journal
		(Sconce *et al.*, 2005)	✓		Blood
		(Kamali *et al.*, 2004)	✓		Clinical Pharmacology Therapeutics
		(Scordo *et al.*, 2002)	✓		Clinical Pharmacology Therapeutics
		(Takahashi *et al.*, 2003)		×	Clinical Pharmacology Therapeutics
2C19	Amitriptyline	(Jiang *et al.*, 2002)	✓		European Journal of Clinical Pharmacology
		(Steimer *et al.*, 2004)	✓		Clinical Chemistry
		(Shimoda *et al.*, 2002)	✓		Journal of Clinical Psychopharmacology
	Carisoprodol	(Dalen *et al.*, 1996)	✓		Pharmacogenetics
	Citalopram	(Sindrup *et al.*, 1993)	✓		Therapeutic Drug Monitoring
	Cyclophosphamide	(Timm *et al.*, 2005)	✓		Pharmacogenomics Journal
	Diazepam	(Sohn *et al.*, 1992)	✓		Clinical pharmacology & Therapeutics
		(Bertilsson *et al.*, 1989)	✓		Clinical pharmacology & Therapeutics
		(Zhang *et al.*, 1990)		×	Clinical pharmacology & Therapeutics
		(Wan *et al.*, 1996)	✓		British Journal of Clinical Pharmacology
		(Qin *et al.*, 1999)	✓		Clinical pharmacology & Therapeutics
	Doxepin	(Kirchheiner *et al.*, 2002d)	✓		Pharmacogenetics
	Fluoxetine	(Liu *et al.*, 2001)	✓		British Journal of Clinical Pharmacology
	Fluvoxamine	Jann *et al.*, 2002		×	Drug Metabolism & Drug Interactions
		(Spigset *et al.*, 1997)		×	European Journal of Clinical Pharmacology
	Glyburide	(Yin *et al.*, 2005b)		×	Clinical pharmacology & Therapeutics
	Hexobarbital	(Knodell *et al.*, 1988)	✓		Journal of Pharmacology & Exp Ther

	(Adedoyin *et al.*, 1994)	✓		Pharmacogenetics
Imatinib	(Gardner *et al.*, 2006)		×	Clinical pharmacology & Therapeutics
Imipramine	(Morinobu *et al.*, 1997)	✓		Psychiatry & Clinical Neuroscience
	(Koyama *et al.*, 1996)	✓		Journal of Clinical Psychopharmacology
Lansoprazole	(Qiao *et al.*, 2006)	✓		European Journal of Clinical Pharmacology
	(Miura *et al.*, 2004)	✓		European Journal of Clinical Pharmacology
	(Kim *et al.*, 2002)	✓		Clinical pharmacology & Therapeutics
	(Katsuki *et al.*, 1997)	✓		European Journal of Clinical Pharmacology
	(Hu *et al.*, 2005)	✓		Acta Pharmacologica Sinica
	(Ieiri *et al.*, 2001)	✓		European Journal of Clinical Pharmacology
	(Sohn *et al.*, 1997)	✓		Clinical pharmacology & Therapeutics
	(Andersson *et al.*, 1998)	✓		British Journal of Clinical Pharmacology
Mephobarbital	(Kobayashi *et al.*, 2004)	✓		Pharmacogenetics
	(Kupfer & Branch, 1985)	✓		Clinical pharmacology & Therapeutics
Methadone	(Crettol *et al.*, 2005)		×	Clinical pharmacology & Therapeutics
Metoprolol	(Taguchi *et al.*, 2004)		×	Biological & Pharmaceutical Bulletin
Moclobemide	(Yu *et al.*, 2001)	✓		Clinical pharmacology & Therapeutics
Nelfinavir	(Burger *et al.*, 2006)		×	British Journal of Clinical Pharmacology
Omeprazole	(Qiao *et al.*, 2006)	✓		European Journal of Clinical Pharmacology
	(Fu *et al.*, 2004)		×	Chinese Pharmaceutical Journal
	(Fu *et al.*, 2003)	✓		Chinese Journal of Pharmacol Toxicol
	(Hu *et al.*, 2005)	✓		Chinese Pharmacological Bulletin
	(Ohnishi *et al.*, 2005)	✓		Journal of Clinical Pharmacology
	(Zhou *et al.*, 1999)	✓		European Journal of Clinical Pharmacology
	(Chang *et al.*, 1995)	✓		Pharmacogenetics
	(Yasuda *et al.*, 1995)	✓		Clinical pharmacology & Therapeutics
	(Andersson *et al.*, 1998)	✓		Journal of Clinical Pharmacology
	(Furuta *et al.*, 1999b)	✓		Clinical pharmacology & Therapeutics

	(Kita *et al.*, 2001)	✓		Pharmaceutical Research
	(Shirai *et al.*, 2001)	✓		Alimentary Pharmacology & Therapeutics
	(Yin *et al.*, 2004)	✓		Journal of Clinical Pharmacology
	(Tybring *et al.*, 1997)	✓		Clinical Pharmacology & Therapeutics
	(Sakai *et al.*, 2001)	✓		Pharmaceutical Research
Pantoprazole	(Andersson *et al.*, 1998)	✓		Journal of Clinical Pharmacology
Phenobarbital	(Hadama *et al.*, 2001)		×	Therapeutic Drug Monitoring
	(Mamiya *et al.*, 2000)	✓		European Journal of Clinical Pharmacology
Phenytoin	(Allabi *et al.*, 2005)		×	Pharmacogenetics & Genomics
	(Mamiya *et al.*, 1998)	✓		Epilepsia
	(Odani *et al.*, 1997)	✓		Clinical pharmacology & Therapeutics
	(Ieiri *et al.*, 1997)	✓		British Journal of Clinical Pharmacology
	(Schellens *et al.*, 1990)		×	British Journal of Clinical Pharmacology
	(Watanabe *et al.*, 1998)		×	Clinical Neuropharmacology
	(Rosemary *et al.*, 2006)		×	Indian Journal of Medical Research
Proguanil	(Hoskins *et al.*, 1998)	✓		British Journal of Clinical Pharmacology
	(Brosen *et al.*, 1993)	✓		British Journal of Clinical Pharmacology
	(Kaneko *et al.*, 1999b)	✓		Pharmacogenetics
	(Kaneko *et al.*, 1999a)	✓		Journal of Infectious Diseases
Propafenone	(Kuang *et al.*, 1994)	✓		Chinese Journal of Pharmacol & Toxicology
Propranolol	(Xie *et al.*, 1997)		×	Zhongguo Yao Li Xue Bao
Rabeprazole	(Qiao *et al.*, 2006)	✓		European Journal of Clinical Pharmacology
	(Horai *et al.*, 2001)	✓		Alimentary Pharmacology & Therapeutics
	(Hu *et al.*, 2005)	✓		Acta Pharmacologica Sinica
	(Ieiri *et al.*, 2001)	✓		European Journal of Clinical Pharmacology
	(Shirai *et al.*, 2001)	✓		Alimentary Pharmacology & Therapeutics
Selegiline	(Laine *et al.*, 2001)		×	European Journal of Clinical Pharmacology
Sertraline	(Wang *et al.*, 2001)	✓		Clinical pharmacology & Therapeutics

	Ticlopidine	(Ieiri *et al.*, 2005)		×	Pharmacogenetics & Genomics
	Tolbutamide	(Shon *et al.*, 2002)		×	Pharmacogenetics
		(Kirchheiner *et al.*, 2002b)		×	Pharmacogenetics
2D6	Amiflamine	(Alvan *et al.*, 1984)	✓		Clinical pharmacology & Therapeutics
	Amitriptyline	(Steimer *et al.*, 2004)	✓		Clinical Chemistry
		(Shimoda *et al.*, 2002)		×	Journal of Clinical Psychopharmacology
	Carvedilol	(Honda *et al.*, 2005)	✓		Biological & Pharmaceutical Bulletin
		(Takekuma *et al.*, 2006)		×	J Pharmacy & Pharmaceutical Sciences
	Codeine	(Caraco *et al.*, 1999)	✓		J Pharmacology Exp Ther
	Debrisoquine	(Dorado *et al.*, 2005)	✓		Clinical Chemistry & Laboratory medicine
	Dextromethorphan	(Casner, 2005)	✓		Journal of Clinical Pharmacology
		(Desmeules *et al.*, 1999)	✓		J Pharmacology & Exp Therapeutics
		(Abdul Manap *et al.*, 1999)	✓		British Journal Clinical Pharmacology
		(Pope *et al.*, 2004)	✓		Journal of Clinical Pharmacology
		(Capon *et al.*, 1996)	✓		Clinical Pharmacology & Therapeutics
	Diazepam	(Bertilsson *et al.*, 1989)		×	Clinical Pharmacology & Therapeutics
	Diltiazem	(Molden *et al.*, 2002)		×	Clinical pharmacology & Therapeutics
	Donepezil	(Varsaldi *et al.*, 2006)		×	European Journal of Clinical Pharmacology
	Doxepin	(Kirchheiner *et al.*, 2002d)	✓		Pharmacogenetics
	Flecanide	(Tenneze *et al.*, 2002)		×	Clinical pharmacology & Therapeutics
	Fluvoxamine	(Ohara *et al.*, 2003)		×	European Journal of Clinical Pharmacology
		(Spigset *et al.*, 1997)	✓		European Journal of Clinical Pharmacology
	Gefitinib	(Swaisland *et al.*, 2006)		×	Clinical Pharmacokinetics
	Haloperidol	(Brockmoller *et al.*, 2002)	✓		Clinical pharmacology & Therapeutics
		(Pan *et al.*, 1999)	✓		Therapeutic Drug Monitoring
	Hydrocodone	(Otton *et al.*, 1993)	✓		Clinical pharmacology & Therapeutics
	Imatinib	(Gardner *et al.*, 2006)	✓		Clinical pharmacology & Therapeutics
	Loratadine	(Yin *et al.*, 2005a)	✓		Drug Metabolism & Disposition

Metoprolol	(Taguchi *et al.*, 2004)	✓		Biological & Pharmaceutical Bulletin
	(Zineh *et al.*, 2004)	✓		Clinical pharmacology & Therapeutics
	(Kirchheiner *et al.*, 2004a)	✓		Clinical Pharmacology & Therapeutics
	(Fux *et al.*, 2005)	✓		Clinical pharmacology & Therapeutics
	(Rau *et al.*, 2002)	✓		Pharmacogenetics
Mianserin	(Mihara *et al.*, 1997)	✓		Journal of Clinical Psychopharmacology
	(Dahl *et al.*, 1994)	✓		Clinical pharmacology & Therapeutics
Nateglinide	(Kirchheiner *et al.*, 2004b)		×	Clinical Pharmacokinetics
Nortriptyline	(Kvist *et al.*, 2001)	✓		Clinical Pharmacokinetics
Paroxetine	(Yoon *et al.*, 2000)	✓		Clinical pharmacology & Therapeutics
Perhexiline	(Davies *et al.*, 2006)	✓		British Journal of Clinical Pharmacology
Phenformin	(Oates *et al.*, 1983)	✓		Clinical Pharmacology & Therapeutics
Propafenone	(Cai *et al.*, 2002)	✓		Acta Pharmacolgica Sinica
Propranolol	(Huang *et al.*, 2003)		×	Pharmacogenetics
	(Huang *et al.*, 2003)	✓		Journal of the Chinese Medical Association
Propafenone	(Kuang *et al.*, 1994)		×	Chinese Journal of Pharmacol & Toxicology
Quinidine	(Nielsen *et al.*, 1995)		×	European Journal of Clinical Pharmacology
Risperidone	(Yasui-Furukori *et al.*, 2003)	✓		Journal of Clinical Pharmacology
	(Riedel *et al.*, 2005)	✓		European Arch Psychiatry & Neuroscience
	(Kakihara *et al.*, 2005)		×	International Clinical Psychopharmacology
Selegiline	(Scheinin *et al.*, 1998)		×	Clinical pharmacology & Therapeutics
Tamoxifen	(Jin *et al.*, 2005)	✓		Journal of the National Cancer Institute
	(Borges *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
Timolol	(Nieminen *et al.*, 2005)	✓		European Journal of Clinical Pharmacology
Tolterodine	(Brynne *et al.*, 1998)	✓		Clinical pharmacology & Therapeutics
Tramadol	(Pedersen *et al.*, 2006)	✓		European Journal of Clinical Pharmacology
Trimipramine	(Kirchheiner *et al.*, 2003d)	✓		Pharmacogenetics

	Venlafaxine	(Fukuda *et al.*, 1999)	✓		British Journal of Clinical Pharmacology
3A4	Cyclosporine	(Hesselink *et al.*, 2004)	✓		Clinical pharmacology & Therapeutics
	Gefitinib	(Swaisland *et al.*, 2006)		×	Clinical Pharmacokinetics
	Imatinib	(Gardner *et al.*, 2006)		×	Clinical pharmacology & Therapeutics
	Irinotecan	(Mathijssen *et al.*, 2003)		×	Clinical Cancer Research
	Midazolam	(Lepper *et al.*, 2005)		×	Clinical Cancer Research
		(Eap *et al.*, 2004b)		×	European Journal of Clinical Pharmacology
		(Floyd *et al.*, 2003)		×	Pharmacogenetics
	Paclitaxel	(Henningsson *et al.*, 2005)		×	Clinical Cancer Research
		(Nakajima *et al.*, 2005)		×	Journal of Clinical Pharmacology
		(Nakajima *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
	Saquinavir	(Mouly *et al.*, 2005)		×	Clinical pharmacology & Therapeutics
	Sirolimus	(Anglicheau *et al.*, 2005)	✓		American Journal of Transplantation
3A5	Alprazolam	(Park *et al.*, 2006)	✓		Clinical pharmacology & Therapeutics
	Cyclosporine	(Hesselink *et al.*, 2004)		×	Clinical pharmacology & Therapeutics
		(Min *et al.*, 2004)	✓		Therapeutic Drug Monitoring
		(Zhao *et al.*, 2005)	✓		Transplantation Proceedings
		(Haufroid *et al.*, 2004)	✓		Pharmacogenetics
		(Anglicheau *et al.*, 2004)		×	Clinical pharmacology & Therapeutics
	Docetaxel	(Goh *et al.*, 2002)		×	Journal of Clinical Oncology
	Gefitinib	(Swaisland *et al.*, 2006)		×	Clinical Pharmacokinetics
	Imatinib	(Gardner *et al.*, 2006)		×	Clinical pharmacology & Therapeutics
	Irinotecan	(Mathijssen *et al.*, 2003)		×	Clinical Cancer Research
	Midazolam	(Lepper *et al.*, 2005)		×	Clinical Cancer Research
		(Goh *et al.*, 2002)	✓		Journal of Clinical Oncology
		(Wong *et al.*, 2004)	✓		Clinical pharmacology & Therapeutics
		(Shih & Huang, 2002)		×	Drug Metabolism & Disposition
		(Eap *et al.*, 2004b)		×	European Journal of Clinical Pharmacology

	(Floyd *et al.*, 2003)		×	Pharmacogenetics
	(Yu *et al.*, 2004)	✓		Clinical Pharmacology & Therapeutics
Paclitaxel	(Henningsson *et al.*, 2005)		×	Clinical Cancer Research
Saquinavir	(Mouly *et al.*, 2005)	✓		Clinical pharmacology & Therapeutics
	(Frohlich *et al.*, 2004)		×	British Journal of Clinical Pharmacology
Sirolimus	(Anglicheau *et al.*, 2005)	✓		American Journal of Transplantation
	(Le Meur *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
Tacrolimus	(Tada *et al.*, 2005)	✓		Transplantation Proceedings
	(Tsuchiya *et al.*, 2004)	✓		Transplantation
	(Thervet *et al.*, 2003)	✓		Transplantation
	(Zhang *et al.*, 2005)	✓		Clinical Transplantation
	(Macphee *et al.*, 2005)	✓		Transplantation
	(Haufroid *et al.*, 2004)	✓		Pharmacogenetics
Tamoxifen	(Jin *et al.*, 2005)		×	Journal of the National Cancer Institute

Table 2.11 Summary of studies recovered that have investigated the effect of CYP phenotype/genotype on the pharmacodynamics of drugs. The highlighted studies indicate drugs for which both positive and negative outcomes were reported in the literature.

			Significant relationship?		
CYP	**Drug**	**Reference**	**Yes**	**No**	**Journal**
1A2	Clozapine	(van der Weide *et al.*, 2003)		×	Pharmacogenetics
		(Eap *et al.*, 2004a)	✓		Journal of Clinical Psychopharmacology
2B6	Methadone	(Crettol *et al.*, 2005)		×	Clinical Pharmacology & Therapeutics
2C8	Repaglinide	(Niemi *et al.*, 2003)		×	Clinical Pharmacology & Therapeutics
2C9	Acenocoumarol	(Morin *et al.*, 2004)	✓		Clinical Pharmacology & Therapeutics
		(Visser *et al.*, 2004)	✓		Pharmacogenetics
		(Tassies *et al.*, 2002)	✓		Haematologica
		(Hermida *et al.*, 2002)	✓		Blood
		(Bodin *et al.*, 2005)	✓		Blood
		(Verstuyft *et al.*, 2001)	✓		Pharmacogenetics
	Diclofenac	(Kirchheiner *et al.*, 2003c)		×	British Journal of Clinical Pharmacology
	Fluvastatin	(Kirchheiner *et al.*, 2003b)		×	Clinical Pharmacology & Therapeutics
	Glibenclamide	(Holstein *et al.*, 2005)	✓		British Journal of Clinical Pharmacology
	Glyburide	(Kirchheiner *et al.*, 2002b)	✓/×		Clinical Pharmacology & Therapeutics
		(Yin *et al.*, 2005b)	✓		Clinical Pharmacology & Therapeutics
	Glimepiride	(Suzuki *et al.*, 2006)	✓		Diabetes Research & Clinical Practice
		(Niemi *et al.*, 2002)		×	Clinical Pharmacology & Therapeutics
	Ibuprofen	(Kirchheiner *et al.*, 2002c)	✓		Clinical Pharmacology & Therapeutics
	Irbesartan	(Chen *et al.*, 2006)	✓		Methods & Findings in Exp & Clin Pharmacol
		(Hallberg *et al.*, 2002)	✓		Journal of Hypertension
	Losartan	(Sekino *et al.*, 2003)	✓		European Jounrnal of Clinical Pharmacology

Methadone	(Crettol *et al.*, 2005)		×	Clinical Pharmacology & Therapeutics
Nateglinide	(Kirchheiner *et al.*, 2004b)		×	Clinical Pharmacokinetics
Phenprocoumon	(Schalekamp *et al.*, 2006)		×	Clinical Pharmacology & Therapeutics
	(Visser *et al.*, 2004)		×	Pharmacogenetics
	(Schwabedissen *et al.*, 2006)		×	European Journal of Clinical Pharmacology
Phenytoin	(Soga *et al.*, 2004)		×	Life Sciences
	(Tate *et al.*, 2005)	✓		**
	(van der Weide *et al.*, 2001)	✓		Pharmacogenetics
Tolbutamide	(Kirchheiner *et al.*, 2002a)		×	Pharmacogenetics
	(Shon *et al.*, 2002)	✓		Pharmacogenetics
	(Lee *et al.*, 2002)		×	Clinical Pharmacology & Therapeutics
Warfarin	(Aquilante *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
	(Khan *et al.*, 2004)	✓		British Journal of Haematology
	(Joffe *et al.*, 2004)		✓/×	Thrombosis & Haemostasis
	(Aithal *et al.*, 1999)	✓		The Lancet
	(Tabrizi *et al.*, 2002)	✓		Journal of the American College of Surgeons
	(Loebstein *et al.*, 2001)		✓/×	Clinical Pharmacology & Therapeutics
	(Peyvandi *et al.*, 2004)	✓		Clinical Pharmacology & Therapeutics
	(Margaglione *et al.*, 2000)	✓		Thrombosis & Haemostasis
	(Higashi *et al.*, 2002)	✓		Journal of the American Medical Association
	(Lindh *et al.*, 2005)	✓		Clinical Pharmacology & Therapeutics
	(Hillman *et al.*, 2004)	✓		Pharmacogenetics
	(Taube *et al.*, 2000)		✓/×?	Hemostasis, Thrombosis & Vascular Biology
	(Sconce *et al.*, 2005)	✓		Hemostasis, Thrombosis & Vascular Biology
	(Kamali *et al.*, 2004)	✓		Clinical Pharmacology & Therapeutics
	(Takahashi *et al.*, 2003)		×	Hemostasis, Thrombosis & Vascular Biology
	(Chern *et al.*, 2006)	✓		Clinica Chimica Acta
	(King *et al.*, 2004)	✓		Pharmacogenetics

		(Scordo *et al.*, 2002)	✓		Clinical Pharmacology & Therapeutics
2C19	Cyclophosphamide	(Takada *et al.*, 2004)	✓		Arthritis & Rheumatism
		(Yin *et al.*, 2005b)	✓		Clinical Pharmacology & Therapeutics
	Lansoprazole	(Furuta *et al.*, 2005)		×	Clinical Gastroenterology & Hepatology
		(Ieiri *et al.*, 2001)	✓		European Journal of Clinical Pharmacology
		(Adachi *et al.*, 2000)	✓		Alimentary Pharmacology & Therapeutics
		(Schwab *et al.*, 2004)	✓		Clinical Pharmacology & Therapeutics
		(Furuta *et al.*, 2002)	✓		Clinical Pharmacology & Therapeutics
	Methadone	(Crettol *et al.*, 2005)		×	Clinical Pharmacology & Therapeutics
	Mephenytoin	(Sim *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
	Omeprazole	(Shimatani *et al.*, 2003)	✓		Alimentary Pharmacology & Therapeutics
		(Chang *et al.*, 1995)	✓		British Journal of Clinical Pharmacology
		(Hu *et al.*, 2005)	✓		Chinese Pharmacological Bulletin
		(Egan *et al.*, 2003)	✓/×		Alimentary Pharmacology & Therapeutics
		(Ohkusa *et al.*, 2005)		×	Alimentary Pharmacology & Therapeutics
		(Miyoshi *et al.*, 2001)		×	Journal of Gastroenterology & Hepatology
		(Kita *et al.*, 2001)	✓		Pharmaceutical Research
		(Furuta *et al.*, 1999a)	✓		Clinical Pharmacology & Therapeutics
		(Tanigawara *et al.*, 1999)	✓		Clinical Pharmacology & Therapeutics
		(Sim *et al.*, 2006)	✓		Clinical Pharmacology & Therapeutics
		(Kita *et al.*, 2001)	✓		Pharmaceutical Research
		(Shirai *et al.*, 2001)	✓		Alimentary Pharmacology & Therapeutics
	Phenytoin	(Soga *et al.*, 2004)		×	Life Sciences
	Proguanil	(Skjelbo *et al.*, 1996)		×	Clinical Pharmacology & Therapeutics
		(Kaneko *et al.*, 1999a)	✓/×		Pharmacogenetics
		(Kaneko *et al.*, 1999b)		×	Journal of Infectious Diseases
	Rabeprazole	(Horai *et al.*, 2001)	✓		Alimentary Pharmacology & Therapeutics
		(Hu *et al.*, 2005)		×	Acta Pharmacologica Sinica

		(Furuta *et al.*, 2001)	✓		Pharmacogenetics
		(Hokari *et al.*, 2001)		×	Alimentary Pharmacology & Therapeutics
		(Adachi *et al.*, 2000)		×	Alimentary Pharmacology & Therapeutics
		(Miyoshi *et al.*, 2001)		×	Journal of Gastroenterology & Hepatology
		(Niu *et al.*, 2004)		×	Chinese Journal of Gastroenterology
		(Ieiri *et al.*, 2001)	✓		European Journal of Clinical Pharmacology
		(Ariizumi *et al.*, 2006)		×	Journal of Gastroenterology & Hepatology
	Tolbutamide	(Kirchheiner *et al.*, 2002a)		×	Pharmacogenetics
		(Shon *et al.*, 2002)		×	Pharmacogenetics
2D6	Clomipramine	(Bouchez *et al.*, 1995)		×	European Psychiatry*
	Codeine	(Desmeules *et al.*, 1991)	✓		European Journal of Clinical Pharmacology
		(Sindrup *et al.*, 1993)	✓		Pain
	Dextromethorphan	(Abdul Manap *et al.*, 1999)		×	British Journal of Clinical Pharmacology
		(Capon *et al.*, 1996)		×	Clinical Pharmacology & Therapeutics
	Donepezil	(Varsaldi *et al.*, 2006)		×	European Journal of Clinical Pharmacology
	Flecanide	(Tenneze *et al.*, 2002)		×	Clinical Pharmacology & Therapeutics
	Fluvoxamine	(Suzuki *et al.*, 2006)	✓		Neuropsychopharmacology
	Haloperidol	(Brockmoller *et al.*, 2002)	✓		Clinical Pharmacology & Therapeutics
		(Pan *et al.*, 1999)		×	Therapeutic Drug Monitoring
	Metoprolol	(Zineh *et al.*, 2004)		×	Clinical Pharmacology & Therapeutics
		(Wuttke *et al.*, 2002)	✓		Clinical Pharmacology & Therapeutics
		(Kirchheiner *et al.*, 2004a)		✓/×	Clinical Pharmacology & Therapeutics
		(Fux *et al.*, 2005)		×	Clinical Pharmacology & Therapeutics
	Mianserin	(Mihara *et al.*, 1997)	✓		Journal of Clinical Psychopharmacology
	Mirtazapine	(Murphy *et al.*, 2003)		×	American Journal of Psychiatry
	Nateglinide	(Kirchheiner *et al.*, 2004b)		×	Clinical Pharmacokinetics
	Paroxetine	(Murphy *et al.*, 2003)		×	American Journal of Psychiatry
	Phenformin	(Oates *et al.*, 1983)	✓		Clinical Pharmacology & Therapeutics

		(Fletcher *et al.*, 1986)		×	British Journal of Clinical Pharmacology
	Piroxicam	(Perini *et al.*, 2005)	✓		Clinical Pharmacology & Therapeutics
	Propafenone	(Jazwinska-Tarnawska *et al.*, 2001)	✓		Clinical Pharmacology & Therapeutics
		(Cai *et al.*, 2002)	✓		Acta Pharmacologica Sinica
		(Lee *et al.*, 1990)	✓		New England Journal of Medicine
	Propranolol	(Huang *et al.*, 2003)		×	Journal of the Chinese Medical Association
	Risperidone	(Lane *et al.*, 2006)	✓		Journal of Clinical Psychopharmacol
		(Riedel *et al.*, 2005)		×	Archives of Psychiatry & Clinical Neuroscience
		(Kakihara *et al.*, 2005)		×	International Clinical Psychopharmacology
	Timolol	(Nieminen *et al.*, 2005)		×	European Journal of Clinical Pharmacology
	Tolterodine	(Brynne *et al.*, 1998)		×	Clinical Pharmacology & Therapeutics
	Venlafaxine	(Shams *et al.*, 2006)	✓/×		Journal of Clinical Pharmacy & Therapeutics
3A4	Atorvastatin	(Kajinami *et al.*, 2004)	✓		American Journal of Cardiology
	Simvastatin	(Fiegenbaum *et al.*, 2005)		×	Clinical Pharmacology & Therapeutics
3A5	Alprazolam	(Park *et al.*, 2006)		×	Clinical Pharmacology & Therapeutics
	Atorvastatin	(Kivisto *et al.*, 2004)	✓		Pharmacogenetics
	Lovastatin	(Kivisto *et al.*, 2004)	✓		Pharmacogenetics
	Simvastatin	(Kivisto *et al.*, 2004)	✓		Pharmacogenetics
		(Fiegenbaum *et al.*, 2005)		×	Clinical Pharmacology & Therapeutics

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CHAPTER 3

General Methods for the Prediction of Drug Clearance from *In Vitro* Data

3 GENERAL METHODS

This thesis is concerned with the use of *in vitro* drug metabolism data to simulate and predict pharmacokinetic differences between CYP phenotypes/genotypes. Pharmacokinetic parameters were then used in pharmacokinetic-pharmacodynamic models to simulate drug response. With some minor variations, the processes involved in predicting drug clearance and the use of clearance to predict elimination rate constants are common to each of the 5 drugs investigated in this thesis. This chapter will describe the process of extrapolation of human drug clearances (and elimination rate constants) from *in vitro* data using Simcyp® algorithms (www.simcyp.com). The use of elimination rate constant data within full pharmacokinetic-pharmacodynamic models to simulate and predict human concentration- and response-time profiles is specific to the 5 drugs studied and is described in the methods sections of the relevant chapters (Chapters 4 to 8).

Simcyp® algorithms allow the creation of populations of virtual individuals with genetic, physiological, and demographic characteristics that are derived from literature sources using Monte Carlo Simulation. The latter involves the generation of 'pseudo random' values, which can take on one of a range of parameters defined by a population distribution. Parameters relevant to the scaling process of *in vitro in vivo* extrapolation (IVIVE) are obtained for each virtual individual in the population (incorporating population variability) and are then used, together with the relevant *in vitro* metabolism data, to obtain values for the whole liver intrinsic clearance. These data are then converted into values for hepatic clearance (CL_H) using a liver model (Section 3.3.2.1). Between subject variability in first pass gut metabolism (where applicable) and renal clearance were estimated as described in Sections 3.3.1.2. & 3.3.3, and combined with the estimate of hepatic clearance to generate overall clearance values following intravenous (i.v.) or oral (p.o.) administration (CL, $CL_{p.o.}$).

Table 3.1 Primary drug specific parameters used in the IVIVE scaling process. The secondary parameters, which the primary parameters are used to calculate, are also indicated and the section in which further information is given is listed in the 3rd column.

Primary Parameter	Secondary Parameter	Section
fu	fu_B	3.1.1.3
fu_B	F_H; E_H; $CL_{i.v}$; $CL_{p.o.}$	3.3.2.1; 3.3.3
B:P	fu_B; $CL_{i.v}$; $CL_{p.o.}$	3.1.1.3; 3.3.3
f_a	$CL_{p.o.}$	3.3.3
V_{max} [CYP] [route]	$CLu_{H,int}$; $CLu_{G,int}$; E_H; F_H; F_G; $CL_{p.o./i.v.}$	3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.3
K_m	$CLu_{H,int}$; $CLu_{G,int}$; E_H; F_H; F_G; $CL_{p.o./i.v.}$	3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.3
fu_{mic}	$CLu_{H,int}$; $CLu_{G,int}$; E_H; F_H; F_G; $CL_{p.o/i.v.}$	3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.3

fu = fraction unbound in plasma; fu_B = fraction unbound in blood; B:P = blood to plasma partition ratio; f_a = fraction absorbed;

3.1 Drug Specific Information

Drug-specific data (Table 3.1) were collated from the published literature after identifying sources using OVID MEDLINE (1966–2006) and WEB OF SCIENCE (1981–2006). Drugs were selected based on the criteria outlined in Chapter 2; Section 2.4.3. In addition to these conditions, the substrate also had to fulfil several other criteria: (i) that the primary route of elimination is catalysed by a CYP enzyme, (ii) that the substrate is a recognized probe for a specific CYP enzyme and, (iii) that the drug does not exhibit non-linear kinetics *in vivo*.

3.1.1 *In Vitro* Data Collection and Manipulation

3.1.1.1 *In Vitro* Metabolism Data

Studies providing metabolic data in the form of values of V_{max} (pmol min^{-1} $pmol^{-1}$ CYP) and K_m (mM) were selected. Data obtained from experiments using microsomes prepared from recombinant systems expressing human CYPs (e.g. Escherichia coli, lymphoblastoid, baculovirus or yeast cells) were preferred over those from experiments carried out in human liver microsomes (HLM). In experiments in recombinant enzymes, V_{max} values are expressed in the units of 'per pmol CYP'. In contrast, V_{max} values derived from HLM experiments are expressed 'per mg protein'. Conversion of the values from HLM experiments to a rate 'per pmol CYP' is achieved by using CYP abundance data. However, since very few HLM studies have reported CYP abundances in individual livers, it was necessary to estimate this parameter in each individual using known population distributions. This involves making the additional assumption, that the HLM samples were pooled from large enough numbers to be representative of the population average. Assuming this to be the case, mean population abundance values could be used to estimate clearance, but this introduces a possible source of error in the scaling process. Because adequate recombinant data were available for all 5 drugs studied, HLM data were not used.

Where K_m and V_{max} data were available for a particular metabolic pathway from several different studies, it was necessary to find the weighted mean (WX) of these values. This was achieved using Equation 3.1, or when a drug had multiple metabolic pathways, kinetic data were combined to give 'global' values expressing the overall contribution of each CYP to net metabolism using Equations 3.2 and 3.3:

$$WX = \frac{\sum_{j=1}^{n} n_j \times x_j}{\sum_{j=1}^{n} n_j} \qquad \text{Equation 3.1}$$

where n_j is the number of observations and x_j is the mean value of the jth study.

$$CLu_{int}(global) = \sum_{x=1}^{n} CLu_{int} = \sum_{x=1}^{n} \left(\frac{V_{max}}{K_m} \right)_x \qquad \text{Equation 3.2}$$

where subscripts x (1 to n) are the different metabolic routes.

3.1.1.2 Microsomal Binding

Non-specific binding of substrates to the experimental apparatus and to proteins in *in vitro* enzyme kinetic experiments may lead to an underprediction of intrinsic clearance. Thus, values of the fraction unbound in microsomes (fu_{mic}) were used to adjust data to account for such non-specific binding. These data were kindly provided by Simcyp Ltd. In the absence of experimentally determined values, Equation 3.3 was used to estimate fu_{mic} (Austin *et al.*, 2002):

$$fu_{mic} = \frac{1}{C \times 10^{0.56 \log P/D - 1.41} + 1} \qquad \text{Equation 3.3}$$

where C is the microsomal protein concentration (mg ml^{-1}) and log P/D is the log P of the drug if it is a base, or the log $D_{7.4}$ of the drug if it is neutral or an acid. Experimental Log P and $logD_{7.4}$ values were experimental values reported in the literature.

In the absence of experimental data for microsomal protein concentration, a default value of 0.25 mg/mL was chosen, as it is the most commonly used concentration in rCYP experiments.

3.1.1.3 Fraction Unbound in Blood

Values of the fraction of unbound drug in blood (fu_B) were calculated from the fraction unbound in plasma (fu) divided by the blood to plasma concentration drug ratio (B/P;). Values of fu and B:P were obtained from *ex-vivo* studies reported in the literature. Where B:P values were not available, a default value of 0.55 (representing no drug uptake into erythrocytes at an haematocrit of 45%) was used.

3.2 Population Specific Information

Plasma drug clearance values ($CL_{i.v.}$, $CL_{p.o.}$) and their variances were generated for virtual populations with age ranges and male to female ratios consistent with those documented in the *in vivo* studies with which the simulated data were to be compared. If *in vivo* data were not available, a default age range of 20 to 50 years was used based on that commonly observed in the *in vivo* studies, and a default male to female ratio of 0.5 was chosen to correspond with the general population. The population specific parameters necessary for IVIVE simulation are listed in Table 3.2.

3.2.1 *Generation of Populations*

3.2.1.1 Demographic Parameters.

For a given population where the age distribution is known, it is necessary to estimate height and weight based on age, to aid subsequent assignment of anatomical and physiological parameters. In the current model, height was calculated from age and body weight from height.

Briefly, height and weight data based on 19,564 individuals were obtained from the Health Survey for England 1998 (Erens & Primatesta, 1999) (Figure 3.1). Height was modelled based on age and quadratic relationships were derived for both men and women (Figure 3.1). A normal distribution of height was assumed and variability was added at each age to generate individual values. Weight was modelled against height using an exponential function (Figure 3.1).

Since body surface area (BSA) is known to be better correlated with many physiological functions, organ sizes and blood flows, than with height or weight alone (Barger-Lux & Heaney, 2005; Seo *et al.*, 2000; Wu *et al.*, 2004), a value of BSA was calculated for each individual based on the Du Bois and Du Bois equation (Du Bois & Du Bois, 1916):

$$BSA_i\ (m^2) = weight_i\ (kg)^{0.425} \times Height_i\ (cm)^{0.725} \times 0.007184$$

Table 3.2 Primary population specific parameters used in the IVIVE scaling process. The secondary parameters, estimated from the primary parameters, are also listed, together with references to the appropriate sections of this thesis.

Primary Parameter	Secondary Parameters	Section
n	Used to generate population	
Male:Female ratio	Height; BW; BSA; LV; LW etc.	3.2; 3.2.1.1; 3.2.1.2
Age	Height; BW; BSA; LV; crCL etc.	3.2; 3.2.1.1; 3.2.1.2; 3.3.3
Height	BW; BSA; LV; LW; CI etc.	3.2; 3.2.1.1; 3.2.1.2; 3.2.1.3
Body Weight	BSA; LV; LW; CI; CO; crCL etc.	3.2.1.1; 3.2.1.2; 3.2.1.3; 3.3.3
BSA	LV; LW; CI; CO; Q_{port} ; Q_{Art} etc.	3.2.1.1; 3.2.1.2; 3.2.1.3

BSA = body surface area; CI = cardiac index; CO = cardiac output; crCL = creatinine clearance.

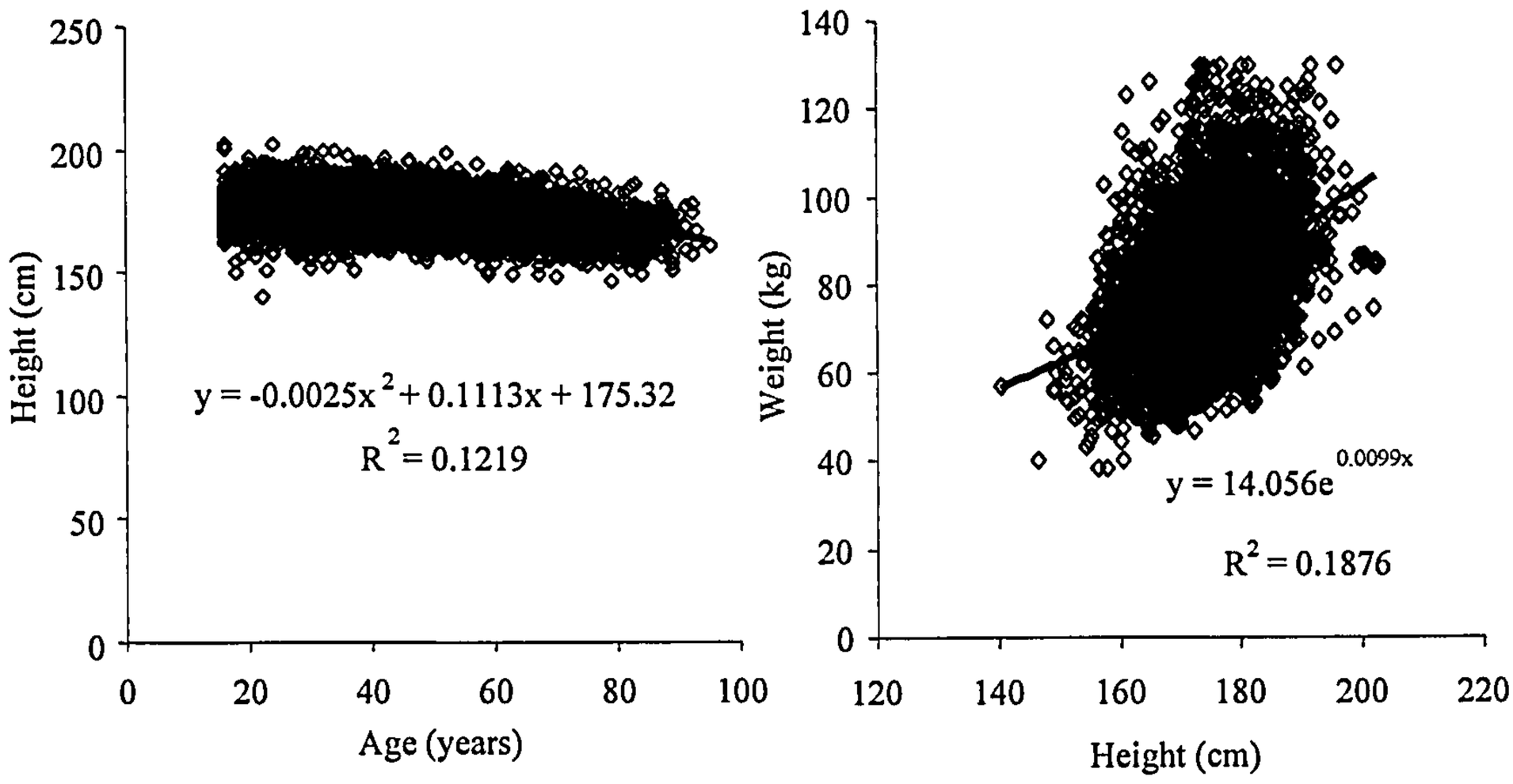


Figure 3.1 Data from 19-564 individuals showing the relationship between age and height, and height and body weight in males (females not shown).

3.2.1.2 Anatomical Parameters

Individual liver volume (LV) was estimated from BSA using the following equation, which relates liver volume (LV) to BSA, based on data from 1332 autopsy livers from German subjects (Heinemann *et al.*, 1999):

$$LV_i = 1.0728 \times BSA_i\,(m^2) - 0.3457 \qquad \text{Equation 3.5}$$

LV was then converted to liver weight (LW) by multiplying by liver density:

$$LW_i\,(g) = LV_i \times \text{density}\,(g/L) \qquad \text{Equation 3.6}$$

where density is 1,080 g/L (Heinemann *et al.*, 1999).

3.2.1.3 Physiological Parameters

It was necessary to calculate cardiac output in order to estimate liver blood flow (for use in the well-stirred liver model; Section 3.3.2.1). The equation that describes cardiac index (CO m^{-2} BSA) as a function of age (for adults aged greater than 20 years) was based on the relationship reported by Guyton *et al.* (1973):

$$\text{Cardiac index}_i\,(l\,min^{-1}m^{-2}) = 3 + (-0.01(age_i\,(\text{years}) - 20)) \qquad \text{Equation 3.7}$$

Individual cardiac index values were then converted to CO by multiplying by BSA:

$$CO_i\,(l\,h^{-1}) = \text{cardiac index}_i\,(l\,min^{-1}m^{-2}) \times 60 \times BSA_i\,(l\,m^{-2}) \qquad \text{Equation 3.8}$$

Total CO was subsequently used to estimate the portal and hepatic artery blood flows (Q_{Port} and Q_{Art}, respectively) in each individual. Q_{Port} and Q_{Art} were assumed to constitute 6 and 21% of the total CO, respectively (Valetin, 2002). Total blood flow to the liver (Q_H) was calculated from the sum of Q_{Port} and Q_{Art}:

$$Q_H = Q_{Port} + Q_{Art} \qquad \text{Equation 3.9}$$

Table 3.3 Primary physiological parameters used in the IVIVE scaling process. The secondary parameters, estimated from the primary parameters, are also listed, together with references to the appropriate sections of this thesis.

Primary Parameter	Secondary Parameter	Section
LV	LW; V_{max}; $CLu_{H,int}$; $CLu_{G,int}$; F_H; F_G; $CL_{p.o./i.v.}$	3.2.1.2; 3.1.1.1; 3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.2.2; 3.3.3
Liver density	LW; V_{max}; $CLu_{H,int}$; $CLu_{G,int}$; F_H; F_G; $CL_{p.o./i.v.}$	3.2.1.2; 3.1.1.1; 3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.2.2; 3.3.3
LW	V_{max}; $CLu_{H,int}$; $CLu_{G,int}$; F_H; F_G; $CL_{p.o./i.v.}$	3.1.1.1; 3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.2.2; 3.3.3
MPPGL	V_{max}; $CLu_{H,int}$; $CLu_{G,int}$; F_H; F_G; $CL_{p.o./i.v.}$	3.1.1.1; 3.3.1.1; 3.3.1.2; 3.3.2.1; 3.3.2.2; 3.3.3
$[CYP]_H$ abund.	V_{max}; $CLu_{H,int}$; F_H; $CL_{p.o./i.v.}$	3.1.1.1; 3.3.1.1; 3.3.2.1; 3.3.3
$[CYP]_G$ abund.	V_{max}; $CLu_{G,int}$; F_G; $CL_{p.o.}$	3.1.1.1; 3.3.1.2; 3.3.2.2; 3.3.3
CI	CO; Q_{Port}; Q_{Art}; Q_H; F_H; $CL_{p.o./i.v.}$	3.2.1.3; 3.3.2.1; 3.3.3
CO	Q_{Port}; Q_{Art}; Q_H; F_H; $CL_{p.o./i.v.}$	3.2.1.3; 3.3.2.1; 3.3.3
Q_{Port}	Q_H; F_H; $CL_{p.o./i.v.}$	3.2.1.3; 3.3.2.1; 3.3.3
Q_{Art}	Q_H; F_H; $CL_{p.o./i.v.}$	3.2.1.3; 3.3.2.1; 3.3.3
Q_H	F_H; $CL_{p.o./i.v.}$	3.3.2.1; 3.3.3
$CLu_{H,int}$	F_H; $CL_{p.o./i.v.}$	3.3.2.1; 3.3.3
$CLu_{G,int}$	F_G ; $CL_{p.o.}$	3.3.2.2; 3.3.3
GFR	CL_R ; $CL_{p.o./i.v.}$	3.3.3
Creatinine CL	CL_R ; $CL_{p.o./i.v.}$	3.3.3
Renal Function	CL_R ; $CL_{p.o./i.v.}$	3.3.3
CL_R	$CL_{p.o./i.v.}$	3.3.3
Q_{gut}	F_G ; $CL_{p.o.}$	3.3.2.2; 3.3.3
F_H	$CL_{p.o.}$	3.3.3
F_G	$CL_{p.o.}$	3.3.3

MPPGL = milligrams microsomal protein per gram of liver; $[CYP]_H$ abund. = hepatic CYP abundances; $[CYP]_G$ abund. = gut CYP abundances; CI = cardiac index; CO = cardiac output; Q_{Port} = portal vein blood flow ; Q_{Art} = hepatic artery blood flow ; Q_H = hepatic blood flow; $CLu_{H,int}$ = unbound hepatic intrinsic clearance; $CLu_{G,int}$ = unbound gut intrinsic clearance; GFR = glomerular filtration rate.

3.3 Scaling of Human *in vivo* Clearance

3.3.1.1 Hepatic Metabolism

In order to scale intrinsic *in vitro* clearance data to whole liver values, a number of scaling factors are required such as enzyme abundances, liver weights and the number of milligrams of microsomal protein per gram of liver (MPPGL). The calculation of individual intrinsic hepatic clearance ($CLu_{H,int,i}$) was achieved using the following equation:

$$CLu_{H,int,i} = \left[\sum_{j=1}^{m}\left(\frac{ISEF_j \times CLu_{H,int}(rCYP_j) \times CYP_j\ abundance_i}{fu_{mic}}\right)\right] \times MPPGL_i \times LW_i \qquad \text{Eq 3.10}$$

where there are *j* CYPs with corresponding CLu_{int}(global) values, calculated from enzyme kinetic parameters for different pathways in each recombinant system (Section 3.1.1.1; Equation 3.2).

It is widely accepted that CYPs have different turnover numbers in recombinant systems and HLM. Accordingly, inter-system extrapolation factors (ISEFs) (Proctor *et al.*, 2004), which account for differences in intrinsic activity per unit enzyme, were applied to the in vitro metabolism data generated by rCYP systems. Median values specific to the expression system and each particular CYP were used. Where no CYP-specific data were available, the median ISEF value for that expression system was applied (Proctor *et al.*, 2004).

The CYPj abundance is the amount of the jth CYP enzyme per mg microsomal protein (pmol/mg) in the livers of each individual within the virtual population. As rates of metabolism arising from in vitro experiments in recombinant enzymes are expressed per unit enzyme (pmol P450), values for abundance are required to convert this value to the rate of metabolism per mg of microsomal protein.

Abundance values (pmolCYP per mg protein) for each enzyme in HLM (mean and coefficient of variation - CV) were derived from a meta-analysis of published data (Rowland-Yeo *et al.*, 2004) [mean values ($pmolmg^{-1}$): CYP1A2 (52), CYP2B6 (11), CYP2C8 (24), CYP2C9 (73), CYP2C18 (1), CYP2C19 (14), CYP2D6 (8), CYP2E1 (61), CYP3A (155)], and were used with their variabilities to generate values for each individual in the virtual population. The abundance of a particular CYP enzyme for a genotypic "poor metaboliser" (PM) was assumed to be zero (unless otherwise stated in Chapters 4 to 8). In the case of CYP2D6 ultra-rapid metabolisers

(UMs), the abundance of the enzyme was assumed to be twice that in extensive metabolisers (EMs). Frequencies of PM phenotypes for relevant CYP enzymes in Caucasians were accounted for, and abundance values were adjusted accordingly CYP2B6 (0.1), CYP2C9 (0.06), CYP2C19 (0.03), CYP2D6 (0.06)) and that for the CYP2D6 ultrarapid metabolizer (UM) phenotype (0.01).

MPPGL is required to scale the rate of metabolism per mg of microsomal protein to the rate per gram of liver. The rate per whole liver is obtained by multiplying the latter by liver weight (LW). The mean (29.3) and CV (25%) of MPPGL measured in 20 livers (Wilson *et al.*, 2003) was used to assign a value to each individual.

3.3.1.2 Gut Metabolism

Intestinal metabolism contributes significantly to the clearance of many CYP3A substrates (Masica *et al.*, 2004). Although other CYP enzymes such as CYP2C9 and CYP2D6 have been detected in the gut (Paine et al., 2006), the amounts are negligible compared to that of CYP3A (which contributes up to 82% of the total in the gut) (Paine *et al.*, 2006). Gut intrinsic clearance ($CLu_{G,int}$) was calculated using Equation 1.22:

$$CLu_{G,int,i} = \left(\frac{CLu_{int}(CYP3A) \times CYP3A\ abundance,\ i\ (gut)}{fu_{mic}} \right) \qquad \text{Equation 3.11}$$

where CLu_{int} and fu_{mic} are the *in vitro* parameters used to calculate hepatic clearance (Section 3.1.1).

The assumption that the intrinsic clearance per pmol CYP is the same in both gut and liver is supported by the observation that the rates of formation (per pmol CYP) of verapamil metabolites in HLM were not significantly different to those observed in intestinal microsomes (Yang *et al.*, 2004). Because CYP3A abundance represents the total amount of enzyme in the gut (pmol/total gut), no additional scaling factors were required.

Using a mean value of 70 000 pmol P450/total gut (Paine *et al.*, 1997), CYP3A abundance in gut was calculated using the equation:

$$\text{Individual CYP3A gut abundance} = \text{Average CYP3A gut abundance} \times \frac{\text{Individual GSA}}{\text{Average GSA}} \qquad \text{Equation 3.12}$$

where GSA is the surface area of the small intestine, calculated by multiplying the lengths of the ileum, jejunum and duodenum [3.5, 2.5 and 0.205 meters, respectively (Martini, 1998) by their diameters [0.025, 0.025 and 0.033 meters, respectively (Marieb, 1997)] and by π (assuming the sections of gut are, on average, perfectly cylindrical). Variability was added to the mean values of the lengths of the three sections of gut (Martini, 1998).

3.3.2 *Liver and Gut Clearance Models*

3.3.2.1 Liver model

Several liver models have been developed for the estimation of hepatic clearance ($CL_{H,i}$) and the fraction of the amount of drug reaching the liver escaping hepatic metabolism (hepatic availability; $F_{H,i}$). These are the well-stirred model (Wilkinson & Shand, 1975), the parallel tube model and the dispersion model. The performance of each of these models is drug dependent. However, the well-stirred model appears to be the most consistent and is recommended for use when predicting *in vivo* drug clearance from *in vitro* data (Ito & Houston, 2004). Accordingly, this model was chosen for the present work.

In the well-stirred model, instantaneous and complete mixing of the drug is assumed to occur within the liver (Wilkinson & Shand, 1975):

$$CL_{H,i} = \frac{Q_{H,i} \times fu_{B,i} \times CLu_{H,int,i}}{Q_{H,i} + (fu_{B,i} \times CLu_{H,int,i})} \qquad \text{Equation 3.13}$$

$$F_{H,i} = \frac{Q_{H,i}}{Q_{H,i} + (fu_{B,i} \times CLu_{H,int,i})} \qquad \text{Equation 3.14}$$

$$E_{H,i} = \frac{fu_{B,i} \times CLu_{H,int,i}}{Q_{H,i} + (fu_{B,i} \times CLu_{H,int,i})} \qquad \text{Equation 3.15}$$

where $Q_{H,i}$ is the hepatic blood flow in the i^{th} individual (Section 3.2.1.3), $fu_{B,i}$ is the free fraction of drug in blood in each individual (Section 3.1.1.3) and $CLu_{H,int,i}$ is calculated as described in 3.1.1.1.

3.3.2.2 Gut model

To estimate the individual fraction of drug escaping metabolism in the intestinal wall (intestinal availability; $F_{G,i}$), a model of 'first-pass' gut metabolism (Rostami-Hodjegan & Tucker, 2002), similar to the 'well-stirred liver' model was used for CYP3A substrates (Equation 3.15).

The gut model, in contrast to the liver model incorporates a flow term (Q_{gut}), which represents a nominal blood flow and is a hybrid parameter reflecting drug absorption rate from the gut lumen, removal of drug from the enterocyte by the enterocytic blood supply, and the volume of enterocytes (Rostami-Hodjegan & Tucker, 2002). The free fraction of drug within the enterocyte is represented by the fu_{gut} term:

$$F_{G,i} = \frac{Q_{gut,i}}{Q_{gut,i} + (fu_{gut} \times CLu_{G,int,i})}$$ Equation 3.16

In the absence of any information on active drug uptake into the enterocyte, fu_{gut} was set at a default value of 1 (which assumes that there is insufficient time for binding to plasma protein or erythrocyte uptake before the drug is removed from the basolateral side of the enterocyte).

A Q_{gut} value of 20 l h^{-1} was calculated for midazolam based on *in vivo* data obtained after both intravenous and oral administration (Yang *et al.*, 2004). This value approximates the blood flow to enterocytes (10–15% of mesenteric blood flow). Assuming that a proportional relationship exists between Q_{gut} and permeability (P_{app}) obtained using Caco-2 cells, Q_{gut} values for other drugs were estimated by comparing their P_{app} at concentrations estimated to occur in the gut lumen with that of midazolam (data kindly provided by Simcyp Ltd). Any information on the affinity of the drug for P-glycoprotein was also taken account of when deciding on the value of Q_{gut} value. A default value of 10 l h^{-1} was assumed in the absence of any permeability data.

3.3.3 *Human Plasma Drug Clearance*

Renal clearance values were obtained from the literature or were calculated from the fraction excreted unchanged in the urine (fe) multiplied by $CL_{i.v.}$. Renal clearance was individualized by adjusting for renal function. This involved the estimation of creatinine clearance as follows (Cockcroft & Gault, 1976):

$$\text{Creatinine Clearance}_i = (140 - \text{Age}_i) \times \frac{BW_i}{70} \qquad \text{Equation 3.17}$$

where BW_i is the individual body weight.

Renal function was expressed as a function of creatinine clearance relative to average glomerular filtration rate (GFR = 120 ml min^{-1}; Rowland and Tozer, 1995):

$$\text{Renal Function}_i = \frac{\text{Creatinine clearance}_i}{GFR} \qquad \text{Equation 3.18}$$

Renal clearance value was then multiplied by renal function.

Total plasma clearances after oral and intravenous administration were calculated for each individual subject using the corresponding organ clearances and associated availabilities, after correction for B:P:

$$CL_{i.v.,i} = (CL_{H,i} + CL_{R,i}) \times B:P \qquad \text{Equation 3.19}$$

$$CL_{p.o.,i} = \left(\frac{CL_{H,i} + CL_{R,i}}{f_a \times F_{G,i} \times F_{H,i}}\right) \times B:P \qquad \text{Equation 3.20}$$

where f_a is the fraction of drug available for absorption from the dosage form and $CL_{R,i}$ is the individual renal clearance. In the absence of lower values indicated in the literature, a default value for f_a of 1, representing complete absorption from the gut lumen into the gut wall was assumed for each of the 5 drugs studied.

3.3.4 *Conversion of CL Into Rate Constants*

Individual values of $CL_{i.v.,i}$ were converted to elimination rate constants (k_{10}) for use in the pharmacokinetic-pharmacodynamic model (Equations 3.21 and 3.22). Two different approaches were applied, depending on the availability of data. Values of volume of distribution (mean and standard deviation) linked to bodyweight (V/kg) were available from the literature for *(S)*-warfarin, MDZ, OMZ and TLB and thus the following equation was used:

$$k_{10,i} = \frac{CL_{i.v,i}}{V_i \times BW_i} \qquad \text{Equation 3.21}$$

where V_i is the initial volume of distribution in the i^{th} individual.

However, for DEX, no data for V were available in this form. Since no correlation exists between V and CL (Chapter 4; Section 4.2.2.1), it was deemed appropriate to use a non-weight normalised value for V (taken from the literature) and the following equation was used:

$$k_{10,i} = \frac{CL_{i\,v,i}}{V_i}$$ Equation 3.22

3.4 Defining Parameter Distributions for Monte Carlo Simulation

Population values are generally considered to be log normally distributed (Aitchison & Brown, 1966). For such a distribution, the geometric mean (GM) and geometric standard deviation (GSD) represent the central tendency and variation, respectively. Algebraically, the GM and the median are identical for a log normal distribution. A 90% confidence interval can also then be calculated using μ and σ, the natural logarithms of GM and GSD. These values were determined from the weighted mean (WX) and overall SD as follows:

$$\mu = \ln \overline{x} - (0.5 * \sigma^2) \qquad \text{Equation 3.23}$$

$$\sigma = \sqrt{\ln(1 + CV^2)} \qquad \text{Equation 3.24}$$

where:

$$\text{Coefficient of variation (CV)} = \frac{SD}{\overline{x}} \qquad \text{Equation 3.25}$$

3.5 Reference Values for Validating Predicted Clearance

To evaluate predictions of clearance, the simulated outcome (predicted) was compared with experimental values (observed). However, in some cases, experimental values were obtained from several reports. These were combined to calculate an overall reference value, as described below.

Clearance data (oral and/or intravenous) were obtained from reported *in vivo* studies performed in healthy Caucasian subjects. Where data from more than one study were available for the same route of administration, weighted mean values (WX) were calculated using Equation 3.1 (Section 3.1.1.1) and the overall SD was calculated from the equation:

$$\text{Overall SD} = \sqrt{\frac{\text{Overall sum of squares}}{N}} \qquad \text{Equation 3.26}$$

where

$$\text{Overall sum of squares} = \sum_{j=1}^{J} \left[\left[(SD_j)^2 + (\bar{x}_j)^2\right] \times n_j\right] - N \cdot (WX)^2 \qquad \text{Equation 3.27}$$

where N is the total number of observations from all studies, and SD_j is the standard deviation from the *j*th study.

CHAPTER 4

The Propagation of Pharmacogenetic Differences in Cytochrome P450 into Pharmacokinetic & Pharmacodynamic Measures: The Example of CYP2D6 & Dextromethorphan

4 DEXTROMETHORPHAN

4.1 Introduction

Dextromethorphan (DEX) is widely available over-the-counter as a cough suppressant. Its efficacy has been confirmed in both clinical cough (Aylward *et al.*, 1984; Cass & Frederik, 1953; Cass & Frederik, 1956; Matthys *et al.*, 1983) and in experimental cough challenge studies (Grattan *et al.*, 1995; Kartunnen *et al.*, 1987). CYP2D6 is responsible for most of the *O*-demethylation of DEX to its active metabolite, dextrorphan (DOR). DOR then undergoes glucuronide formation (Barnhart, 1980) (Figure 4.1). DEX is also *N*-demethylated to form 3-methoxymorphinian (Figure 4.1). This pathway is primarily catalysed by CYP3A4 but CYPs 2C9 and 2C19 also contribute (Gorski *et al.*, 1994; Schmider *et al.*, 1997; Von Moltke *et al.*, 1998a; Von Moltke *et al.*, 1998b) (Figure 4.1). Both DOR and 3-methoxymorphinian are further metabolised to 3-hydroxymorphinian, by CYP3A4 and CYP2D6, respectively (Jacqz-Aigrain *et al.*, 1993) (Figure 4.1). DEX is commonly used as an *in vitro* probe to provide estimates of kinetic parameters for CYP2D6 (Tucker *et al.*, 2001). It has also been used widely in *in vivo* phenotyping studies of CYP2D6 and CYP3A4 (Evans & Relling, 1991; Streetman *et al.*, 2000).

As discussed in Chapter 2 (Section 2.4.3.1), a number of studies have investigated the effect of CYP2D6 phenotype on the pharmacokinetics and pharmacodynamics of DEX. At least three have observed a significant difference in the AUC of DEX between (i) CYP2D6 EM and PM phenotypes (Capon *et al.*, 1996) or, (ii) a group of EM individuals before and after treatment with quinidine to produce PM phenocopies (Abdul Manap *et al.*, 1999; Capon *et al.*, 1996; Pope *et al.*, 2004). However, studies have not detected differences in the antitussive AUEC of DEX between CYP2D6 phenotypes. (Abdul Manap *et al.*, 1999; Capon *et al.*, 1996). The reason for this disparity may be related to the relatively small size of the studies. Larger numbers of subjects may be necessary to observe differences in pharmacodynamics than in pharmacokinetics (Chapter 2; Section 2.4.2).

The aim of the present study was to simulate the pharmacokinetics and pharmacodynamics of DEX in virtual populations of human subjects. These simulations were used to estimate the power of *in vivo* studies to differentiate the

pharmacokinetics and pharmacodynamics of DEX between CYP2D6 phenotypes. Whereas DOR is generally considered to be pharmacologically active, there are some concerns regarding its potency and how this may affect the DEX concentration-response relationship. Because of this, the models described in this chapter were also used to investigate the outcome of studies in which the proportional contribution of DEX and DOR to the antitussive activity was altered. The sensitivity of study power to pharmacodynamic variability and to changes in the contribution of CYP2D6 to the overall elimination of DEX, were also investigated. The overall aim of the work was to explain the failure of *in vivo* studies to detect differences in the pharmacodynamics of DEX between CYP2D6 phenotypes by assessing the effect of several factors on study power.

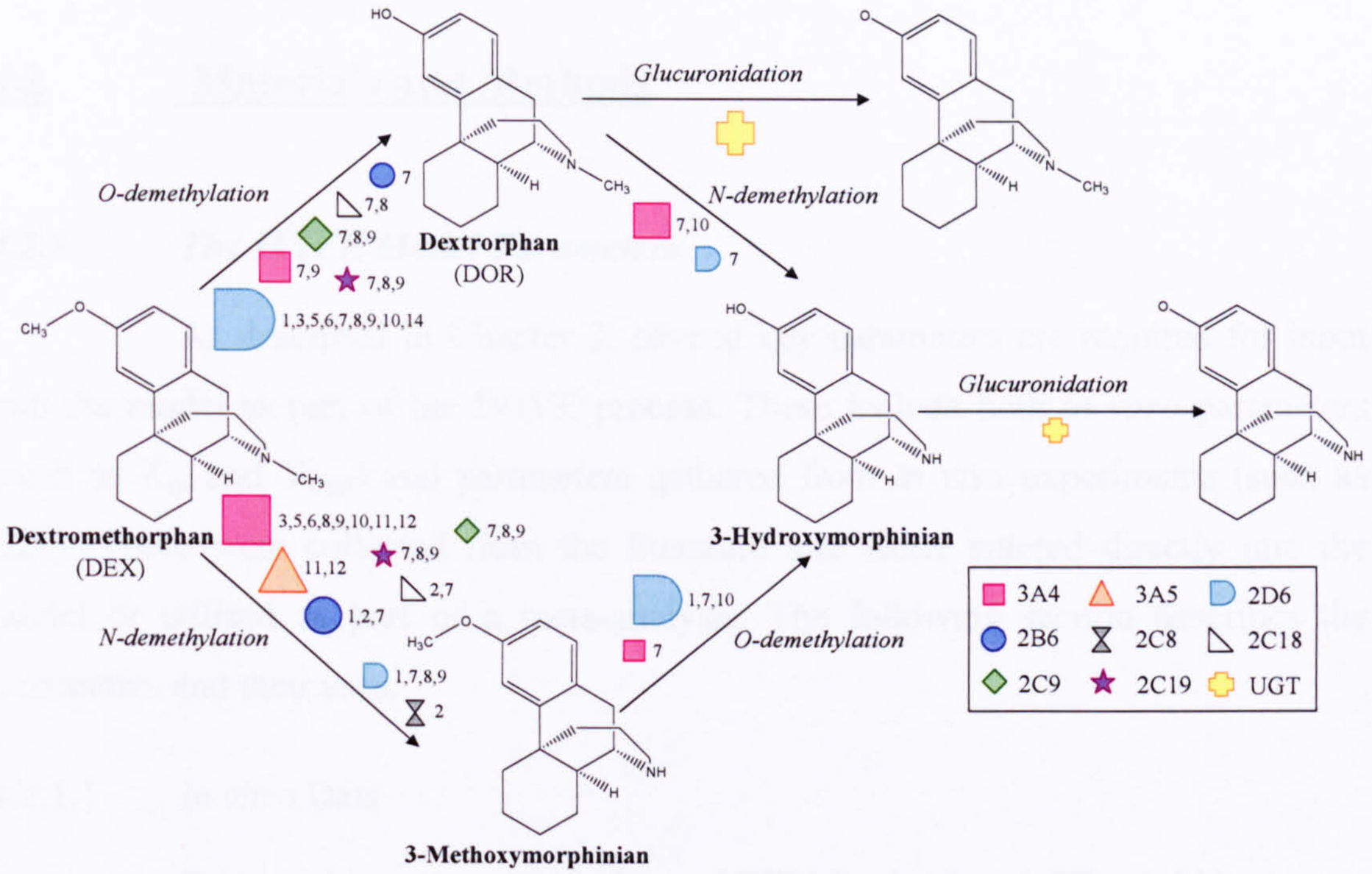


Figure 4.1 The metabolism of DEX in humans with all known pathways being shown. Each symbol represents an enzyme and the size of the symbol is indicative of the relative contribution of that enzyme to the metabolic route. Data were taken from Yu *et al.* (2001)[1], Wang & Unadkat (1999)[2], Schmider *et al.* (1997) [3], Schadel *et al.* (1995)[4], Jacqz-Aigrain *et al.* (1993)[5], Jacqz-Aigrain & Cresteil (1992)[6], Yu & Haining (2001)[7], Von Moltke *et al.* (1998b)[8], McGinnity *et al.* (2000)[9], Kerry *et al.* (1994)[10], Gorski *et al.* (1994)[11], Jones *et al.* (1996)[12], Lutz *et al.* (2004)[13], Ching *et al.* (1995)[14].

4.2 Materials and Methods

4.2.1 The IVIVE Model Parameters

As described in Chapter 3, several key parameters are required for input into the model as part of the IVIVE process. These include both *in vitro* parameters (such as K_m and V_{max}) and parameters gathered from *in vivo* experiments (such as CL_R). These were collected from the literature and either entered directly into the model or utilised as part of a meta-analysis. The following section describes the parameters and their uses.

4.2.1.1 *In vitro* Data

Data on the *in vitro* metabolism of DEX (in the form of K_m and V_{max}) were collected from published reports (Delaporte *et al.*, 2001; Hayhurst *et al.*, 2001; Mankowski *et al.*, 2000; Von Moltke *et al.*, 1998b; Yu *et al.*, 2001; Yu & Haining, 2001) (Table 4.1).

Intersystem extrapolation factors (ISEFs), were applied as described by Proctor *et al.,* (2004) to correct for any differences between the intrinsic activities of recombinantly expressed CYP enzymes and human liver microsomes (Table 4.2). To account for the non-specific binding of DEX to microsomal protein, a value of fu_{mic} was calculated and applied to the data as described in Chapter 3; Sections 3.1.1.2 and 3.3. Values of ISEF and fu_{mic} are shown in Table 4.2. A meta-analysis was then conducted to determine the overall V_{max} and K_m values for the metabolism of DEX by each CYP isoform.

4.2.1.2 Additional Parameters Required for IVIVE

The parameters listed in Table 4.3 were required for scaling the *in vitro* data to human whole body clearance and to the elimination rate constant (Chapter 3). These values were the entered into the pharmacokinetic-pharmacodynamic model described below (Sections 4.2.2 & 4.2.3).

Table 4.1 Parameters describing the *in vitro* metabolism of DEX.

		DOR formation		3-MM formation	
Reference	**CYP**	**Vmax**	**Km**	**Vmax**	**Km**
Von Moltke *et al.*, 1998	2D6	10.8	5.20	4.07	838
	2C9	7.16	229	2.10	254
	2C19	0.84	32.9	1.45	49.2
Mankowski *et al.*, 2000	2D6	36.0	0.70	-	-
Delaporte *et al.*, 2001	2D6	27.0	1.60	-	-
Hayhurst *et a*l., 2001	2D6	8.16	2.00	-	-
Yu *et al.*, 2001	2D6	8.50	1.90	-	-
Yu and Haining, 2001	2D6	11.9	3.70	38.5	1290
	3A4	5.40	157	21.3	232
	2B6	0.50	243	29.9	105
	2C9	3.40	222	1.00	343
	2C19	1.30	219	1.90	237
	2C18	2.40	453	17.2	354

DOR – dextrorphan; 3-MM – 3-methoxymorphinian; V_{max} - pmol/min/pmol P450; K_m – μM.

Table 4.2 ISEF and fu_{mic} values that were applied to the data shown in Table 4.1, resulting in revised V_{max} and K_m values. The final values entered into the IVIVE algorithms are indicated in bold type at the bottom of the table.

						DOR formation		**3-MM formation**	
Reference	Expression System	CYP	Protein Conc (mg/mL)	fu_{mic}	ISEF	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)
Von Moltke *et al.*, 1998	Lymphoblastoid	2D6	1	0.396	1.57	16.9	2.06	6.39	331.8
		2C9	1	0.396	6.82	48.8	90.7	14.3	100.6
		2C19	1	0.396	7.69	6.46	13.0	11.2	19.5
Mankowski *et al.*, 2000	Baculovirus	2D6	0.5	0.567	0.90	32.4	0.40	-	-
Delaporte *et al.*, 2001	Baculovirus	2D6	0.25	0.724	0.90	24.3	1.16	-	-
Hayhurst *et al.*, 2001	Yeast	2D6	0.25*	0.724	4.63	37.8	1.45	-	-
Yu *et al.*, 2001	Baculovirus	2D6	0.25	0.724	0.90	7.65	1.38	-	-
Yu and Haining,, 2001	Baculovirus	2D6	0.25	0.724	0.90	10.7	2.68	34.7	934.0
		3A4	0.25	0.724	0.34	1.84	113.7	7.24	168.0
		2B6	0.25	0.724	1.09	0.55	175.9	32.6	76.0
		2C9	0.25	0.724	2.64	8.98	160.7	2.64	248.3
		2C19	0.25	0.724	18.3	23.8	158.6	34.8	171.6
		2C18	0.25	0.724	1.09	2.62	328.0	18.7	256.3
Weighted Mean		**2D6**				**20.5**	**1.49**	**25.3**	**733.3**
		3A4				**1.84**	**113.7**	**7.24**	**168.0**
		2B6				**0.55**	**175.9**	**32.6**	**76.0**
		2C9				**22.3**	**137.4**	**6.5**	**199.1**
		2C19				**18.0**	**110.1**	**26.9**	**120.9**
		2C18				**2.62**	**328.0**	**18.7**	**256.3**

*A default value of 0.25 mg/mL was assumed as none was available (Chapter 3; Section 3.1.1.2).

Table 4.3 Mean pharmacokinetic parameter values for DEX obtained directly or calculated from the literature.

Parameter	Value	References
fu	0.5	Capon *et al.,* 1996 ; Section 3.1.1.3.
B:P	0.55	Default value† (Section 3.1.1.3).
fu_B	0.90	Section 3.1.1.3.
CL_R (L/h)	0.375	Barnhart, 1980; Moghadamnia *et al.*, 2003; Schadel *et al.*, 1995; Tenneze *et al.*, 1999; Section 3.3.3.
V (L)	961 (167)*	Moghadamnia *et al.,* 2003; Section 3.3.4.

*Mean (SD); fu = fraction unbound in plasma; B:P = Blood to Plasma concentration drug ratio; fu_B = fraction unbound in blood; CL_R = renal clearance; †No reported values were available, and thus, no distribution of drug into erythrocytes was assumed.

Table 4.4 Additional literature values describing the pharmacokinetics of DEX and DOR.

Parameter	**Value***	**Reference**
$V_{(DOR)}$ (L)	3533 (1201)	Unpublished data†
k_a (h-1)	2.6 (0.9)	Moghadamnia *et al.*, 2003
tlag (h)	0.8 (0.1)	Moghadamnia *et al.*, 2003
k_{12}	0.126 (2.14×10-4)	Unpublished data†
k_{21}	0.062 (4.32×10-5)	Unpublished data†
k_{ofDOR} (h-1)	0.51 (0.21)	Moghadamnia *et al.*, 2003

*Mean (SD); †From work carried out within the Department of Pharmacology – University of Sheffield

Table 4.5 Literature data describing the pharmacodynamics (antitussive effect) of DEX and DOR used in the modelling of response to DEX.

Parameter	**Value***	**Reference**
E_{max} (%)	37.7 (18.47) (CV = 49)	Moghadamnia *et al.*, 2003
EC_{50} (ng/mL)	3.2 (0.64) (CV = 20)	Moghadamnia *et al.*, 2003
n	3.9 (7.61) (CV = 195)	Moghadamnia *et al.*, 2003
k_{e0} (h^{-1})	0.41	Moghadamnia *et al.*, 2003
$Potency_{(DOR)}$ (%)	38 (26) (CV = 69)	Moghadamnia *et al.*, 2003
BaseCough (no. coughs)	10.7 (1.5)	Rostami-Hodjegan *et al.*, 2001
ScaleCough	7.56 (4.79)	Rostami-Hodjegan *et al.*, 2001
tlagCough (h)	1.09 (1.03)	Rostami-Hodjegan *et al.*, 2001
k_{cough} (males) (h^{-1})	0.29 (0.19)	Rostami-Hodjegan *et al.*, 2001
k_{cough} (females) (h^{-1})	0.34 (0.16)	Rostami-Hodjegan *et al.*, 2001

*Mean(SD)

4.2.2 The Pharmacokinetic Model

4.2.2.1 Concentrations of DEX in the Systemic Compartment

Plasma concentrations of DEX in each individual ($C(t)_i$) were generated using a two-compartment model with first order absorption and a lag time:

$$C(t)_i = \frac{f_a \cdot F_{Hi} \cdot F_{Gi} \cdot k_{ai} \cdot D}{V_i} \times (A1_i + A2_i + A3_i) \qquad \text{Equation 4.1}$$

Where f_a is the oral bioavailability of DEX (set to 1), F_{Hi} is the fraction of the amount of DEX reaching the liver escaping hepatic metabolism in each individual (calculated using Simcyp® algorithms; Equation 3.14; Chapter 3), D is the oral dose of DEX (30mg of DEX hydrochloride), $k_{a,i}$, and V_i are the first order absorption rate constant and the central volume of distribution in the i^{th} individual, respectively (Table 4.4). $A1_i$, $A2_i$ and $A3_i$ are given by:

$$A1_i = \frac{k_{21,i} - \alpha_i}{(k_{a,i} - \alpha_i) \cdot (\beta_i - \alpha_i)} \times e^{-\alpha_i \cdot (t - tlag_i)} \qquad \text{Equation 4.2}$$

$$A2_i = \frac{k_{21,i} - \beta_i}{(k_{a,i} - \beta_i) \cdot (\alpha_i - \beta_i)} \times e^{-\beta_i \cdot (t - tlag_i)} \qquad \text{Equation 4.3}$$

$$A3_i = \frac{k_{21,i} - k_{a,i}}{(\beta_i - k_{a,i}) \cdot (\alpha_i - k_{a,i})} \times e^{-k_{a,i} \cdot (t - tlag_i)} \qquad \text{Equation 4.4}$$

where $k_{21,i}$ and $k_{12,i}$ are the transfer rate constants from the peripheral to the central compartment and central to peripheral compartment, respectively and α_i and β_i are the hybrid rate constants associated with the distribution and elimination phases, respectively (Equations 4.5 and 4.6; Gabrielsson & Weiner, 2000).

$$\alpha_i = 0.5 \times \left[(k_{12,i} + k_{21,i} + k_{10,i}) - \sqrt{(k_{12,i} + k_{21,i} + k_{10,i})^2 - 4 \cdot k_{21,i} \cdot k_{10,i}} \right] \qquad \text{Equation 4.5}$$

$$\beta_i = 0.5 \times \left[(k_{12,i} + k_{21,i} + k_{10,i}) + \sqrt{(k_{12,i} + k_{21,i} + k_{10,i})^2 - 4 \cdot k_{21,i} \cdot k_{10,i}} \right] \qquad \text{Equation 4.6}$$

where $k_{10,i}$ is the individual elimination rate constant estimated using Simcyp® algorithms as outlined in Chapter 3.

Throughout this thesis, values of V for the compounds investigated are expressed in the form of weight normalised values (L/kg). However, in the case of DEX, such information was not available. If an absolute value of V (L) is used, this may lead to an unrealistic, lack of correlation between V and CL (since clearance is also related to body weight). This may result in possible over-estimation of the variability in kinetic parameters such as half life. Although Moghadamnia *et al.* (2003) did not report any weight normalised values of V, individual kinetic parameter values from that study were available to us. Therefore, a preliminary investigation was carried out to define any relationship between oral CL and V. No significant correlation was found (data not shown) and hence, absolute values of V (together with random variability) were incorporated into our simulations.

4.2.2.2 Concentrations of DOR in the Systemic Compartment

Equation 4.6 describes the plasma concentration – time course of DOR:

$$C(t)_{DOR,i} = C(t)_{DOR(1stpass),i} + C(t)_{DOR(systemic),i} \quad \text{Equation 4.7}$$

where $C(t)_{DOR(1st\ pass)}$ and $C(t)_{DOR(systemic)}$ are first-pass and systemic components described by Equations 4.7 and 4.12, respectively:

$$C(t)_{DOR(systemic),i} = \frac{D \cdot f_a \cdot F_{G,i} \cdot F_{H,i} \cdot fm_i \cdot k_{10,i} \cdot k_{a,i}}{V_{(DOR),i}} \times [A1m_i + A2m_i + A3m_i + A4m_i] \quad \text{Equation 4.8}$$

A value for $V_{(DOR)}$ was calculated as part of the studies by Moghadamnia *et al.* (2003) and although it was not provided in the published report, it was available to us (Table 4.4). The fraction of DEX converted to DOR after an *iv* bolus dose in the i^{th} individual (fm_i) was calculated from:

$$fm_i = \frac{CLm_{DOR,i}}{CL_i} \quad \text{Equation 4.9}$$

where $CLm_{DOR,i}$ is the metabolic clearance of DEX to DOR in the i^{th} individual, and CL_i is the total clearance of DEX (including renal clearance) in the i^{th} individual (both values were estimated using the Simcyp® algorithm). $A1m_i$, $A2m_i$, $A3m_i$ and $A4m_i$ are given by:

$$A1m_i = \frac{k_{21,i} - \alpha_i}{(k_{a,i} - \alpha_i) \cdot (\beta_i - \alpha_i) \cdot (k_{(DOR),i} - \alpha_i)} \times e^{-\alpha_i \cdot (t - lag_i)} \quad \text{Equation 4.10}$$

$$A2m_i = \frac{k_{21,i} - \beta_i}{(k_{a,i} - \beta_i) \cdot (\alpha_i - \beta_i) \cdot (k_{(DOR),i} - \beta_i)} \times e^{-\beta_i \cdot (t - lag_i)} \quad \text{Equation 4.11}$$

$$A3m_i = \frac{k_{21,i} - k_{a,i}}{(\beta_i - k_{a,i}) \cdot (\alpha_i - k_{a,i}) \cdot (k_{(DOR),i} - k_{a,i})} \times e^{-\beta_i \cdot (t - lag_i)} \quad \text{Equation 4.12}$$

$$A4m_i = \frac{k_{21,i} - k_{(DOR),i}}{(k_{a,i} - k_{(DOR),i}) \cdot (\beta_i - k_{(DOR),i}) \cdot (\alpha_i - k_{(DOR),i})} \times e^{-\beta_i \cdot (t - lag_i)} \quad \text{Equation 4.13}$$

where $k_{(DOR)i}$ is the elimination rate constant of DOR (Table 4.4).

The plasma concentration of DOR as a result of its first-pass formation is given by:

$$C(t)_{DOR(1stpass),i} = \frac{D \cdot k_{a,i} \cdot E_{HDOR,i}}{V_{(DOR),i}} \times \left(\frac{e^{-k_{(DOR),i} \cdot (t - tlag_i)}}{(k_{a,i} - k_{(DOR),i})} + \frac{e^{-k_{a,i} \cdot (t - tlag_i)}}{(k_{(DOR),i} - k_{a,i})} \right) \quad \text{Equation 4.14}$$

4.2.2.3 Concentrations of DEX in the Effect Compartment

The following equation was used to calculate the concentration of DEX in a hypothetical effect compartment:

$$Ce(t)(DEX)_i = \frac{f_a \cdot F_{Hi} \cdot F_{Gi} \cdot k_{ai} \cdot D \cdot k_{e0i}}{V_i} \times (A1_i + A2_i + A3_i + A4_i) \quad \text{Equation 4.15}$$

where k_{e0i} is the rate constant defining removal of DEX from the effect compartment (Table 4.5). $A1_i$, $A2_i$, $A3_i$ and $A4_i$ are given by:

$$A1_i = \frac{k_{21,i} - \alpha_i}{(k_{a,i} - \alpha_i)\cdot(\beta_i - \alpha_i)\cdot(k_{e0,i} - \alpha_i)} \times e^{-\alpha_i\cdot(t\text{-}tlag_i)}$$ Equation 4.16

$$A2_i = \frac{k_{21,i} - \beta_i}{(k_{a,i} - \beta_i)\cdot(\alpha_i - \beta_i)\cdot(k_{e0,i} - \beta_i)} \times e^{-\beta_i\cdot(t\text{-}tlag_i)}$$ Equation 4.17

$$A3_i = \frac{k_{21,i} - k_{a,i}}{(\beta_i - k_{a,i})\cdot(\alpha_i - k_{a,i})\cdot(k_{e0,i} - k_{a,i})} \times e^{-k_{a,i}\cdot(t\text{-}tlag_i)}$$ Equation 4.18

$$A4_i = \frac{k_{21,i} - k_{e0,i}}{(k_{a,i} - k_{e0,i})\cdot(\beta_i - k_{e0,i})\cdot(\alpha_i - k_{e0,i})} \times e^{-k_{e0,i}\cdot(t\text{-}tlag_i)}$$ Equation 4.19

4.2.2.4 Concentrations of DOR in the Effect Compartment

Equation 4.18 was used to calculate the concentration of DOR in the same hypothetical effect compartment as DEX:

$$Ce(t)_{DOR(sys),i} = \frac{D\cdot f_a\cdot F_{G,i}\cdot F_{H,i}\cdot k_{a,i}\cdot fm_i\cdot k_{10,i}\cdot k_{e0i}}{V_{(DOR)i}} \times (A1m_i + A2m_i + A3m_i + A4m_i + A5m_i)$$ 4.20

where the rate of removal of DOR from the effect compartment was assumed to be the same as that for DEX (k_{e0} - Table 4.5). $A1m_i$, $A2m_i$, $A3m_i$ and $A4m_i$ are given by:

$$A1m_i = \left[\frac{(k_{21,i} - \alpha_i)}{(k_{a,i} - \alpha_i)\cdot(\beta_i - \alpha_i)\cdot(k_{(DOR),i} - \alpha_i)\cdot(k_{e0,i} - \alpha_i)}\right] \times e^{-\alpha_i\cdot(t\text{-}tlag_i)}$$ Equation 4.21

$$A2m_i = \left[\frac{(k_{21,i} - \beta_i)}{(k_{a,i} - \beta_i)\cdot(\alpha_i - \beta_i)\cdot(k_{(DOR),i} - \beta_i)\cdot(k_{e0,i} - \beta_i)}\right] \times e^{-\beta_i\cdot(t\text{-}tlag_i)}$$ Equation 4.22

$$A3m_i = \left[\frac{(k_{21,i} - k_{a,i})}{(\alpha_i - k_{a,i})\cdot(\beta_i - k_{a,i})\cdot(k_{(DOR),i} - k_{a,i})\cdot(k_{e0,i} - k_{a,i})}\right] \times e^{-k_{a,i}\cdot(t\text{-}tlag_i)}$$ Equation 4.23

$$A4m_i = \left[\frac{(k_{21,i} - k_{(DOR),i})}{(k_{a,i} - k_{(DOR),i})\cdot(\beta_i - k_{(DOR),i})\cdot(\alpha_i - k_{(DOR),i})\cdot(k_{e0,i} - k_{(DOR),i})}\right] \times e^{-k_{(DOR),i}\cdot(t\text{-}tlag_i)}$$ Eq 4.24

$$A5m_i = \left[\frac{(k_{21,i} - k_{e0,i})}{(k_{a,i} - k_{e0,i}) \cdot (\beta_i - k_{e0,i}) \cdot (\alpha_i - k_{e0,i}) \cdot (k_{(DOR),i} - k_{e0,i})}\right] \times e^{-k_{e0,i} \cdot (t - tlag_i)} \quad \text{Equation 4.25}$$

The contribution of the first-pass formation of DOR to its effect compartment concentration is described by:

$$Ce(t)_{DOR(1stpass),i} = \frac{D \cdot k_{e0,i} \cdot k_{a,i} \cdot E_{HDOR,i}}{V_{(DOR),i}} \times B1_i + B2_i + B3_i \quad \text{Equation 4.26}$$

where:

$$B1_i = \frac{e^{k_{(DOR),i} \cdot (t - tlag_i)}}{(k_{a,i} - k_{(DOR),i}) + (k_{e0,i} - k_{(DOR),i})} \quad \text{Equation 4.27}$$

$$B2_i = \frac{e^{k_{a,i} \cdot (t - tlag_i)}}{(k_{(DOR),i} - k_{a,i}) + (k_{e0,i} - k_{a,i})} \quad \text{Equation 4.28}$$

$$B3_i = \frac{e^{k_{e0,i} \cdot (t - tlag_i)}}{(k_{(DOR),i} - k_{e0,i}) + (k_{a,i} - k_{e0,i})} \quad \text{Equation 4.29}$$

4.2.3 The Pharmacodynamic Model

4.2.3.1 Modelling of the Placebo Response

The placebo effect was differentiated from drug effect, and defined by Equation 4.30, as described by Rostami-Hodjegan *et al.* (2001). Values for $scale_i$, $baseline_i$, $tlag_i$(placebo) in the i^{th} individual and the appearance/disappearance rate constant (k_{coughi}) were included in the model (Table 4.5) and assigned random variability:

$$\text{Placebo effect}_i = \text{BaseCough}_i - (\text{ScaleCough}_i \times k_{cough}) \times [t - tlag_i\text{Cough}] \times e^{-k_{cough} \cdot (t - tlag_i\text{Cough})} \quad 4.30$$

where the first-order rate constant for nonlinear suppression of cough response and return to baseline, k_{cough}, was sex-dependent and was described by Equation 4.31 (Rostami-Hodjegan *et al.*, 2001):

$$k_{cough}(h^{-1}) = 0.049 \times \text{sex}(\text{male} = 1; \text{female} = 2) + 0.238 \quad \text{Equation 4.31}$$

4.2.3.2 Modelling of Overall Response

Concentrations of DEX and DOR were calculated in a hypothetical effect compartment (Equation 4.15, 4.20 and 4.26). The pharmacokinetic and pharmacodynamic data were linked assuming a sigmoidal E_{max} model such that the response to DEX in each individual (E_i) was described by Equation 4.32.

$$E_i = \text{placebo effect}_i \times \left[1 - E_{max,i} \times \frac{(Ce_i/EC_{50,i})^{n_i}}{1+(Ce_i/EC_{50,i})^{n_i}}\right] \qquad \text{Equation 4.32}$$

where Ce_i, E_{maxi}, and $EC_{50,i}$, and n_i are the concentration of the active moiety (DEX and/or DOR) (Equation 4.15, 4.20 and 4.26), maximal antitussive effect (Table 4.5), the concentration of active moiety in the effect compartment associated with half the E_{max} (Table 4.5), and the Hill-coefficient (Table 4.5), respectively, in each individual. The combined effects of DEX and DOR were modelled assuming competitive interaction at the same receptor site:

$$E_i = \text{placebo effect}_i \times \left[1 - E_{max,i} \times \frac{(Ce_{DEX,i}/EC_{50,i} + Pot_{DOR,i} \cdot Ce_{DOR,i}/EC_{50,i})^{n_i}}{1+(Ce_{DEX,i}/EC_{50,i} + Pot_{DOR,i} \cdot Ce_{DOR,i}/EC_{50,i})^{n_i}}\right] \qquad 4.33$$

where $Pot_{DOR,i}$ is the potency of DOR relative to DEX in the i^{th} individual [EC_{50}(DEX)/EC_{50}(DOR)] (Table 4.5). Based on the analysis of Moghadamnia *et al.*, (2003), the potency of DOR was assumed to be 0.38 (+/- 0.26) that of DEX in the default case. This value was varied to assess its effect on the discriminatory power of the different studies. A measurement error in the cough response (assumed to be a normal random distribution with a mean of zero and a standard deviation of 0.5) was used based on studies using the citric acid cough model (Rostami-Hodjegan *et al.*, 2001).

Due to the design of the pharmacokinetic-pharmacodynamic model, it was necessary to generate the pharmacodynamic effect (number of coughs) as a continuous output. However, since it is more realistic for the number of coughs to be integer values, the figure was rounded to the nearest whole number for the purposes of data analysis.

4.2.4 Study Design

Individual plasma DEX and DOR concentration and response *vs.* time profiles following the administration of DEX were simulated for all time points between 0 and 24h. For the purposes of data-analysis, samples were taken every hour (24 samples). The simulated profiles in EMs and PMs (with equal numbers of each phenotype in each study arm) were compared using populations sizes (n) of 2, 4, 6, 8, 22, 50, 100, 200 or 500. Twenty simulations were run for each study size.

4.2.5 Sensitivity of the Study Power to Changes in Drug Specific Parameters

In addition to the simulations and analysis described above (which will now be referred to as the 'default setting'); further simulations were carried out to assess the sensitivity of the study power to:

(a) the relative contribution of the polymorphic pathway to the overall metabolism of the parent compound (Section 4.2.5.1),

(b) the relative pharmacological activity of the polymorphically formed metabolite compared to parent compound (Section 4.2.5.2), and

(c) variability in pharmacodynamics (Section 4.2.5.3).

Overall, 3600 simulations were completed, involving more than 350,000 virtual subjects.

4.2.5.1 Contribution of the Polymorphic Pathway to Metabolism of the Parent Compound

The contribution of CYP2D6 to the overall metabolism of the drug was modified and metabolism was shifted to non-polymorphic pathways. This value was increased to 100% and then decreased to 50%. In the default setting, the CYP2D6 pathway catalysed about 90% of the metabolism of DEX. The Km and Vmax values for CYP2D6 and CYP3A4 were altered such that the CLint due to CYP2D6 increased whereas that due to CYP3A4 decreased, total CLint being made to remain the same.

4.2.5.2 The Relative Potency of Metabolite Compared to Parent Compound.

The relative activity of DOR used in the default setting was 38% that of DEX. The activity of DOR (formed *via* the polymorphic route) was then varied

between 0 (inactive) and 10,000%. The case where the metabolite is the only active compound was also investigated. The sensitivity of study power (to detect differences in the AUEC of the parent compound between CYP2D6 phenotypes) to the potency of the active metabolite was thereby observed.

4.2.5.3 Variability in Pharmacodynamics

In the default setting, the variability in the pharmacodynamic parameters ranged from 20 to 130%. Simulations were also carried out with this value changed to 30% and to 0%.

4.2.6 Data Analysis

Values of the area under plasma drug concentration-time (AUC) and effect-time (decrement in cough response) curves (AUEC) to 24 h were calculated using the trapezoidal rule. The probability of detecting statistically significant differences in pharmacokinetics and pharmacodynamics between CYP2D6 phenotypes was assessed using ANOVA. The number of studies from the 20 simulations that led to a statistically significant difference in AUC (or AUEC) between EMs and PMs was recorded as the power of that particular trial.

Concentration-time and effect-time profiles were compared with those observed *in vivo* by Abdul-Manap *et al.* (1999) and Capon *et al.* (1996). The accuracy of the simulated data was assessed subjectively by a visual comparison of the observed and the simulated concentration- and response-time curves, and statistically by comparing some of the key simulated parameter values with those observed *in vivo*.

4.3 Results

4.3.1 Model Validation

Comparisons of observed (Abdul Manap *et al.*, 1999) plasma concentration - time curves of DEX and DOR (in EMs and PMs) and a representative set of corresponding simulated data are shown in Figure 4.2 and Figure 4.3, respectively.

Figure 4.4 shows a comparison between the mean concentration-time curves of DEX from 10 clinical trial simulations and the mean data from the *in vivo* study (Abdul Manap *et al.*, 1999) in EMs and PMs. The corresponding plots for DOR are shown in Figure 4.5. A similar plot comparing the simulated concentrations with those observed by Capon *et al.,* (1996) is shown in Figure 4.6.

Comparisons of observed (Abdul Manap *et al.*, 1999) and simulated effect - time curves in EMs and PMs are shown in Figure 4.7 and Figure 4.8, respectively.

The comparison between the mean simulated effect-time curves from 10 simulations and the observed data (Abdul Manap *et al.*, 1999) in EMs and PMs is shown in Figure 4.9.

A summary of the mean (± SD) pharmacokinetic parameters that describe the data in EMs and PMs compared with those reported in the literature are given in Table 4.6.

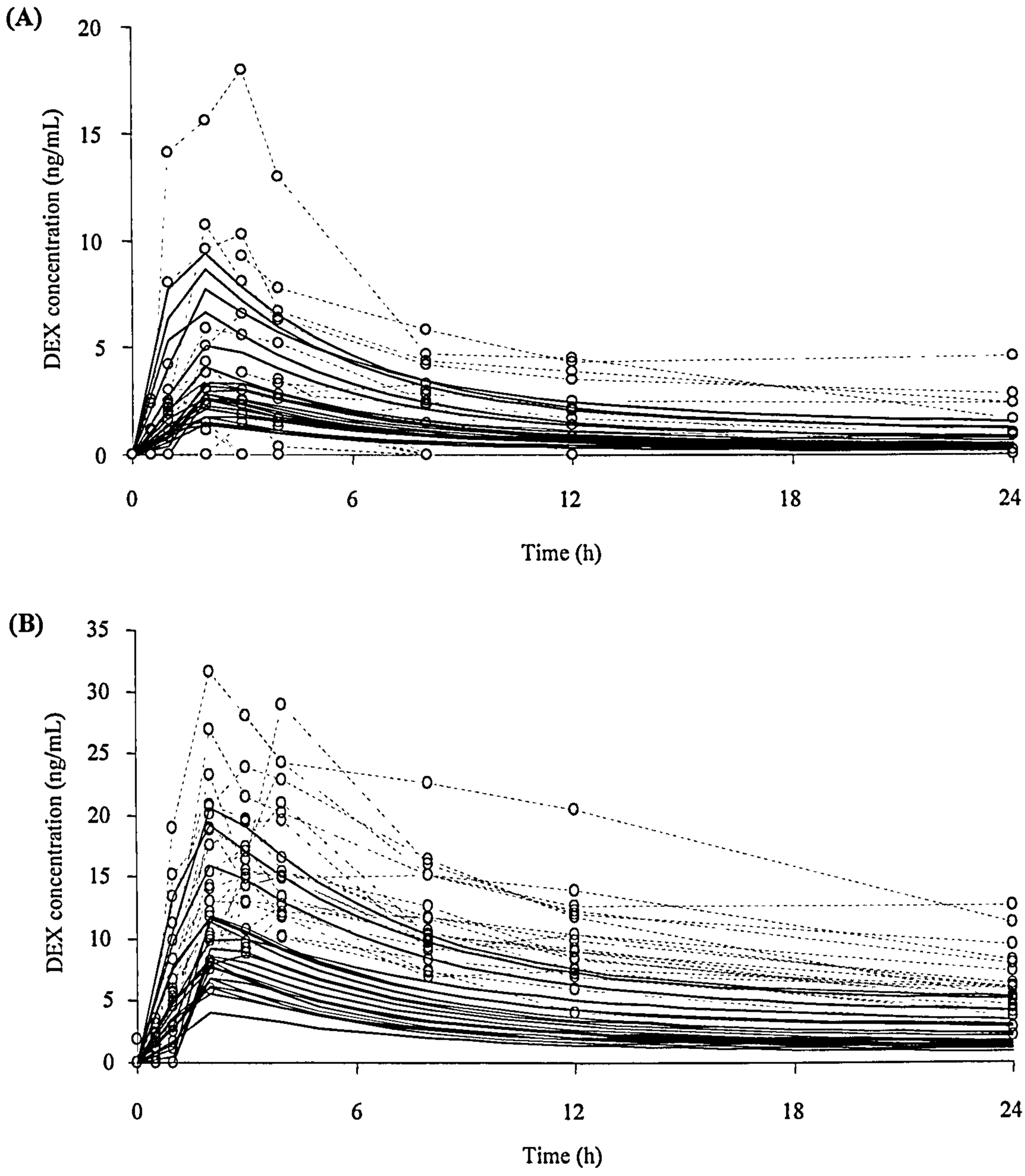


Figure 4.2 Observed (•••⊙••••) (Abdul Manap *et al.*, 1999) and predicted (——) plasma concentration-time profiles of DEX over 24 hours in (A) 22 real and 22 virtual EMs and (B) the same number of PMs.

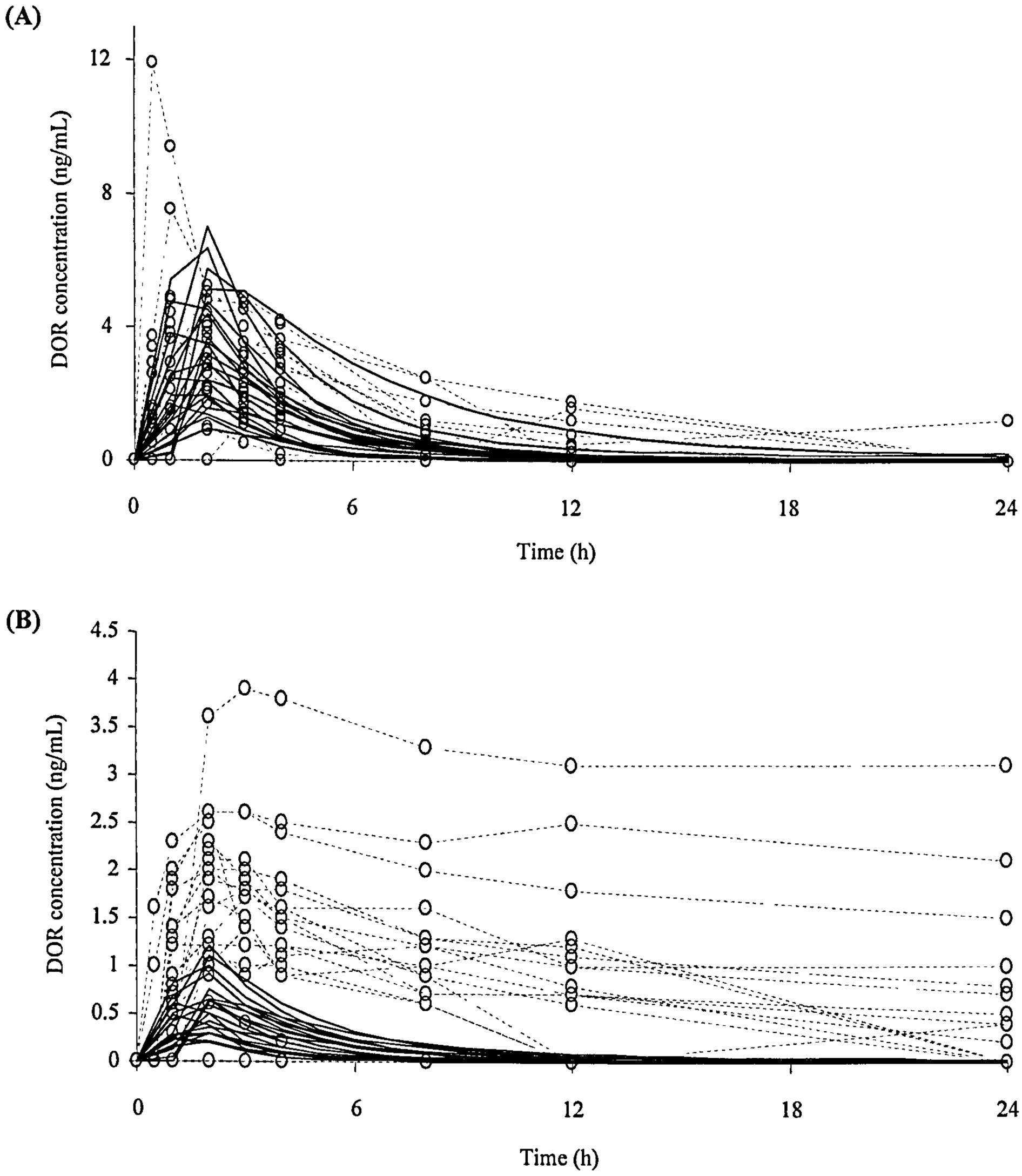


Figure 4.3 Observed (···⊙···) (Abdul Manap *et al.*, 1999)) and predicted (——) plasma concentration-time profiles of DOR over 24 hours in (A) 22 real and 22 virtual EMs and (B) the same number of PMs.

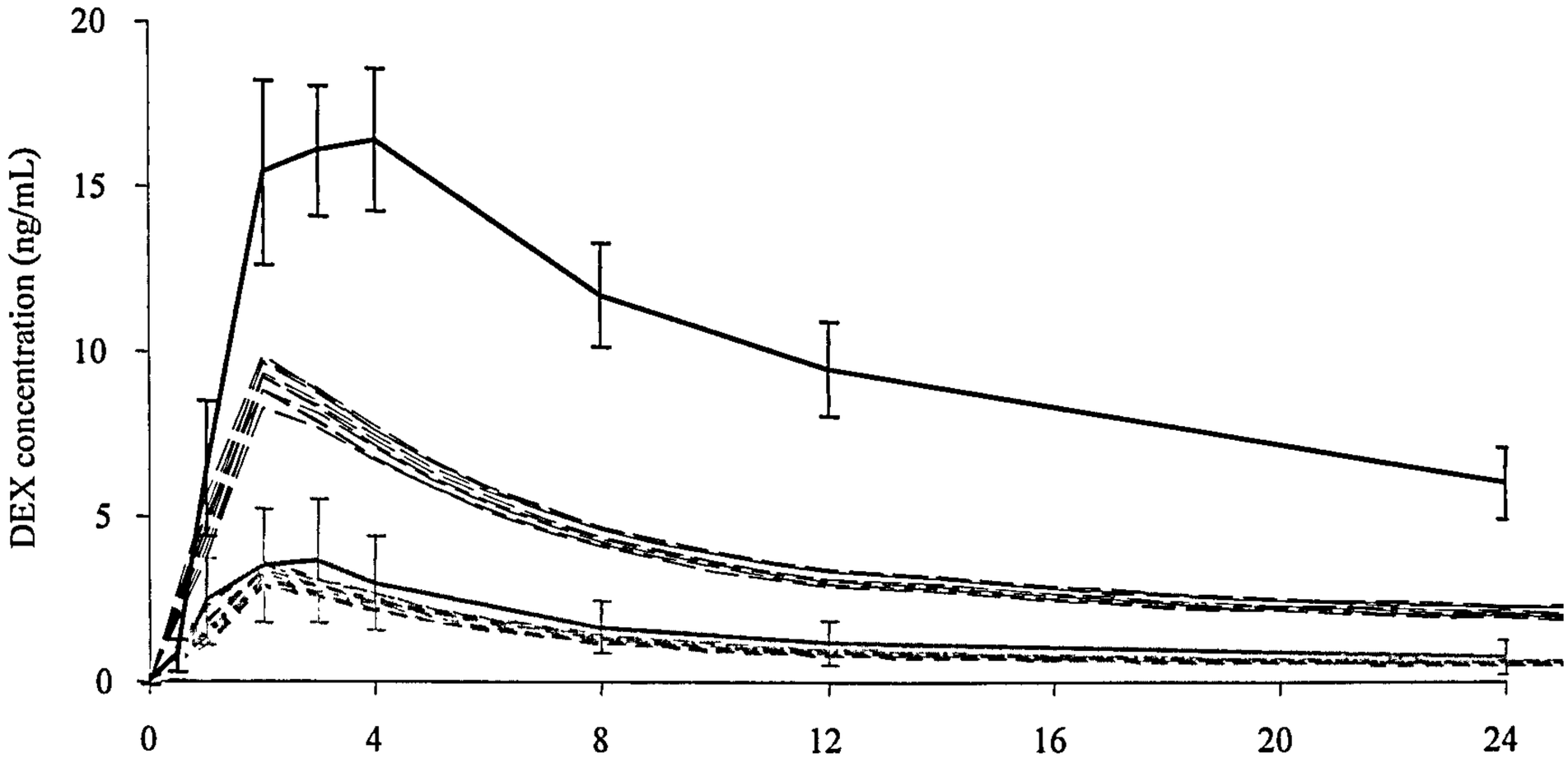


Figure 4.4 Observed (Abdul Manap *et al.*, 1999) and predicted mean concentration-time plots of DEX over 24 hours. The means of ten clinical trial simulations involving 22 EMs (– –) and 22 PMs (– –) are compared with the mean (± 95% CI) *in vivo* data from Abdul-Manap *et al.*, 1999 in EMs (——) and PMs (▬▬) (n = 22 of each).

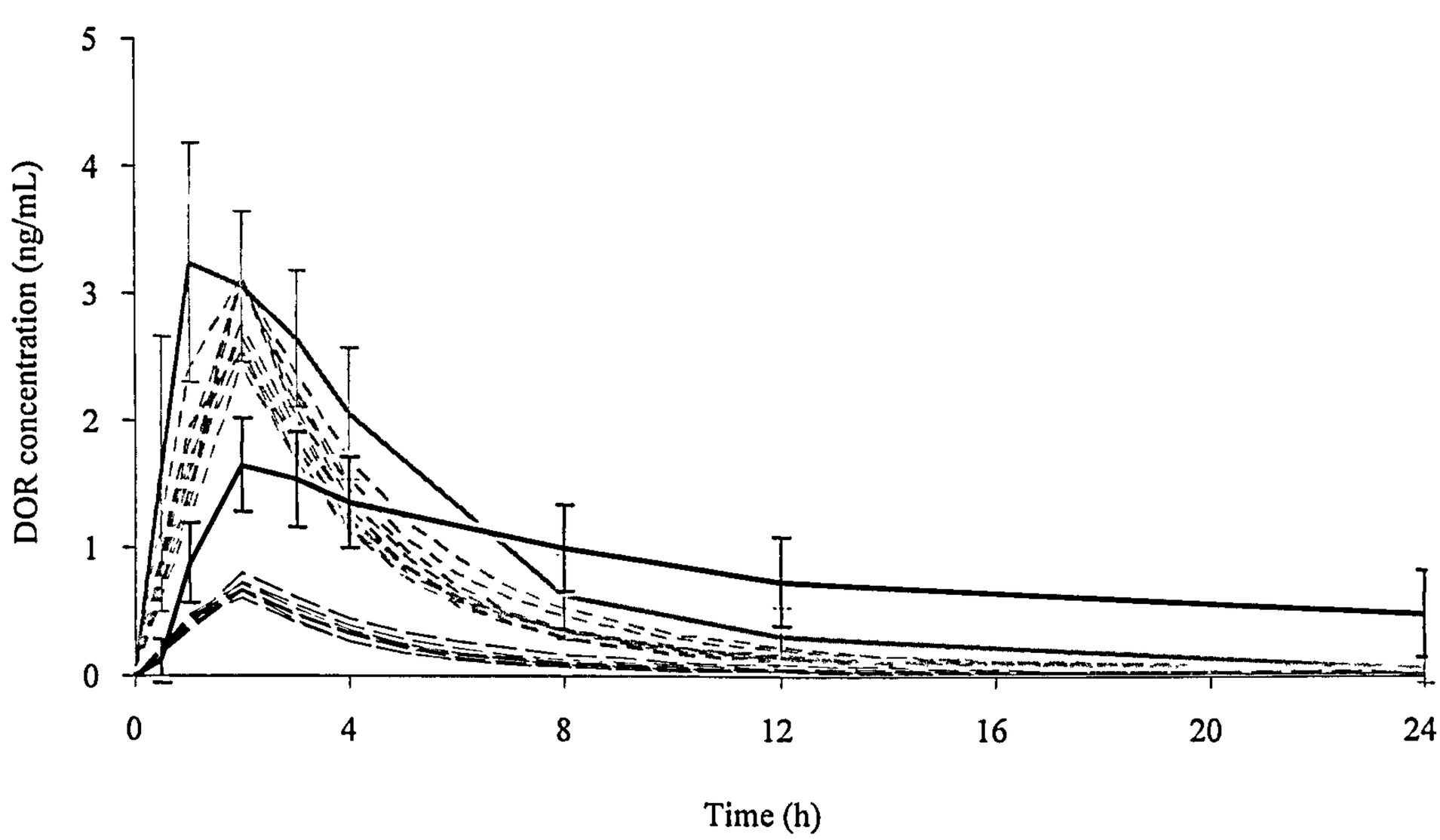


Figure 4.5 Observed (Abdul Manap *et al.*, 1999) and predicted mean concentration-time plots of DOR over 24 hours. The means of ten clinical trial simulations involving 22 EMs (– –) and 22 PMs (– –) are compared with the mean (± 95% CI) *in vivo* data from Abdul-Manap *et al.*, 1999 in EMs (——) and PMs (▬▬) (n = 22 of each).

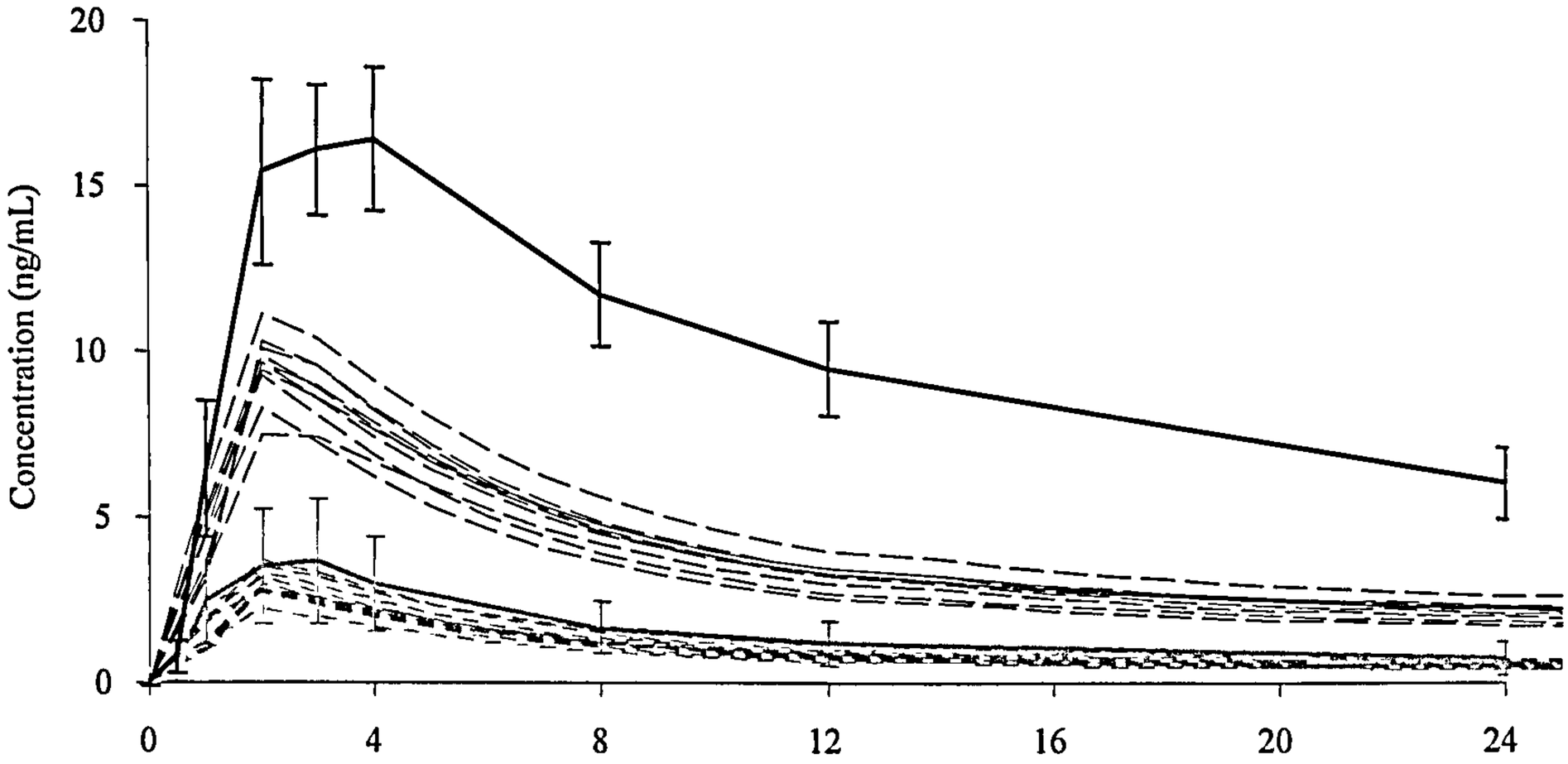


Figure 4.6 Observed (Capon *et al.*, 1996) and predicted mean concentration-time plots of DEX over 24 hours. The means of ten clinical trial simulations involving 6 EMs (– –) and 6 PMs (– –) are compared with the mean (± 95% CI) *in vivo* data from Capon *et al.*, 1996 in EMs (——) and PMs (——) (n = 6 of each).

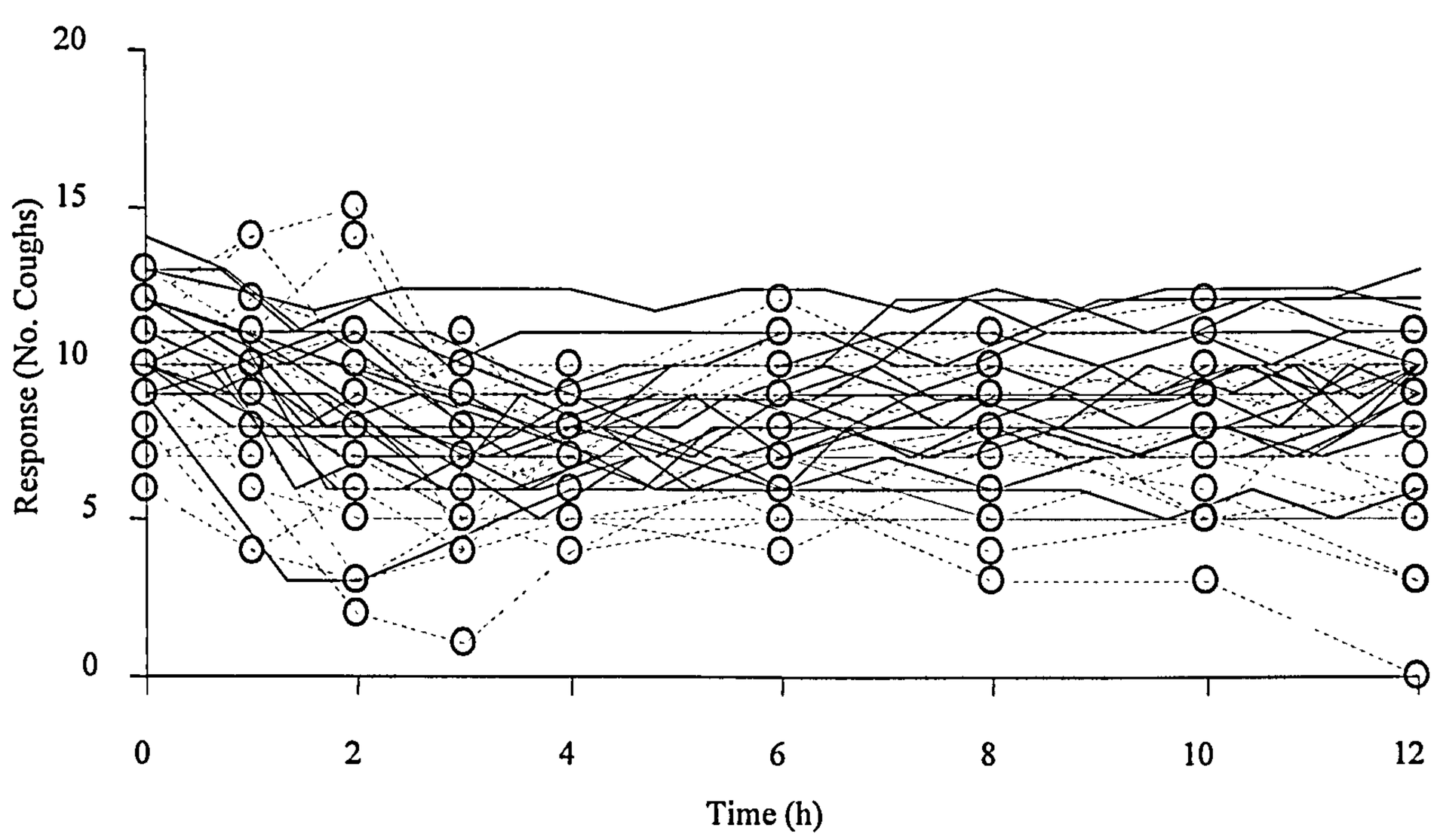


Figure 4.7 Observed (Abdul Manap *et al.*, 1999) and predicted effect-time profiles for cough suppression over 12h after administration of 30mg DEX hydrobromide in 22 real (——) and 22 virtual (····⊙····) EMs.

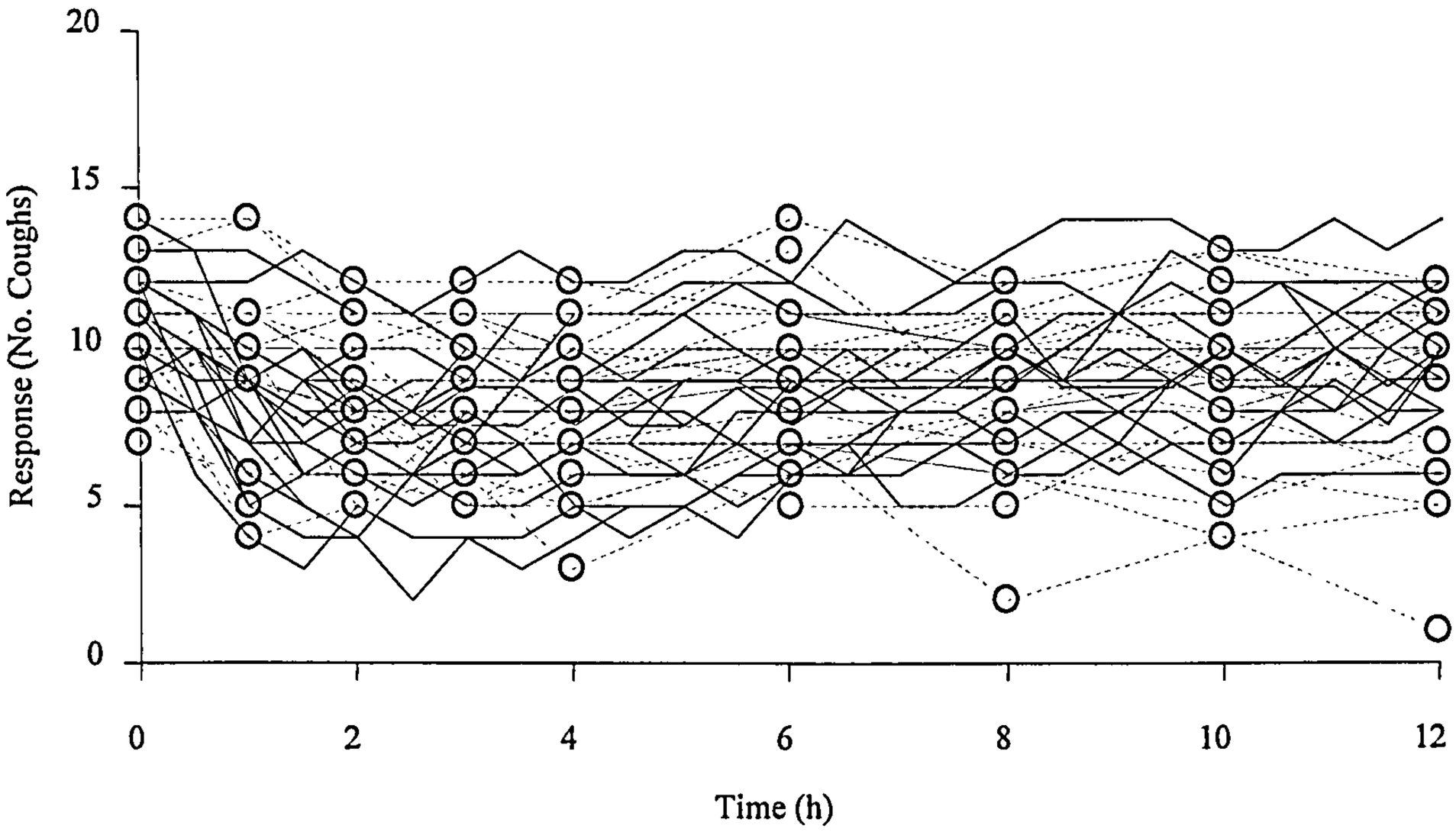


Figure 4.8 Observed (Abdul Manap *et al.*, 1999) and predicted effect-time profiles for cough suppression over 12h after administration of 30mg DEX hydrobromide in 22 real (——) and 22 virtual (····⊙····) PMs.

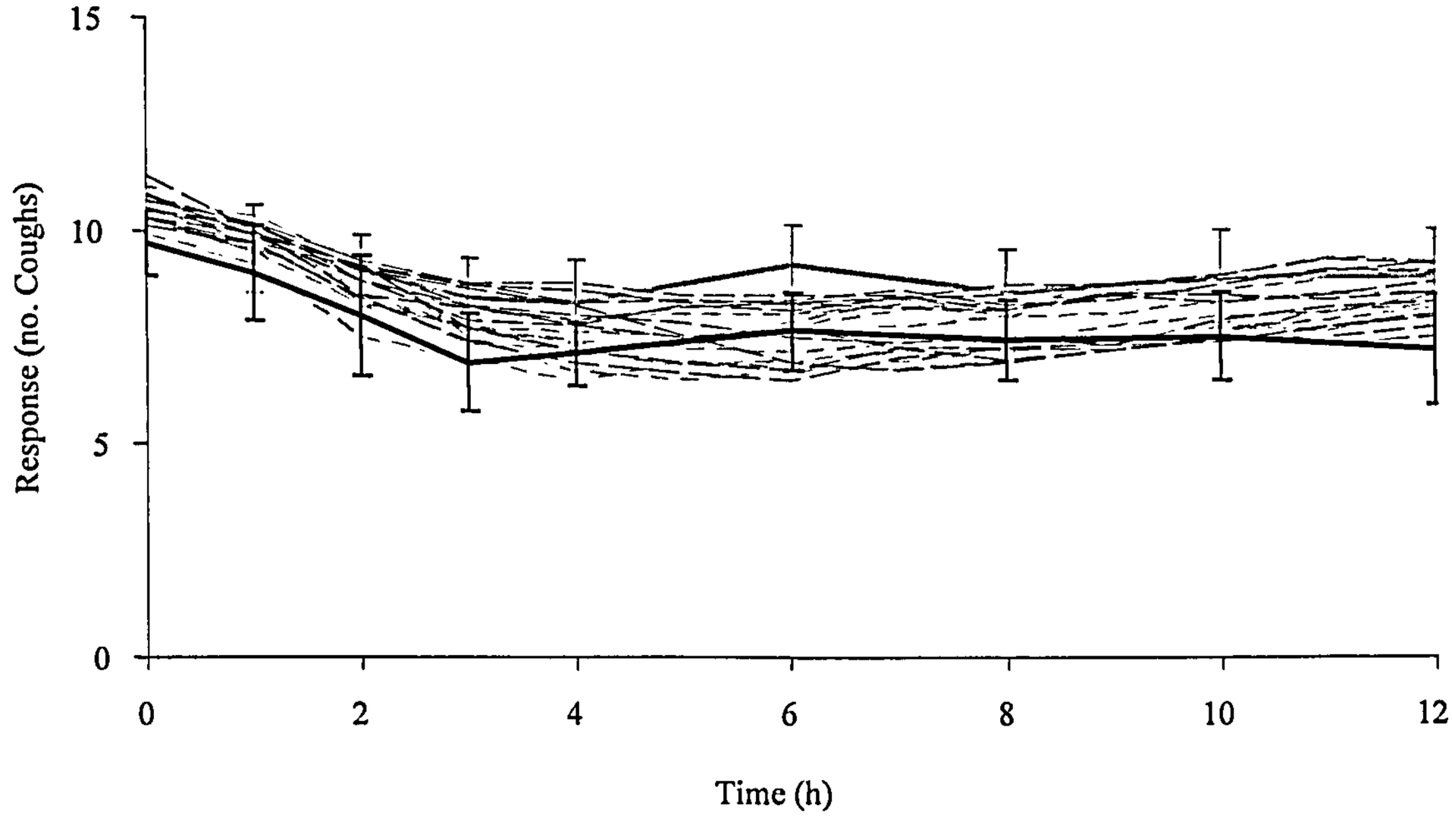


Figure 4.9 Observed (Abdul Manap *et al.*, 1999) and predicted mean effect-time plots over 12 hours. The means of ten clinical trial simulations involving 22 EMs (– –) and 22 PMs (– –) are compared with the mean (± 95% CI) *in vivo* data from Abdul-Manap *et al.*, 1999 in EMs (——) and PMs (——) (n = 22 of each).

Table 4.6 Observed (Abdul Manap *et al.*, 1999) and predicted pharmacokinetic and pharmacodynamic parameter values for DEX and DOR. Predicted values are the average of the mean values from 10 clinical trial simulations.

Phenotype	**Parameter**	**Predicted***	**Observed***
EM	t_{max} (h)	2.00 (0.40)	2.00 (1.70)
	C_{max} (ng/mL)	3.2 (3.2)	4.3 (4.4)
	AUC_{0-12} (ng/mL/h)	19.8 (15.3)	25.6 (28.2)
	$AUCm_{0-12}$ (ng/mL/h)	10.9 (7.5)	16.9 (10.3)
	CL (L/h)	71.6 (10.7)	75.2 (13.9)
	F_H	0.17 (0.07)	0.22 (0.15)
	AUEC	207 (52)	203 (174)
	E_{max}	10.5 (1.6)	10.5 (2.2)
PM	t_{max} (h)	2.00 (0.40)	3.00 (0.90)
	C_{max} (ng/mL)	9.2 (3.5)	18.6 (6.3)
	AUC_{0-12} (ng/mL/hr)	63.3 (27.4)	142.7 (42.4)
	$AUCm_{0-12}$ (ng/mL/hr)	2.81 (2.72)	12.82 (8.62)
	CL (L/h)	43.3 (12.8)	20.4 (9.8)
	F_H	0.53 (0.14)	0.78 (0.12)
	AUEC	208 (58)	299 (194)
	E_{max}	10.6 (2.2)	10.8 (1.7)

*Mean (SD)

4.3.2 Default Setting - Dextromethorphan

The simulations indicated that approximately 5 subjects would be adequate to achieve 80% power to detect a significant difference in the AUC of DEX between the two CYP2D6 phenotypes (Figure 4.10). A power of 100% was obtained when comparing 22 EMs with 22 PMs. The power to observe a difference in the AUEC of DEX between EMs and PMs was 75% at the maximum sample size investigated (500 of each phenotype) (Figure 4.10).

4.3.3 Contribution of the Polymorphic Pathway to Metabolism of the Parent Compound

Changing the proportional contribution of CYP2D6 to the overall metabolism of DEX from 90% in the default model to 50% caused a corresponding decrease in the power to detect a difference in antitussive effect between phenotypes to a maximum of 20% with a study size of 500. Increasing the proportional contribution of CYP2D6 to 100% increased the power to 80% for a study size of 90 of each phenotype (Figure 4.11 (B)). The corresponding plots for differentiating differences in pharmacokinetics between the phenotypes are shown in Figure 4.11 (A).

4.3.4 Changing the Relative Activity of Metabolite Compared to Parent Compound

When the primary metabolite was inactive or its relative potency was increased to 100 or 200%; the power of studies to determine a difference in response between the phenotypes remained relatively unchanged (Figure 4.12). However, when only the metabolite was assumed to be active, the power of studies to detect a statistically significant difference in antitussive effect between CYP2D6 EM and PM phenotypes was re-established. Increasing the relative potency to 1000% decreased the probability of discerning a difference in AUEC between phenotypes to between 0% and 5% for all study sizes (Figure 4.12).

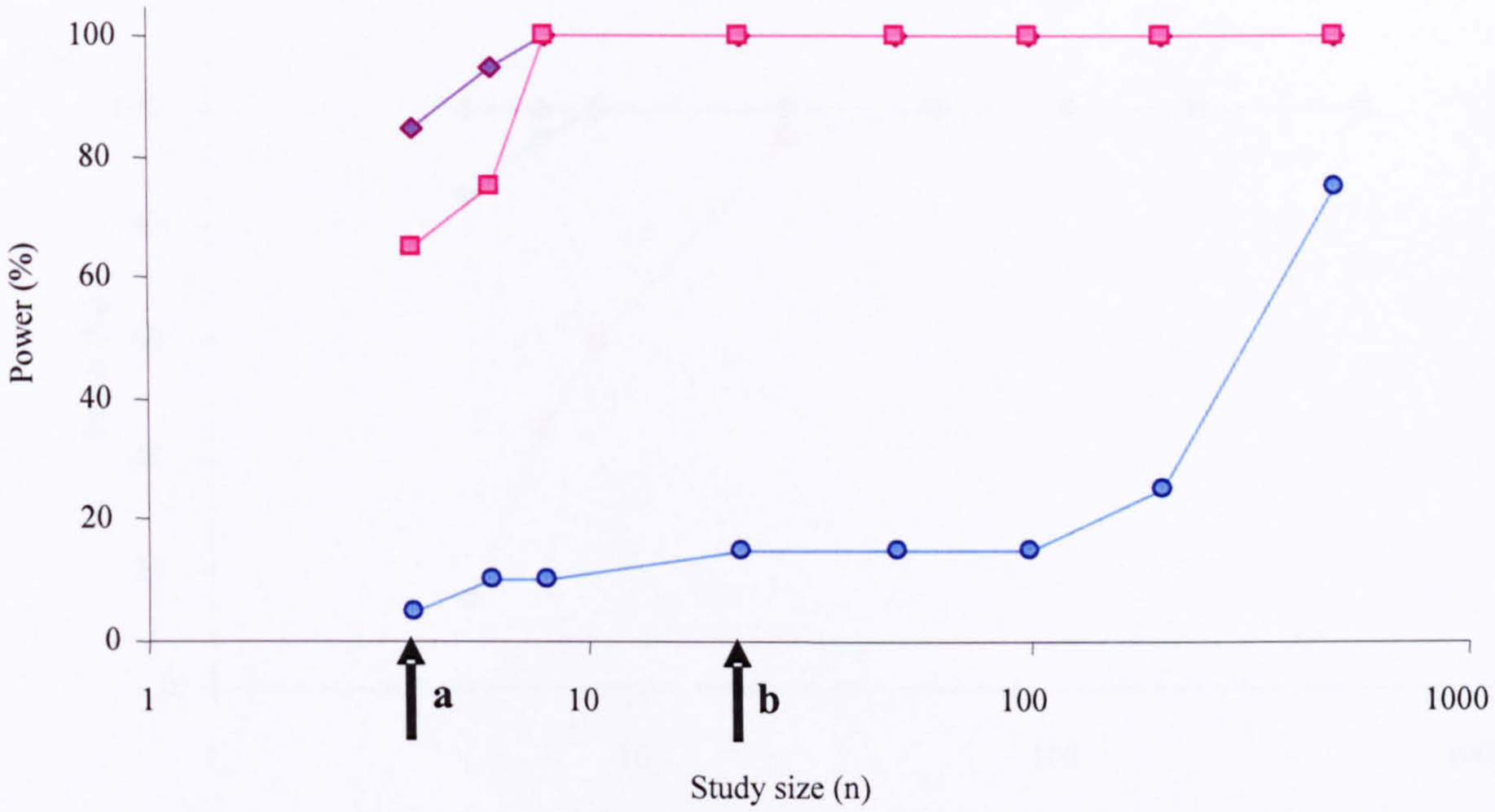


Figure 4.10 The power (%) of simulated studies to show significant differences in the AUC of DEX (◆) and DOR (■) and the AUEC (●) between CYP2D6 phenotypes *vs*. the number of subjects in each study arm (n). The arrows indicate the size of published studies: (a) Capon *et al*., (1999) and (b) Abdul-Manap *et al*., (2003).

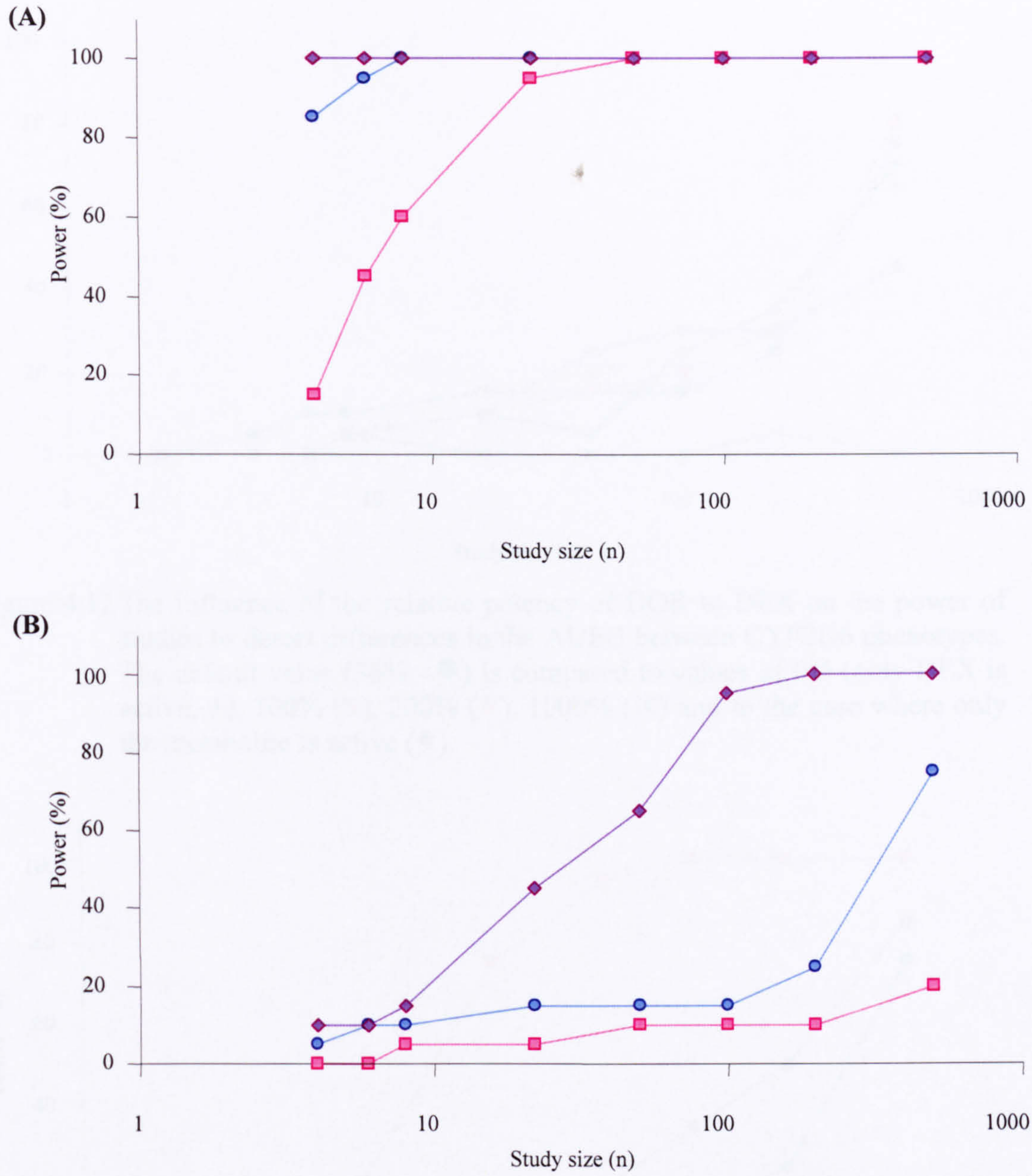


Figure 4.11 The influence of the proportional contribution of CYP2D6 (90% - ●; 50% - ■; 100% - ◆) to the overall clearance of parent drug on the power (%) to detect differences in (A) the AUC of DEX (B) The AUEC of DEX and DOR between CYP2D6 phenotypes.

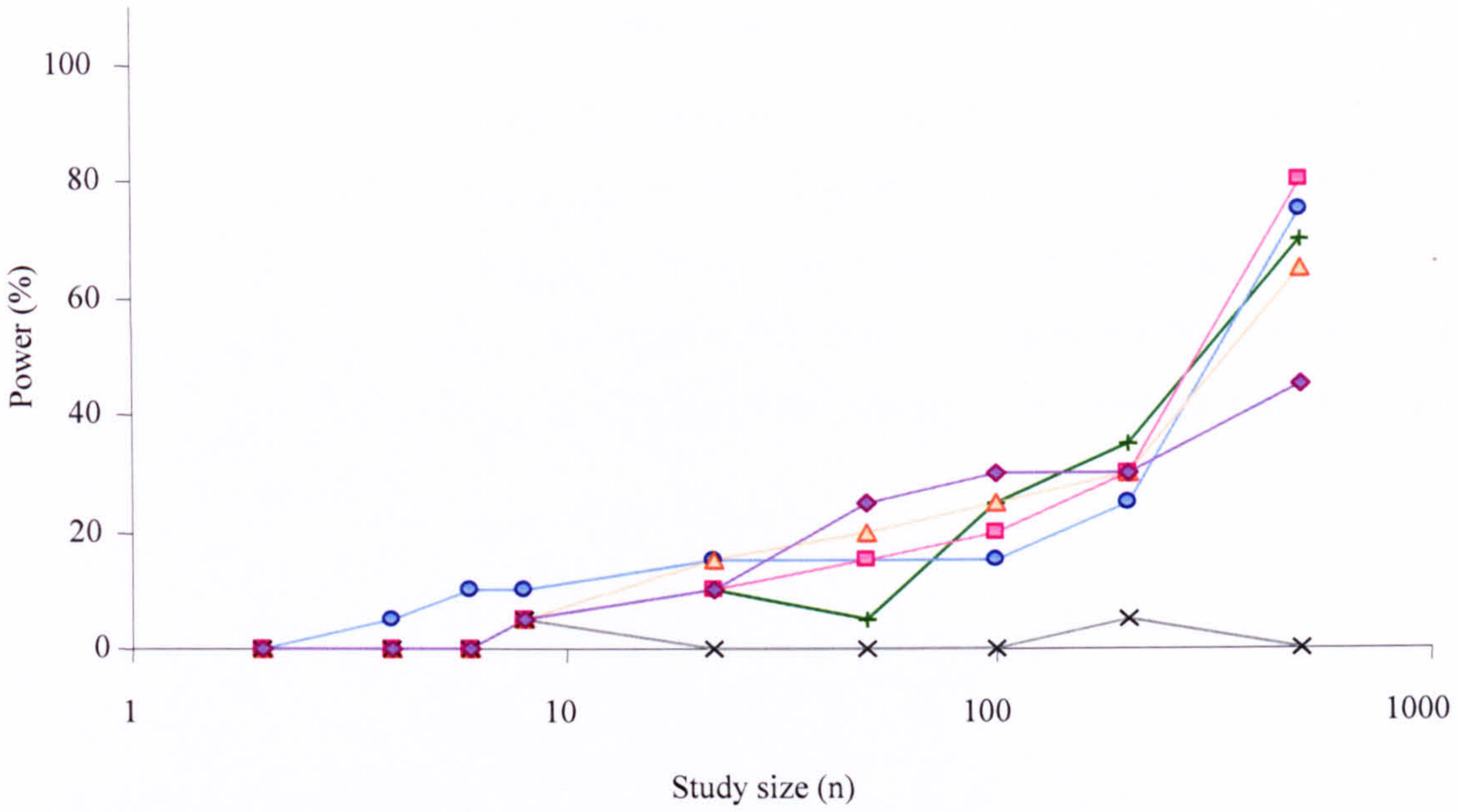


Figure 4.12 The influence of the relative potency of DOR to DEX on the power of studies to detect differences in the AUEC between CYP2D6 phenotypes. The default value (38% - ●) is compared to values of 0% (only DEX is active; +), 100% (■), 200% (△), 1000% (×) and to the case where only the metabolite is active (◆).

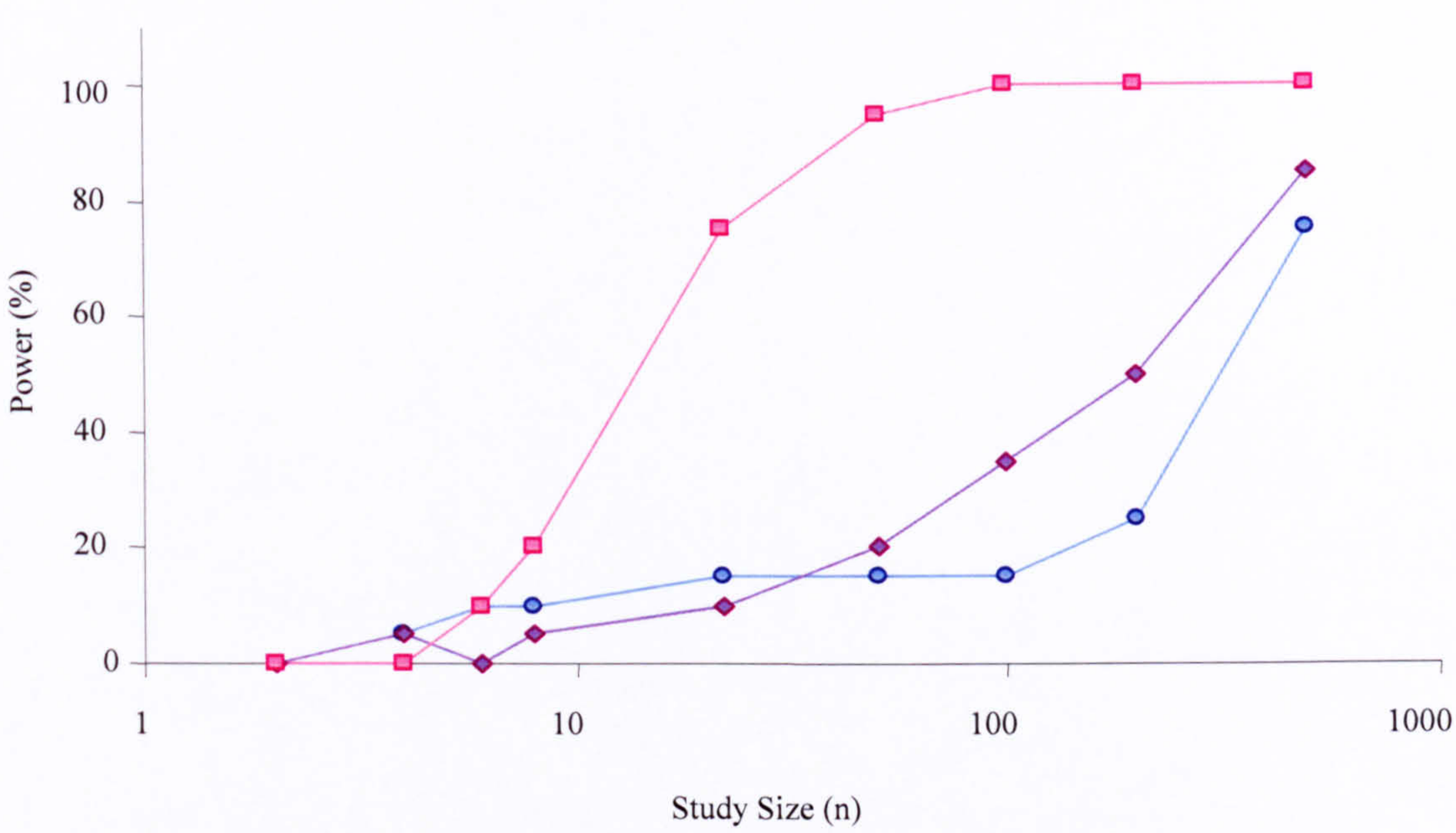


Figure 4.13 The influence of pharmacodynamic variability on the power (%) to detect differences in the AUEC of DEX and DOR between CYP2D6 phenotypes. In the default setting, variability on different pharmacodynamic parameters ranged from 14 to 195% (●); this variability was fixed at either 30% (◆) or 0% (■) to investigate the impact of such variability.

4.3.5 Variability in Pharmacodynamics

In the default model the variability in the pharmacodynamic parameters ranged from 14 to 195% according to the literature values. Assigning a fixed low variability of 30% to all pharmacodynamic parameters, while keeping measurement error unchanged, did not have an appreciable effect on study power (Figure 4.13). However, a further decrease to 0% allowed 80% power to be reached with a sample size of 25 (Figure 4.13).

4.4 Discussion

Using clinical trial simulations the power of *in vivo* studies to determine differences in the disposition of DEX and its antitussive effect between CYP2D6 phenotypes was examined. Using a metabolic clearance derived from *in vitro* data predicted the plasma concentration-time profiles with reasonable accuracy (within 2-fold) both in EM and in PM individuals, although those for PMs were underpredicted to some extent (Figure 4.2 to Figure 4.5). Nevertheless, the estimates of study power were consistent with the findings of published reports. Capon *et al.*, (1996) demonstrated a difference in the AUC of DEX between 6 EM and 6 PM subjects, but failed to detect a significant difference in antitussive response between these groups or between EMs and the same individuals phenocopied to PMs by administration of quinidine. Abdul Manap *et al.* (1999) carried out a similar study with a larger number of 22 EM subjects, who were phenocopied to PMs, and also detected a significant phenotypic difference in pharmacokinetics but not pharmacodynamics. The present work indicates that these two studies had powers of 95 and 100%, respectively, to detect differences in the pharmacokinetics of DEX between EMs and PMs, but powers of only 10 and 15%, respectively to detect differences in pharmacodynamics. Even when all DEX was assumed to be metabolised by CYP2D6 or when pharmacodynamic variability was zero, study sizes remained prohibitive with regard to detecting pharmacodynamic differences between phenotypes. The results suggest that 22 subjects would not have been sufficient to achieve acceptable statistical power in any of the conditions examined, and that between 30 and 500 subjects are necessary to detect significant differences in the AUEC.

The relative potency of DOR had only a small influence on the power of studies to detect differences in the AUEC of DEX. Since the circulating concentrations of DOR are different to those of the parent compound (see Figure 4.2 and Figure 4.3), the lowest power in discriminating between response to DEX does not necessarily occur when the two compounds are equipotent. For example, if a metabolite has 200% the activity of the parent compound but is present in the plasma at 50% of the concentration of the parent drug then the 'effective' concentration of the metabolite is likely to be similar to that of the parent drug, and therefore little difference will be seen in response between EMs and PMs. This is illustrated in Figure 4.12, which indicates

that the lowest power is observed when the metabolite is 10 times more potent than the parent drug.

It might have been expected that setting interindividual pharmacodynamic variability to zero would have resulted in a similar power to detect phenotypic differences in pharmacodynamics to that observed for pharmacokinetic differences. However, this was not the case (Figure 4.13), mainly for two reasons. First, in the absence of any interindividual differences in pharmacodynamic parameters, error is still associated with the measurement of response. Second, because the relationship between concentration and response is not linear, large differences in concentration will not translate to significant variation in response when the latter asymptotes to its maximum.

In conclusion, the results of this study have provided a basis for the discrepancy in the ability of *in vivo* studies to detect differences in the pharmacokinetics and pharmacodynamics of DEX between CYP2D6 phenotypes.

CHAPTER 5

The Propagation of Pharmacogenetic Differences in Cytochrome P450 into Pharmacokinetic & Pharmacodynamic Measures: The Example of CYP2C9 & *(S)*-Warfarin

5 WARFARIN

5.1 Introduction

In the UK warfarin is the anticoagulant treatment of choice for a variety of cardiovascular disorders (Greaves, 2005). Clinically, it is given as a racemic mixture of *(R)*- and *(S)*-warfarin. For the purposes of this work, the *(S)*-isomer of warfarin was assumed to be the major active moiety inhibiting blood coagulation (Breckenridge *et al.*, 1974; Eble *et al.*, 1966; O'Reilly, 1974) although a limited number of reports have suggested similar (or at least appreciable) activity by *(R)*-warfarin (Chan et al., 1994).

The effect of warfarin is mediated through the inhibition of vitamin-K reductases that are linked to the vitamin K-dependent carboxylation of glutamic residues on certain coagulation proteins (e.g. prothrombin) (Holford, 1986). This has the effect of decreasing the concentration of active prothrombin complex, which, is monitored using 'prothrombin time' (PT) (the time taken for blood to clot – after the addition of a thromboplastin-containing mix). The international normalised ratio (INR) was introduced as an alternative to PT in order to standardise the measurement of coagulation and is calculated using the following equation:

$$INR = \left(\frac{\text{patient PT}}{\text{GMNPT}}\right)^{ISI} \qquad \text{Equation 5.1}$$

where PT is the prothrombin time of the test plasma, GMNPT is the geometric mean normal PT, that is, the geometric mean PT of 20 healthy adult plasma samples and ISI is the international sensitivity index, a measure of the sensitivity of the thromboplastin used in that particular laboratory (Guidelines on oral anticoagulation: third edition, 1998).

It is usually recommended that INR values are kept within a therapeutic range of 2.0 to 3.0 (Guidelines on oral anticoagulation: third edition, 1998).

The use of warfarin is associated with a large number of adverse drug reactions (ADRs) which occur as a result of over-anticoagulation (Holford, 1986). These effects can be attributed to the very narrow therapeutic index of the drug and/or the wide interindividual variation in response resulting from similar doses of the drug.

Age (Gurwitz et al., 1992), body weight (Kamali et al., 2004), vitamin K intake (Kamali et al., 2000), concurrent medication (*e.g.* amiodarone) (Heimark et al., 1992; Naganuma et al., 2001) and genetic factors (Loebstein et al., 2001) are thought to contribute to this variability.

(S)-warfarin is metabolised by CYP2C9 (Takahashi et al., 1998). Polymorphisms in the *CYP2C9* gene have been implicated as an important source of genetic variation in the disposition of *(S)*-warfarin and therefore in anticoagulation response to the drug (Sconce *et al.*, 2005; Takahashi *et al.*, 2003; Taube *et al.*, 2000). There are a considerable number of retrospective studies that demonstrate a significant link between *CYP2C9* genotype and therapeutic outcome (e.g. INR or bleeding events) or maintenance dose requirements for warfarin treatment (for references, see summary in Table 5.1). However, observations from these studies are inconsistent, and have not established a similar relationship between *CYP2C9* genotype and the *in vivo* pharmacokinetics of the drug (Table 5.1). This disparity may be due to the small number of subjects employed in the pharmacokinetic studies, thus limiting their power.

More recently, several studies incorporating both *CYP2C9* and vitamin K epoxide reductase complex subunit 1 (*VKORC1*) genotypes have been published demonstrating that interindividual variation in dose requirements of warfarin can be further accounted for by incorporating the latter into regression models (Aquilante *et al.*, 2006; D'Andrea *et al.*, 2005; Li *et al.*, 2006; Tham *et al.*, 2006; Vecsler *et al.*, 2006).

The aim of the study presented in this chapter was to simulate the pharmacokinetics of *(S)*-warfarin and the anticoagulant response to the drug in virtual populations of human subjects. These simulations were used to mimic the *in vivo* studies and to estimate their power to identify differences in the pharmacokinetics and pharmacodynamics of *(S)*-warfarin between *CYP2C9* genotypes. The sensitivity of study power to changes in both the pharmacodynamic variability and the extent of the contribution of CYP2C9 to the overall metabolism of warfarin were also investigated. The ultimate aims of the work were to facilitate a more comprehensive understanding of the relationship between drug pharmacokinetics/pharmacodynamics and *CYP2C9* genotypes, using *(S)*-warfarin as a model drug, and to investigate the impact of study size on the likelihood of success in defining differences in the pharmacokinetics and response of *(S)*-warfarin between *CYP2C9* genotypes.

Assuming that adverse effects resulting from warfarin treatment are related to plasma drug concentrations, it might be beneficial to define a steady state concentration that is associated with a high likelihood of over-anticoagulation. To our knowledge, such information is not available because of practical and ethical issues that prevent the measurement of *(S)*-warfarin in plasma at the time of bleeding complications. Therefore, the IVIVE model has been utilised for the simulation of steady state concentrations in a population of individuals, some of whom experienced adverse effects. This information was then integrated into a threshold model of adverse reactions to warfarin therapy.

Table 5.1 A summary of studies investigating the relationship between *CYP2C9* genotype and warfarin response, maintenance dose and pharmacokinetics.

Relationship studied	References
CYP2C9 genotype and therapeutic outcome	Aithal *et al.*, 1999; Higashi *et al.*, 2002; Joffe *et al.*, 2004; Khan *et al.*, 2004; Lindh *et al.*, 2005; Loebstein *et al.*, 2001; Margaglione *et al.*, 2000; Peyvandi *et al.*, 2004; Taube *et al.*, 2000.
CYP2C9 genotype and maintenance dose	Aithal *et al.*, 1999; Higashi *et al.*, 2002; Hillman *et al.*, 2004; Joffe *et al.*, 2004; Kamali *et al.*, 2004; Khan *et al.*, 2004; King *et al.*, 2004; Margaglione *et al.*, 2000; Peyvandi *et al.*, 2004; Sconce *et al.*, 2005; Scordo *et al.*, 2002; Siguret *et al.*, 2004; Tabrizi *et al.*, 2002; Taube *et al.*, 2000.
CYP2C9 genotype and *(S)*-warfarin pharmacokinetics	Kamali *et al.*, 2004; Sconce *et al.*, 2005; Scordo *et al.*, 2002; Takahashi *et al.*, 2003.

5.2 Materials and Methods

5.2.1 The IVIVE Model Parameters

As described in Chapter 3, as part of the IVIVE process, several key parameters are required for input into the model. These include both *in vitro* parameters (such as K_m and V_{max}) and parameters gathered from *in vivo* experiments (such as CL_R). These were collected from the literature and either entered directly into the model or utilised as part of a meta-analysis. The following section describes the parameters and their uses.

5.2.1.1 *In Vitro* Data

Data on the *in vitro* metabolism of *(S)*-warfarin (in the form of K_m and V_{max}) were collected from published reports (Sullivan-Klose et al., 1996; Takahashi et al., 1999; Takahashi et al., 1998; Takanashi et al., 2000; Yamazaki et al., 1998) (Table 5.2).

Intersystem extrapolation factors (ISEFs), were applied as described by Proctor et al. (2004) to account for any differences between the intrinsic activity of recombinantly expressed CYP enzymes and human liver microsomes (Table 5.3). To account for the non-specific binding of *(S)*-warfarin in the experiments described in Table 5.2, a value of fu_{mic} was calculated and applied to the data as described in Chapter 3; Sections 3.1.1.2 and 3.3. ISEF values and values of fu_{mic} are shown in Table 5.3. A meta-analysis was then conducted to find the overall V_{max} and K_m values for the metabolism of *(S)*-warfarin by CYP2C9.

5.2.1.2 Additional Parameters Required for IVIVE

The additional parameters listed in Table 5.4 were required for scaling the *in vitro* data to human whole body clearance and to the elimination rate constant (Chapter 3). These values were the entered into the pharmacokinetic-pharmacodynamic model described below (Sections 5.2.3 & 5.2.4).

Table 5.2 Parameters describing the *in vitro* metabolism of *(S)*-warfarin and the results of a meta-analysis to determine the relative activity of the enzyme in each of the *CYP2C9* genotypes. All studies were carried out in recombinant CYP2C9 expressed in yeast microsomes.

Allele	No. Observations	V_{max} (pmol/min/ pmol P450)	K_m (μM)	Change in CL_{int} (%)	Reference
*1	2	0.22	18		Yamazaki *et al.* (1998)
*1	3	0.1333	11.6		Sullivan-Klose *et al.* (1996)
*1	3	0.282	1.86		Takahashi *et al.* (1999)
*1	3	0.248	5.8		Takanashi *et al.* (2000)
*1	3	0.28	2.6		Takahashi *et al.* (1998)
*2	2	0.11	22	↓60.6	Yamazaki *et al.* (1998)
*2	3	0.1656	12.5	↑11.4	Sullivan-Klose *et al.* (1996)
*3	2	0.067	53	↓90.9	Yamazaki *et al.* (1998)
*3	3	0.181	92.3	↓83.9	Sullivan-Klose *et al.* (1996)
*3	3	0.111	21.6	↓87.7	Takanashi *et al.* (2000)
*3	3	0.067	10.4	↓94.1	Takahashi *et al.* (1998)

Table 5.3 ISEF and fu_{mic} values that were applied to the data shown in Table 5.2, resulting in the revised V_{max} and K_m values shown here. The final values entered into the IVIVE algorithms are indicated in bold type at the bottom of the table.

Reference	Protein Conc (mg/mL)	fu_{mic}	ISEF	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)
Yamazaki *et al.* (1998)	0.1	0.989	2.66	0.585	17.8
Sullivan-Klose *et al.* (1996)	0.25*	0.973	2.66	0.355	11.3
Takahashi *et al.* (1999)	0.1	0.989	2.66	0.750	1.84
Takanashi *et al.* (2000)	0.25	0.973	2.66	0.660	5.6
Takahashi *et al.* (1998)	0.1	0.989	2.66	0.745	2.6
Weighted Means				**0.62**	**7.12**

*A default value of 0.25 mg/mL was assumed if no specific value was available (Chapter 3; Section 3.1.1.2).

Table 5.4 Mean pharmacokinetic parameter values of *(S)*-warfarin taken or calculated from the literature.

Parameter	Value	References
fu	0.007	Abernethy et al., 1991; Chan *et al.*, 1994; Section 3.1.1.3.
B:P	0.55	Obach, 1999; Section 3.1.1.3.
fu_B	0.013	Section 3.1.1.3.
CL_R (L/h)	0.0032	Abernethy *et al.*, 1991; Heimark *et al.*, 1992; Section 3.3.3.
V (L/kg)	28.9 (5.5)*	Chan *et al.*, 1994; Section 3.3.4.

*Mean (SD); fu = fraction unbound in plasma; B:P = Blood to Plasma concentration drug ratio; fu_B = fraction unbound in blood; CL_R = renal clearance; V = initial volume of distribution.

5.2.2 Genotype Frequencies and Activities

A meta-analysis of the published literature was carried out to determine the relative activity of the enzyme expressed by each *CYP2C9* allele with respect to the *in vitro* metabolism of *(S)*-warfarin. Mean values of the activity of each allelic form were weighted for study size. As shown in Figure 5.1, percentage decreases in intrinsic clearance (CLu_{int}) with respect to wild type (*1/*1) enzyme were calculated assuming that the *in vitro* activities of heterologously expressed variant enzymes represented those in the respective homozygous genotype. Values of CLu_{int} in heterozygous genotypes were assumed to be the average of those for homozygotes (Figure 5.1). A summary of the studies included in the meta-analysis is given in Table 5.2 (Sullivan-Klose *et al.*, 1996; Takahashi *et al.*, 1999; Takahashi *et al.*, 1998; Takanashi *et al.*, 2000; Yamazaki *et al.*, 1998).

The prevalence of each of the 6 established, common *CYP2C9* genotypes in Caucasians were taken from a review by Lee *et al.* (2002) (Table 5.5). *(S)*-warfarin elimination was assumed to be mediated exclusively by CYP2C9 with a small contribution from renal clearance [0.0032 L/h in the average man (Abernethy *et al.*, 1991)]. This information was entered into a physiologically-based pharmacokinetic-pharmacodynamic model.

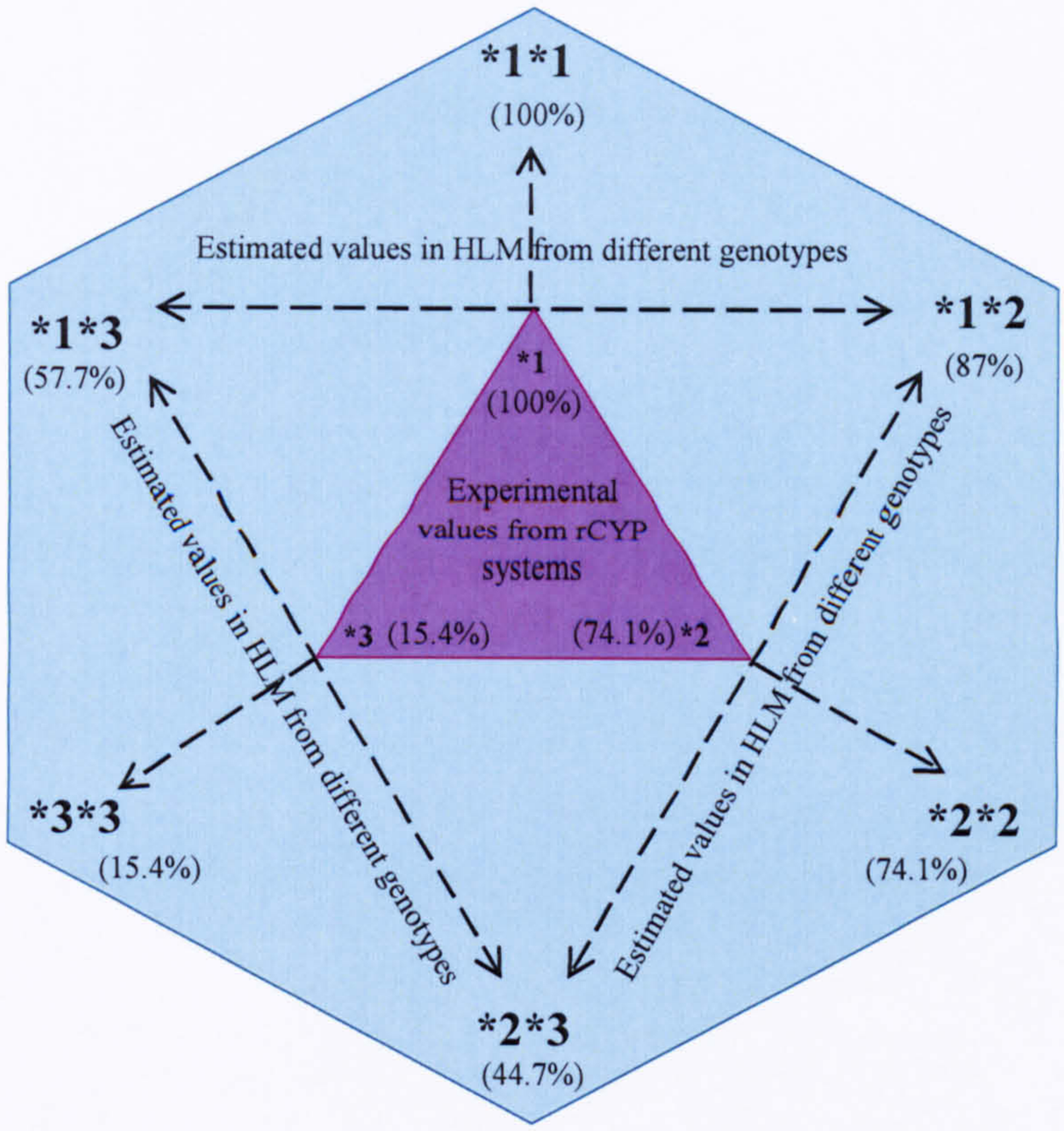


Figure 5.1 A schematic representation of the procedure to calculate the % reduction in CL_{int} for different *CYP2C9* genotypes compared to the wild type. The % CL_{int} of *(S)*-warfarin relative to *1*1 was calculated by assuming the *in vitro* activity of rCYPs represented the respective homozygous genotype in HLM.

Table 5.5 Frequency and relative enzyme activity of each of the common *CYP2C9* genotypes.

Genotype	Frequency (%)*	Relative Activity (%)†
*1/*1	65.3	100
*1/*2	20.4	85
*1/*3	11.6	55
*2/*2	0.9	70
*2/*3	1.4	40
*3/*3	0.4	10

*From (Lee *et al.*, 2002); †Calculated from a meta-analysis of literature data (Sullivan-Klose *et al.*, 1996; Takahashi *et al.*, 1999; Takahashi *et al.*, 1998; Takanashi *et al.*, 2000; Yamazaki *et al.*, 1998).

5.2.3 Development of the Pharmacokinetic Model

Plasma concentrations after oral administration of *rac*-warfarin (assuming that *(S)*-warfarin is responsible for 100% of the activity of the racemate) were generated by inputting values of the elimination rate constant ($k_{10,i}$) and $F_{H,i}$ (both calculated using Simcyp® algorithms; Chapter 3) into a one-compartment model with first order absorption and a lag time (adapted from Chan *et al.*, 1994).

Steady state plasma concentrations of *(S)*-warfarin after multiple doses of *rac*-warfarin, administered at a dosing interval (τ) of 24 hours, were generated using a one compartment model with first order absorption and a lag time (Chan *et al.*, 1994). Thus, values in the i^{th} individual were given by the equation:

$$C_{warf,i}(t) = \left[\frac{f_{a,i} \cdot F_{H,i} \cdot \left(\frac{D_i}{2}\right) \cdot k_{a,i}}{V_i \cdot BW_i \cdot (k_{a,i} - k_{10,i})}\right] \cdot \left(\frac{e^{-k_{10,i} \cdot (t - tlag_i)}}{1 - e^{-k_{10,i} \cdot \tau}}\right) - \left(\frac{e^{-k_{a,i} \cdot (t - tlag_i)}}{1 - e^{-k_{a,i} \cdot \tau}}\right) \qquad \text{Equation 5.2}$$

where $C_{warf,i}(t)$, tlag, $f_{a,i}$, $F_{H,i}$, D_i, $k_{a,i}$, V_i, BW_i and $k_{10,i}$ are, plasma drug concentration at time t, the lag time between administration and appearance of drug in the plasma (Table 5.6), the fraction of the dose absorbed (set to 1), the fraction escaping first-pass metabolism, the dose, a first-order absorption rate constant (Table 5.6), the steady state volume of distribution (Table 5.6), body weight and a first-order elimination rate constant in the i^{th} individual, respectively. The symbol, τ represents the dosing interval.

$F_{H,i}$, BW_i and $k_{10,i}$ were calculated using the Simcyp® algorithm (Chapter 3). Two different dosing regimens were used: (i) where a fixed dose was given to all virtual subjects, or (ii) D was defined as a distribution of likely doses that individuals would receive depending on their genotype (for detail see Section 5.2.5.3). All other parameter values were taken from Chan *et al.* (1996).

Table 5.6 Literature values describing the pharmacokinetics of *(S)*-warfarin.

Parameter	Value*	Reference
tlag (h)	0.8 (0.54)	Chan *et al.*, 1994
k_a (h^{-1})	3.15 (1.68)	Chan *et al.*, 1994
τ	24	Arbitrary

*Numbers in parenthesis indicate standard deviations.

Table 5.7 Different pharmacodynamic models for warfarin.

Model	References
Linear	Sheiner (1969)
Power	Wiegman & Vossepoel (1977)
Log-Linear	Nagashima *et al.* (1969); Svec *et al.* (1985); Theofanous & Barile (1973)
E_{max}	Abbrecht *et al.* (1982); Powers *et al.* (1980)
Sigmoidal E_{max}	Chan *et al.*, 1994

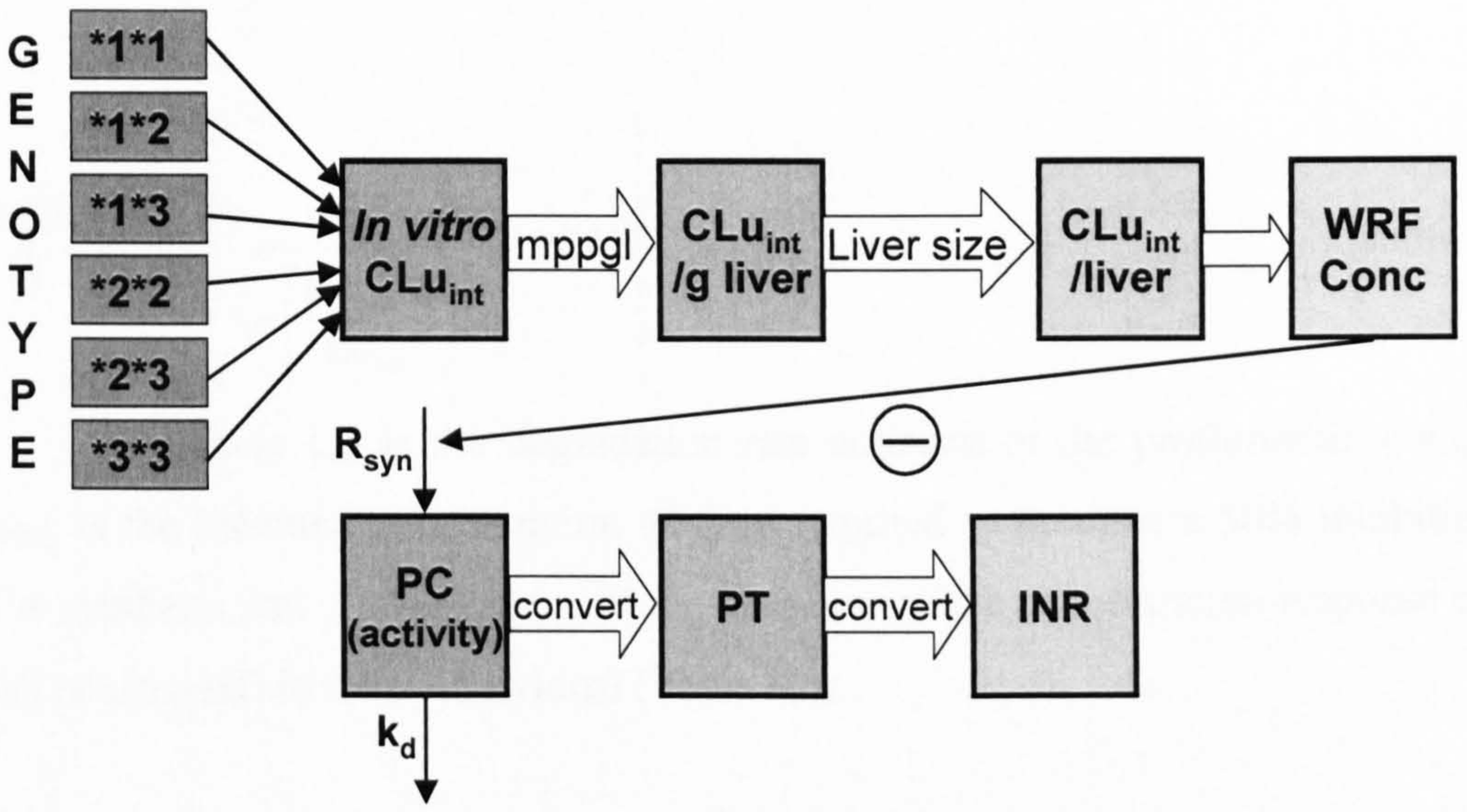


Figure 5.2 Schematic representation of the pharmacokinetic-pharmacodynamic model indicating propagation of metabolic differences between *CYP2C9* genotypes into *(S)*-warfarin clearance and its anticoagulant effect. For a full explanation of the methods used in scaling of *in vitro* data to *in vivo* clearance values see Chapter 3; Materials and Methods. CLu_{int}, unbound intrinsic clearance; mppgl, milligrams microsomal protein per gram of liver; WRF Conc, plasma *(S)*-warfarin concentration; PC, prothrombin complex; R_{syn}, rate of synthesis of PC; k_d, rate constant of degradation of PC; PT, prothrombin time; INR, international normalized ratio.

5.2.4 Selection of the Pharmacodynamic Model

Several pharmacodynamic models for warfarin have been suggested and some of these are summarised in Table 5.7. The sigmoidal E_{max} model suggested by Chan *et al.* (1994) was deemed the most suitable for a number of reasons. Some of the other models are overly simplistic (e.g. the Linear or Power models), some of them are only useful within the range 20 to 80% of the maximum effect (e.g. the Log Linear model) and all models except the sigmoidal E_{max} do not separate the effects of the two isomers of warfarin.

Prothrombin complex activity (PCA) in the blood over time was simulated using an indirect response model in which PCA is the net effect of synthesis and degradation of the complex expressed as a percentage of maximum response (Nagashima *et al.*, 1969) (Figure 5.2).

The change in PCA with time as a function of *(S)*-warfarin concentration was simulated using the following equation:

$$\frac{dPCA_i}{dt} = k_{d,i} \cdot \left[\frac{100}{1 + \left(\frac{C_{warf,i}(t)}{Cu_{50,i}} \right)^{\gamma_i}} - PCA_i \right] \qquad \text{Equation 5.3}$$

where $k_{d,i}$ is the degradation rate constant of the prothrombin complex, $Cu_{50,i}$ is the unbound concentration of drug required to produce a 50% inhibition of PCA synthesis and γ_i is a measure of the steepness of the concentration-response curve (Hill coefficient) in the i^{th} individual (Table 5.8).

5.2.4.1 Conversion of PCA to INR

PCA was converted to Prothrombin Time (PT) using the following equation derived by Chan *et al.* (1994):

$$PCA\ (\%\ normal) = \frac{a}{PT(seconds) - b} \qquad \text{Equation 5.4}$$

In which a and b are constants with variability (Table 5.8).

Table 5.8 Literature values describing the pharmacodynamics (PCA and prothrombin time) of *(S)*-warfarin (from Chan *et al.* 1994).

Parameter	Value*
k_d (h^{-1})	0.054 (0.015)
Cu_{50} (mg/L)	0.0026 (0.0015)
γ	0.90 (0.23)
a	426 (128)
b	7.75 (2.33)

*Numbers in parenthesis indicate standard deviations.

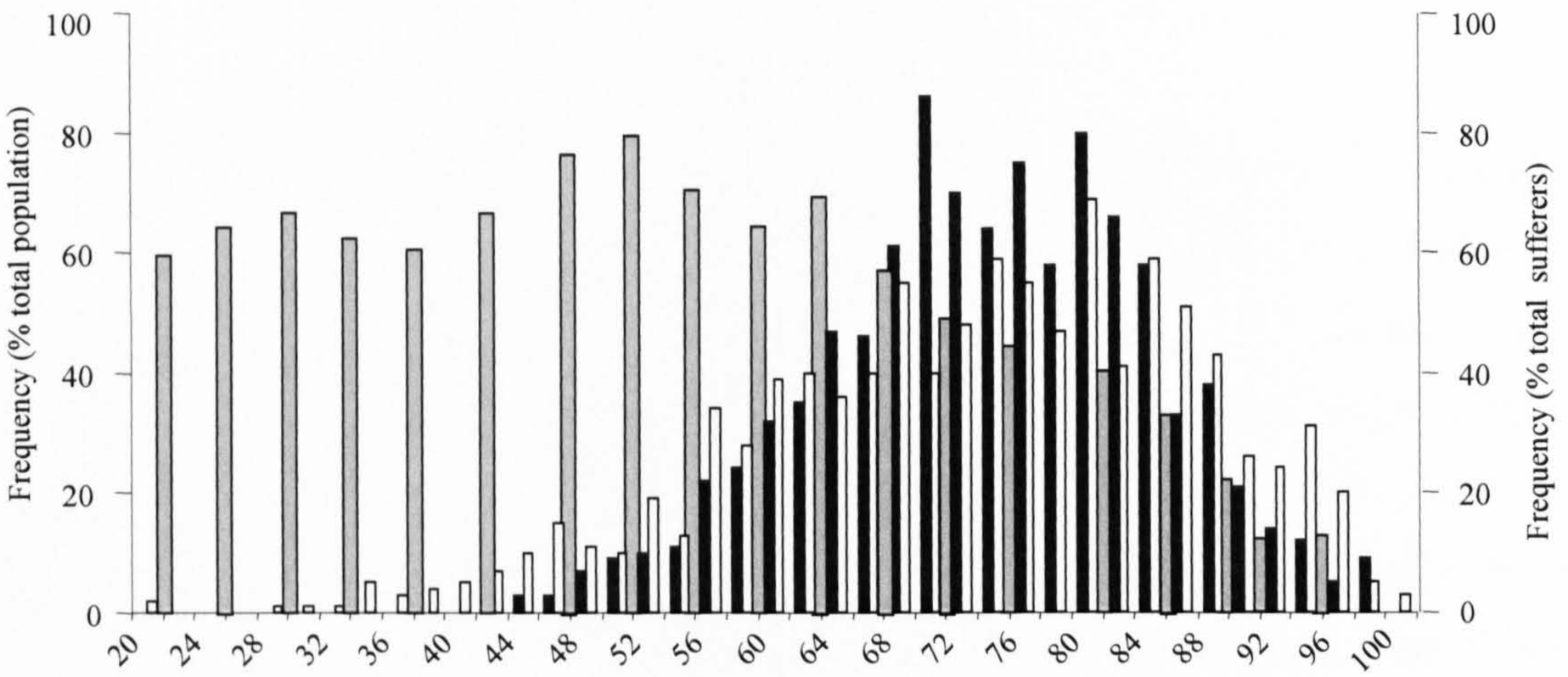


Figure 5.3 Age distribution of a sample of the healthy UK population (Census, 2001) (□) and of male (■) and female (□) stroke patients.

This equation was rearranged to allow calculation of values of PTi in the ith individual from PCAi data.

In turn, PT was converted to INR (Figure 5.2) using the following relationship (determined in a single laboratory) (adapted from Adcock & Duff (2000). An additional 10% arbitrary variability was added to the constants A (0.242) and B (9.5981) to account for differences between laboratories:

$$INR_i = \frac{PT_i + A}{B}$$ Equation 5.5

5.2.5 Study Design

5.2.5.1 Study Population

In contrast to the many pharmacokinetic and pharmacodynamic studies carried out in healthy subjects, the majority of those for warfarin have been performed in patient groups. In the warfarin model, the demography of the virtual patient population was mimicked by matching the age distribution proportion of male to female patients to that which might occur in the real patient population. This was achieved by using data from the Health Survey for England (http://www.dh.gov.uk) to calculate the Weibull distribution for the age ranges of stroke patients (data kindly provided by Trevor Johnson at Simcyp Ltd.). A Weibull distribution is a continuous probability distribution described by two parameters (scale – A; and shape – B), it was used to model the age distribution of the stroke population. The age distribution was described by a Weibull function if the individual was (i) male (A = 2.76; B = 30.3), or (ii) female (A = 2.13; B = 31.8).

Stroke is clearly not the only disorder to be treated with warfarin. However, it was assumed that this age and sex distribution would be closer to the general warfarin-treated population than that of healthy subjects (Figure 5.3).

5.2.5.2 Sampling Schedule

Individual plasma *(S)*-warfarin concentration and response *vs.* time profiles following daily administration of oral warfarin were simulated from hourly samples over 24h. A number of design elements were investigated as described in Section 5.2.5.3, Section 5.2.5.4 and Section 5.2.5.5. As the majority of simulations were undertaken using

'uniform dosage' and 'random recruitment' (see below for definitions), this combination will be referred to as the 'default condition'.

5.2.5.3 Warfarin Dosage Regimens

a) Uniform dosage for each individual regardless of *CYP2C9* genotype: Each individual in the virtual population received 3 mg of *(S)*-warfarin per day. This dose was chosen based on half of the weighted average dose of rac-warfarin calculated from (Holford, 1986). This study design was chosen to simulate a prospective clinical trial where all individuals receive the same dose, assuming that dose requirement for each CYP2C9 genotype is not known.

b) Genotype related dosage: The distribution of commonly prescribed warfarin doses in a typical patient population of 292 patients from two large general hospitals in Merseyside, England, was kindly provided by Prof. M Pirmohammed, of the University of Liverpool (Figure 5.4). No further information (*e.g. CYP2C9* genotype) was available on these patients, but they were assumed to be representative of a general population being treated with warfarin patients. A random number generator operating on a description of these data by a Weibull function was used to assign a maintenance dose to each virtual individual (A = 2.25; B = 4.361; Section 5.2.5.1) (Figure 5.4).

Several retrospective studies have indicated that the average dose of warfarin varies between different CYP2C9 genotypes. Accordingly, a meta-analysis of the data shown in Table 5.9 was carried out to assess the probability of each *CYP2C9* genotype falling within four different dose bands, representing the interquartile ranges of the dose frequency distribution (low (0-3mg), medium-low (3.1-4.5mg), medium-high (4.6-6.4) and high (6.6+)). Thus, the probabilities shown in Figure 5.5 were used to assign a *CYP2C9* genotype to a given individual at a given dose. This study design will be referred to as 'genotype related dosage'.

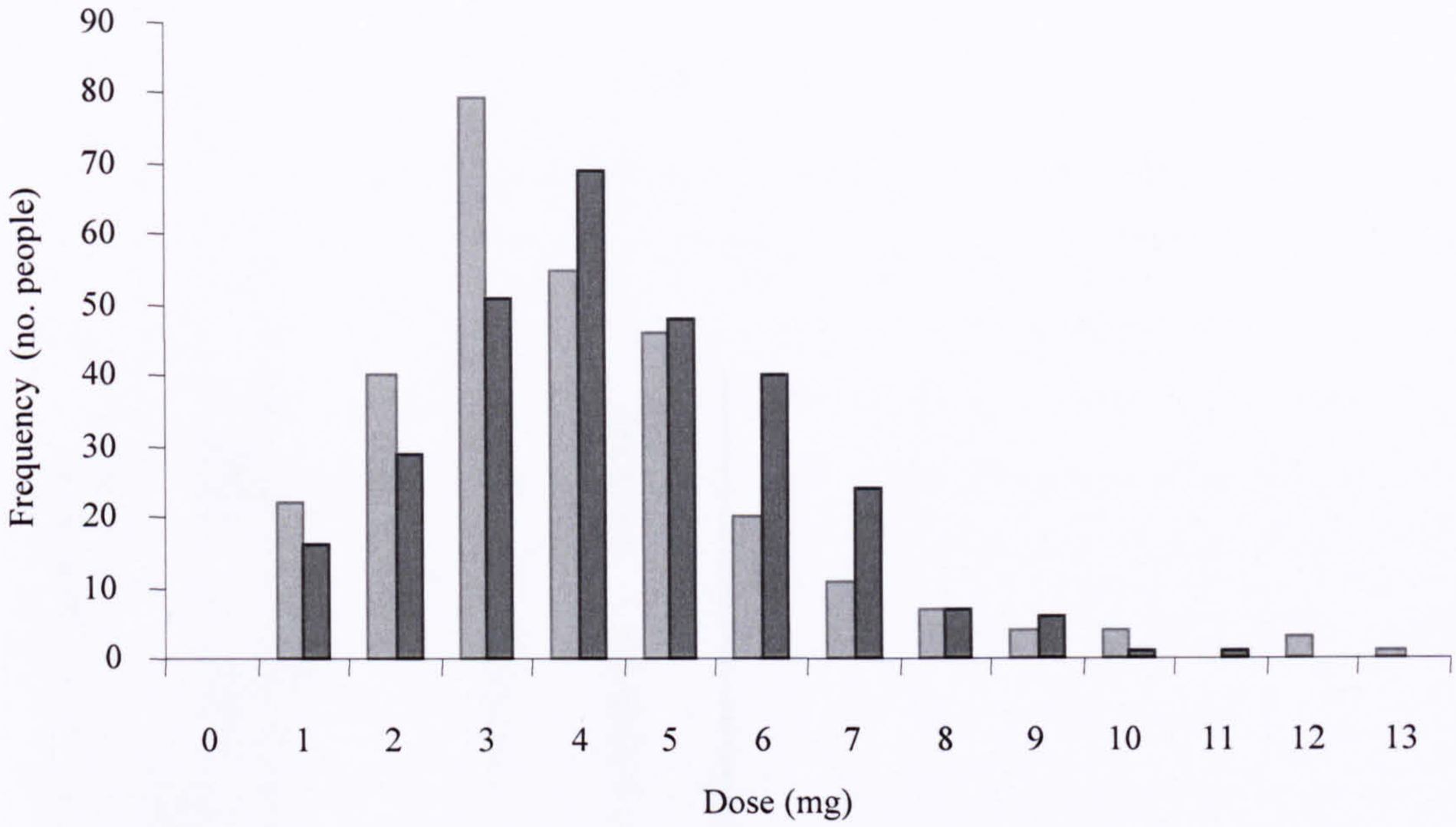


Figure 5.4 Observed (□; n = 292) and predicted (■; n = 292) distribution of warfarin doses in a patient population from two large general hospitals (data supplied by Dr. M. Pirmohamed, University of Liverpool).

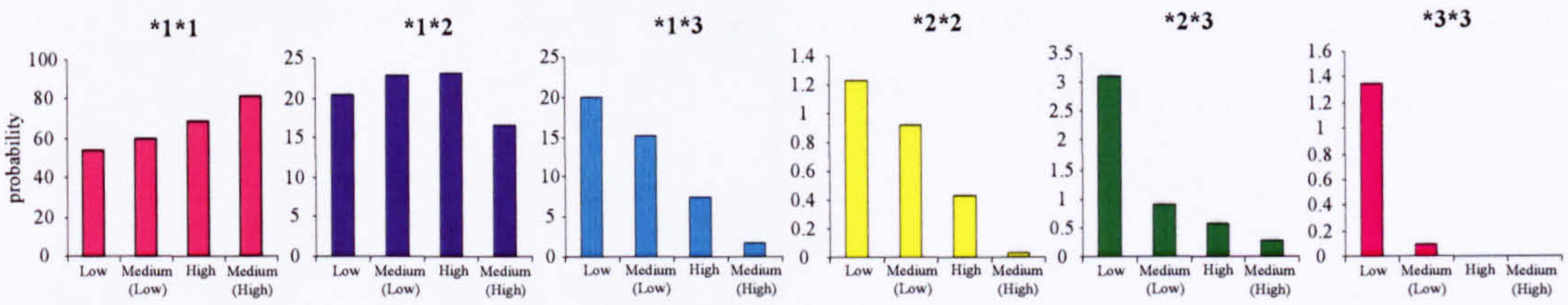


Figure 5.5 The probability (%) of each *CYP2C9* genotype being found in each of the four dose groups described in Section 5.2.5.3, and calculated from a meta-analysis of literature data (Aithal *et al.*, 1999; Higashi *et al.*, 2002; Kamali *et al.*, 2004; Loebstein *et al.*, 2001; Margaglione *et al.*, 2000; Siguret *et al.*, 2004; Taube *et al.*, 2000).

Table 5.9 Summary of a meta-analysis of the literature to find the average dose of warfarin in each *CYP2C9* genotype.

		Average dose in each genotype					
Reference	**n**	***1*1**	***1*2**	***1*3**	***2*2**	***2*3**	***3*3**
Siguret *et al.*, 2004	126	3.1 (1.4)	2.7 (1.3)	2.9 (1.1)	-	2.3 (1.8)	-
Kamali *et al.*, 2004	119	4.06 (1.72)	3.63 (1.78)	2.7 (1.36)	-	-	-
Aithal *et al.*, 1999	155	4.68 (2.1)	3.7 (1.9)	2.7 (1.3)	-	-	-
Taube *et al.*, 2000	561	5.01 (2.43)	4.31 (1.94)	3.97 (1.79)	3.04 (1.29)	4.09 (2.09)	-
Loebstein *et al.*, 2001	156	6.5 (3.2)	5.2 (2.4)	1.6 (0.6)	-	-	-
Higashi *et al.*, 2002	185	5.63 (2.56)	4.88 (2.57)	3.32 (0.94)	4.07 (1.48)	2.34 (0.35)	1.6 (0.81)
Margaglioni *et al.*, 2000	180	6.7 (2.9)	5.2 (2.2)	3.8 (2.0)	-	-	-

*Mean (SD).

5.2.5.4 Subject Recruitment Strategy

a) Random Recruitment: The majority of subjects included in published studies examining the influence of genetic variation in CYP2C9 on warfarin pharmacokinetics or pharmacodynamics were selected randomly from a Caucasian population, and not on the basis of *CYP2C9* genotype. Therefore, the probability of having adequate numbers of a certain genotype in the study population for comparison with other genotypes is dependent on the natural frequency of that genotype in the general population. Virtual populations were simulated based on this 'random recruitment' design.

b) Enriched Recruitment: This type of recruitment involves selecting subjects based on their genotype to give enriched abundances of particular genotypes. In practice, it would require the prior screening of many subjects, particularly with regard to the rarer genotypes, in order to carry out subsequent selective recruitment of small groups of individuals. This trial design was simulated with virtual populations enriched with specific genotypes. For example, a population of 10 *3/*3 individuals was compared to a population of 10 wild type subjects. This design will be referred to as 'enriched recruitment'.

5.2.5.5 Study Size

Simulated plasma drug concentration- and response-time profiles in the different *CYP2C9* genotypes were compared using a range of population sizes (n). For 'random recruitment', n was set at 10, 25, 50, 100, 250 or 550, to correspond approximately to the sizes of the published studies. For 'enriched recruitment' n was set at 2, 3, 5 or 10. No measurement error was assumed.

5.2.5.6 Null Hypothesis

The null hypothesis that there is no true association between genotype and drug clearance was tested using a set of control simulations to evaluate the possibility of false positive outcomes. These simulations used the same study sizes employed in the 'random recruitment' simulations described above (n = 10, 15, 50, 100, 250 or 550) and 'uniform dose' conditions. Half the population were classed as 'extensive

metabolisers' (*1*1) and half were 'poor metabolisers' (*3*3), but no difference in enzyme activity between the genotypes was introduced.

5.2.5.7 Contribution of CYP2C9 to the Metabolism of *(S)*-warfarin

In this section, the default setting was altered and the contribution of CYP2C9 to the overall metabolism of the drug was modified, metabolism being shifted to non-polymorphic pathways. In the default setting, CYP2C9 metabolised about 99% of *(S)*-warfarin. The K_m and V_{max} values for CYP2C9 were altered so that the CL_{int} due to CYP2C9 decreased, whereas renal clearance was made to increase, with total CL_{int} remaining the same.

The contribution of CYP2C9 to the overall metabolism of *(S)*-warfarin was decreased from 99% to 50% and 25%.

5.2.5.8 Influence of *VKORC1* Genotype

Since pharmacodynamic variability in the response to warfarin is known to depend upon *VKORC1* genotype (Aquilante *et al.*, 2006; D'Andrea *et al.*, 2005; Li *et al.*, 2006; Tham *et al.*, 2006; Vecsler *et al.*, 2006) as well as that for *CYP2C9*, further simulations were carried out to assess the impact of the former on study power. In the default model, the variability in k_d was set at 27% (Chan et al., 1994; Table 3). To allow for the increase in certainty arising from a knowledge of VKORC1 genotype, this was decreased to 15%, based on the weighted average of the results of studies indicating that the total variability in warfarin dose due to VKORC1 genotype (after subtracting variability due to *CYP2C9* genotype) is 18 to 26% (Aquilante *et al.*, 2006; D'Andrea *et al.*, 2005; Vecsler *et al.*, 2006). The simulations were carried out under 'random recruitment' and 'uniform dosing' conditions.

5.2.6 Data Analysis

Twenty simulations were run for each study size (800 simulations) involving a total of 119,000 virtual patients. Pharmacokinetic and pharmacodynamic data were available up to 24h post dose in the simulations, values of the areas under the plasma drug concentration-time curves (AUC) and effect (INR)-time curves (AUEC) were calculated up to 12h (trapezoidal rule) with twice-hourly samples in order to maximize consistency with *in* vivo study design in literature reports on

warfarin. The probability of detecting statistically significant differences in pharmacokinetics and pharmacodynamics between the wild type (*1/*1) and the combination of the other *CYP2C9* genotypes was assessed by comparing values of AUC and AUEC, respectively, using ANOVA (SPSS v 12; SPSS Inc, Chicago 2003). The corresponding probabilities of detecting differences in AUC or AUEC between the wild type and any other single genotype were calculated using Tukey's post hoc test for multiple comparisons. The percentage of studies out of the 20 simulations that led to a statistically significant difference in AUC (or AUEC) between *CYPC9* genotypes, was recorded as the power of that comparison.

Clearance values obtained from the model were compared with those values observed *in vivo* (Scordo *et al.*, 2002; Takahashi *et al.*, 2003). The accuracy of the resulting effect-time profiles were assessed by comparing the mean INR values of the virtual populations with those reported *in vivo* (Jiang *et al.*, 2005; Lilja *et al.*, 2005; Lindh *et al.*, 2005; Priskorn *et al.*, 1997; Simonson *et al.*, 2005; Vadher *et al.*, 1999).

5.2.7 Defining a Hypothetical Concentration Threshold for the Occurrence of Adverse Reactions Due to Warfarin

As described in Section 5.2.5.3, the distribution of commonly prescribed warfarin doses in a typical patient population was made available to us (n = 292) (Figure 5.4; Section 5.2.5.3), as was the proportion of patients in this population who were admitted to hospital with adverse drug reactions (ADRs) to warfarin. It is assumed that the latter may occur as a result of elevated plasma *(S)*-warfarin concentrations. Therefore, it was possible to develop a simple 'threshold model', based on steady state *(S)*-warfarin concentrations. Using the IVIVE model for this purpose, a series of steady state plasma concentrations (C_{SS}; n = 292) were simulated 20 times.

Assuming that the proportion of observed ADRs were associated with the highest concentrations of *(S)*-warfarin, the proportion of C_{SS} values that were expected to elicit ADRs was applied and a threshold concentration was estimated in each simulation set. The mean threshold value from twenty simulations was taken as the value of C_{ss} (± SD) above which, serious ADRs are most likely to occur.

5.3 Results

5.3.1 Model Validation

The meta-analysis of the enzyme activities associated with the variant alleles of CYP2C9 indicated mean decreases in CLu_{int} of 15% and 45% compared to wild-type activity, respectively, for every *2 and *3 allele present in an individual (Figure 5.1). The results for the different *CYP2C9* genotypes are shown in Table 5.5 and Figure 5.1. Propagation of these values through the Simcyp® algorithm resulted in mean values of unbound oral clearance for *(S)*-warfarin from 100 individuals from one simulation of 19.9 (*1/*1), 17.1 (*1/*2), 10.8 (*1/*3), 14.8 (*2/*2), 7.6 (*2/*3) and 1.9 (*3/*3) L/h. These predicted values were all within 2-fold of those reported from *in vivo* studies (Kamali *et al.*, 2004; Scordo *et al.*, 2002) as shown in Figure 5.6. The null hypothesis simulations resulted in 0 to 5% power to detect differences in the AUC of (S)-warfarin between genotypes.

A comparison of simulated unbound oral clearance values with those reported by Scordo *et al.* 2002 is shown in Figure 5.7.

The simulated PT- and INR-time profiles obtained from the 'uniform dose' model with 'random recruitment' in 50 virtual subjects are shown in Figure 5.8 and Figure 5.9.

Figure 5.8 and Figure 5.9 also demonstrate direct comparisons between the simulated data and observed data from three different reported studies (Jiang *et al.*, 2005; Lilja *et al.*, 2005; Priskorn *et al.*, 1997).

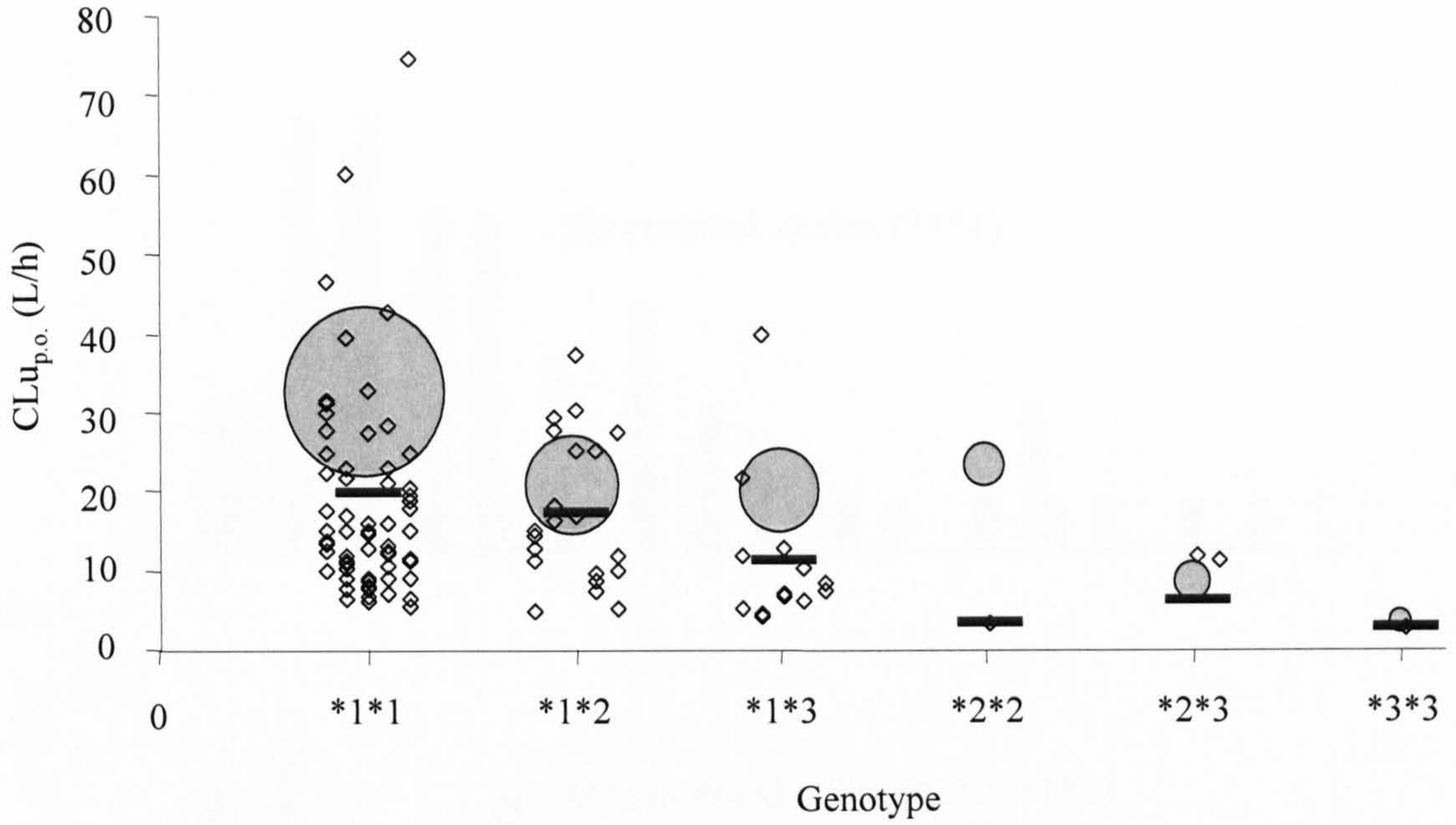


Figure 5.6 Observed [Scordo *et al.,* 2002; Takahashi *et al.,* 2003* (○)] and predicted (◇; n = 100) unbound oral clearance values. Solid lines indicate the medians of one set of simulated data. The size of the circles reflects the number of subjects included in the meta-analysis (described in Chapter 3; Section 3.5).
*The study by Takahashi *et al.* (2003) was conducted in both Japanese and Caucasians, but only the latter population was included in the meta-analysis.

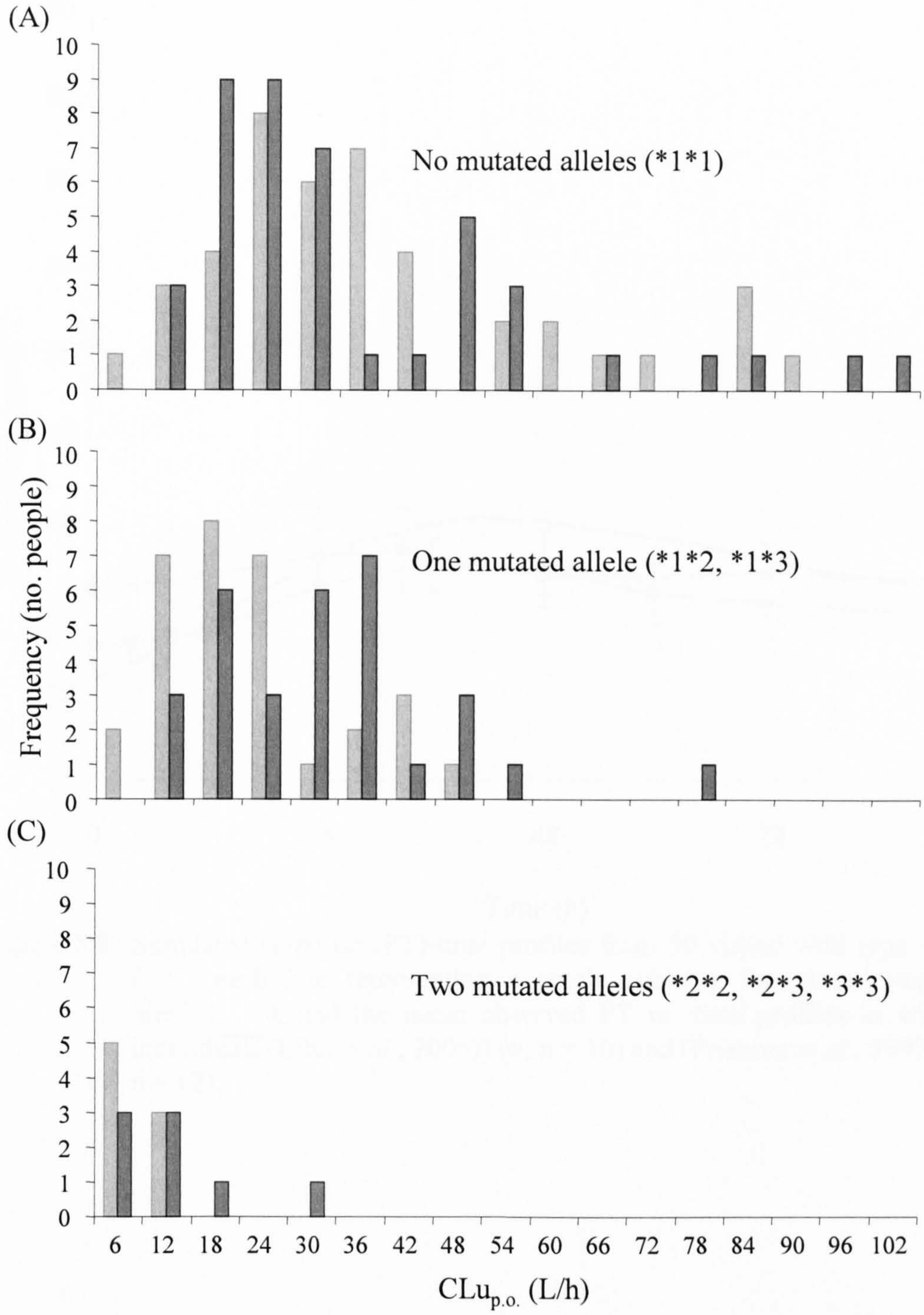


Figure 5.7 Observed [Scordo *et al.,* 2002 (□)] and predicted (■; n = 93) distribution of unbound oral clearances for *(S)*-warfarin in (A) wild type subjects, (B) subjects with one mutated allele, and (C) subjects with two mutated alleles.

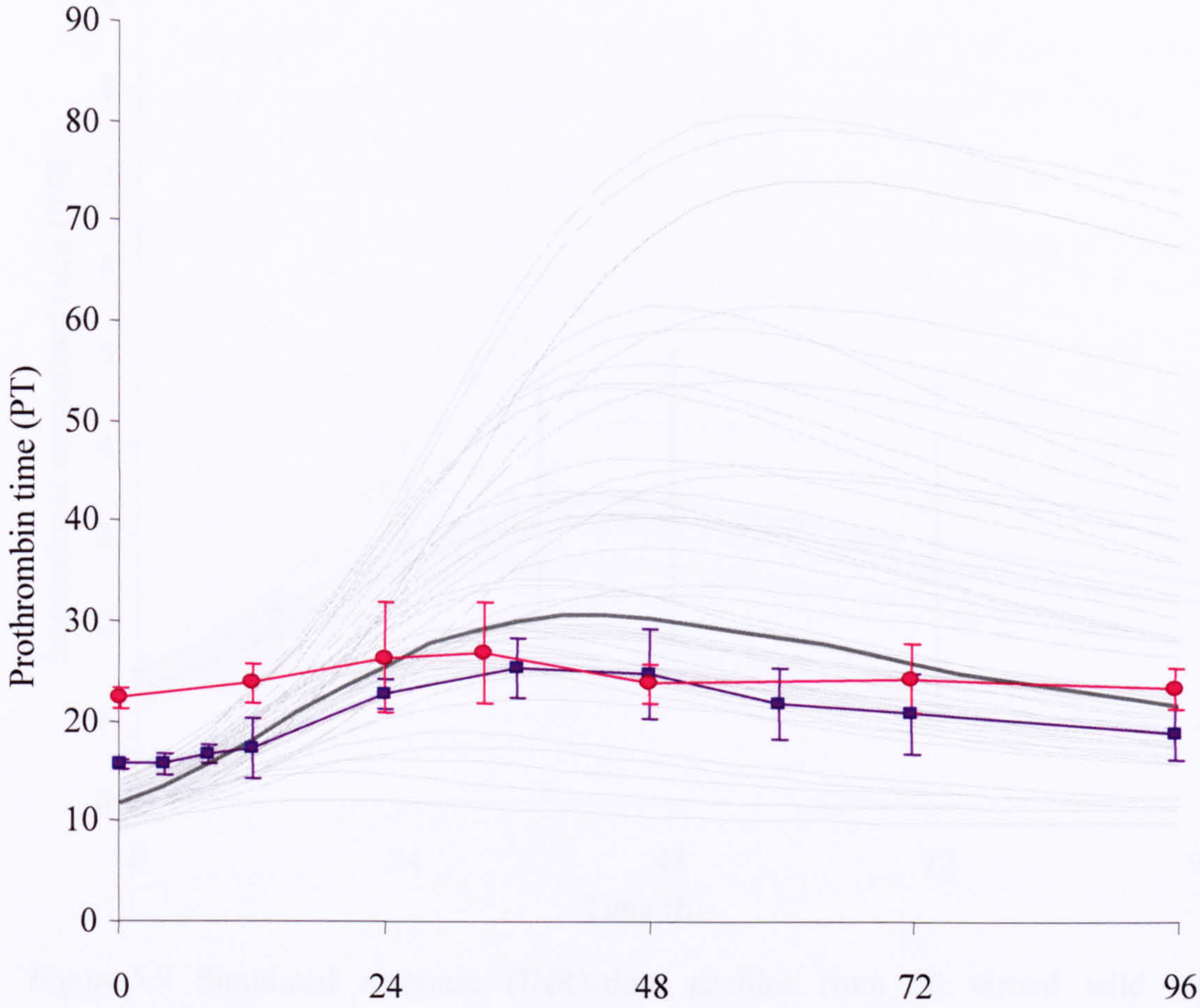


Figure 5.8 Simulated response (PT)-time profiles from 50 virtual wild type subjects (; each line representing a single subject), the virtual population mean ( ), and the mean observed PT *vs.* time profiles in wild type individuals (Lilja *et al.*, 2005)) (●; n = 10) and (Priskorn *et al.*, 1997) (■; n = 12).

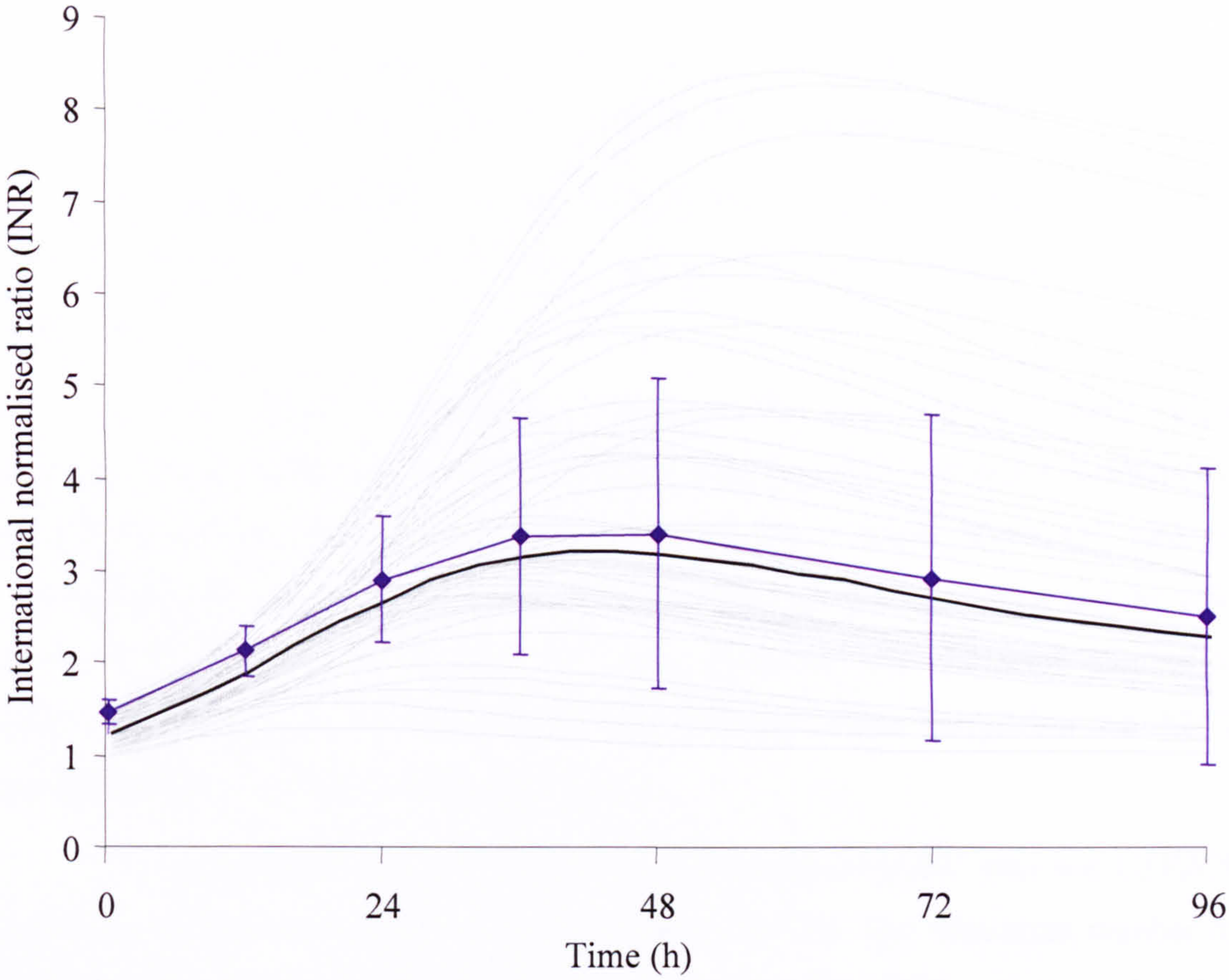


Figure 5.9 Simulated response (INR)-time profiles from 50 virtual wild type subjects (____; each line representing a single subject), the virtual population mean (____), and the mean observed INR *vs.* time profiles in wild type individuals from (Jiang *et al.*, 2005) (◆; n = 50).

5.3.2 Genotypic Differences in (S)-warfarin AUC/AUEC Using the Default Setting ('Uniform Dosage' and 'Random Recruitment')

Approximately 90 subjects were needed to detect a difference in AUC between the wild type (*1/*1) and the combination of all other genotypes (Figure 5.10).

The power to differentiate between wild type and any other single genotype was less than that for the comparison between wild type and a combination of all non-wild type genotypes (Figure 5.11 (A)). For example, about 450 subjects were required to detect a difference in AUC between the *2/*3 genotype and the wild type with a power of 80% (Figure 5.11 (A)). However, with the same number of subjects, a power of only about 25% was achieved when comparing the *2/*2 genotype with the wild type (Figure 5.11 (A)).

Corresponding powers to detect differences in AUEC between *CYP2C9* genotypes are shown in Figure 5.10 and Figure 5.11 (B). The maximum number of subjects investigated of 550 was enough to achieve only 70% power to detect a difference between wild-type and any other genotype (Figure 5.10). The power to detect differences in AUEC between specific genotypes is shown in Figure 5.11 (B). For example, about 100 subjects are required for 80% power to detect a difference between the *1/*3 genotype and the wild type. By comparison, the same number of subjects gave a power of 10% when comparing the *3/*3 genotype with the wild type (Figure 5.11 (B)).

Ninety-five comparisons of the pharmacokinetics or pharmacodynamics of *(S)*-warfarin between the wild type and any single other genotype have been reported in the literature (Table 5.10). Of these, 20 (21%) involved the observation of significant differences (Table 5.10). In the present simulations 19 out of 95 (20%) comparisons were expected to achieve a power of greater than 50% (more likely to succeed than fail) (Table 5.10). A chi squared test indicated no difference between the experimental and simulated proportions ($p = 0.80$). More specifically, in 76 cases out of 95, the expected power matched the observed result (Table 5.10). There were 10 false negative and 9 false positive predictions (Table 5.10).

5.3.3 Genotype Adjusted Dose

Using the genotype adjusted model with 'random recruitment' the power to determine differences in drug clearance between wild type and all the other genotypes was the same as that seen under 'default conditions'. However, this was not the case for AUC (a parameter that is dependent on both dose and clearance) or AUEC, where the powers to determine differences between *CYP2C9* genotypes were decreased under 'genotype related dosage' conditions compared to 'uniform dosage' (Figure 5.10). The power to detect a difference in AUEC (comparing wild type and the combination of other genotypes) with the maximum study size reported in the literature (550 individuals) was 65%.

5.3.4 Enriched Recruitment

To investigate the effect of 'enriched recruitment' under 'uniform dose' conditions, *3/*3 individuals were compared directly with wild type genotypes. As expected, this resulted in much higher power compared to that achieved with 'random recruitment'. Thus, to achieve 80% power in detecting a significant difference in AUC between the two genotypes, only 3 subjects in each group were required (Figure 5.12). The corresponding number needed to detect differences in AUEC was 5 per group (Figure 5.12).

5.3.5 Effect of Changing the Contribution of CYP2C9 to the Metabolism of (S)-warfarin on Study Power.

Changing the proportional contribution of CYP2C9 to the overall metabolism of *(S)*-warfarin from 99% in the default model to 25% caused corresponding decreases in the power to a maximum of 60% with a study size of 550.

5.3.6 Influence of VKORC1 Genotype

Values of study power arising from these simulations were slightly higher than those from the equivalent 'uniform dosage', 'random recruitment' simulations described in Section 5.3.2 (Figure 5.14). For example, with the maximum study size investigated of 550, 100% power was reached when *VKORC1* genotype was taken into account compared to 90% power when it was not.

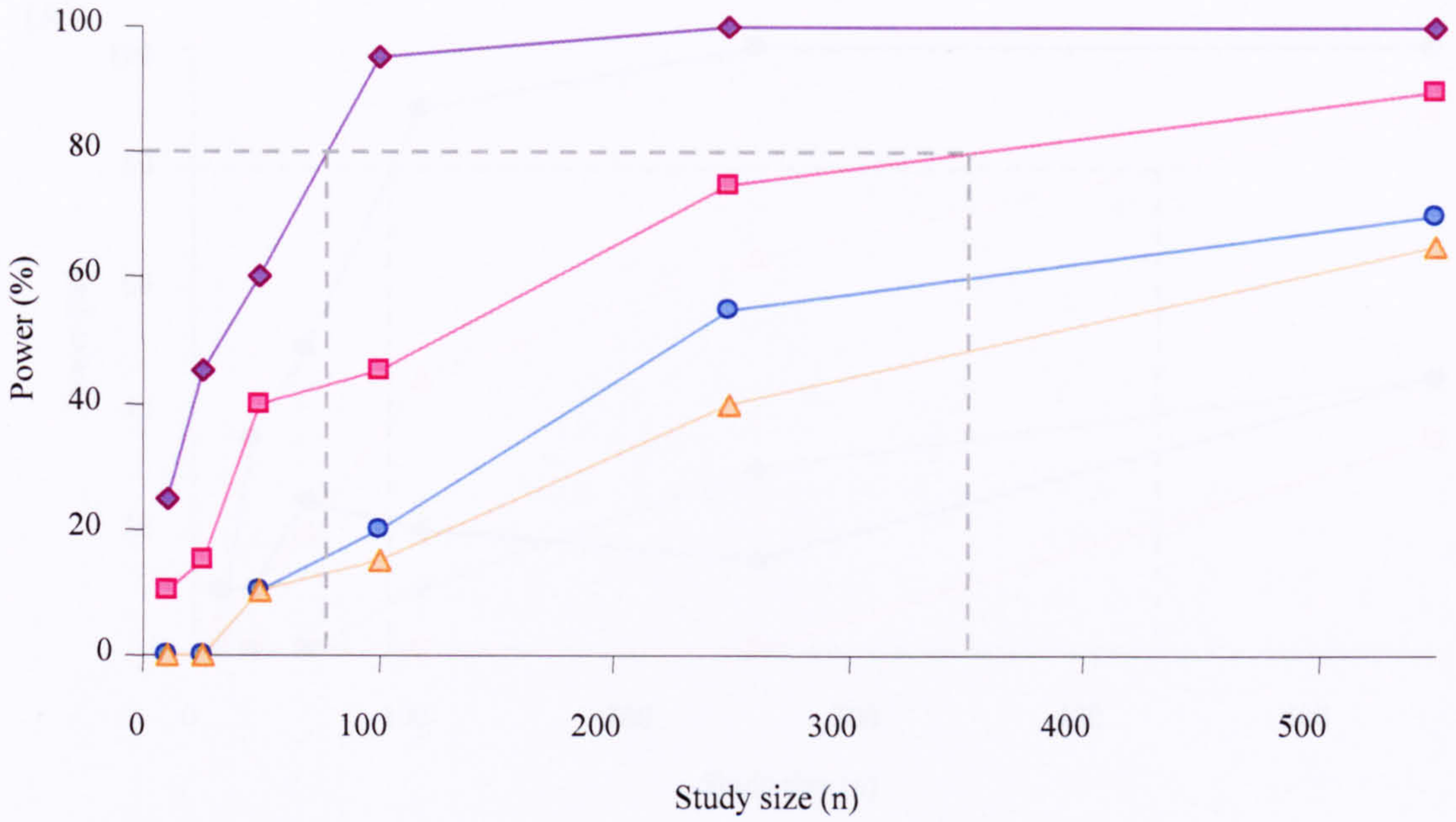


Figure 5.10 Under conditions of 'random recruitment', the power (%) of simulated studies to show significant differences in AUC (under uniform dosage (◆) and adjusted dosage (■) conditions) and in AUEC (under uniform dosage (●) and adjusted dosage (△) conditions) between the wild type and a combination of the other *CYP2C9* genotypes *vs*. the number of subjects in the study population (n). Dashed line represents 80% power which is typically used in designing clinical studies.

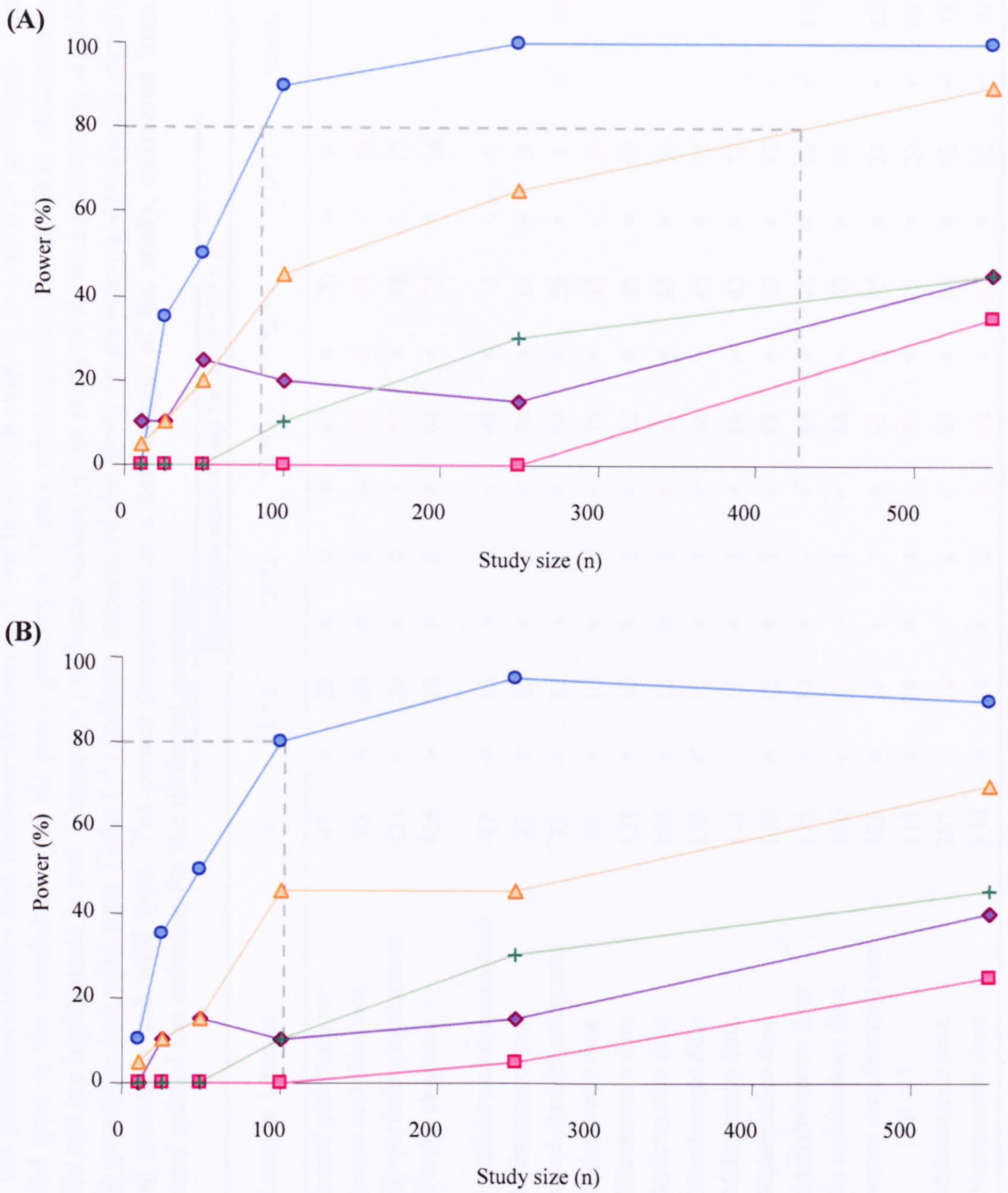


Figure 5.11 Under conditions of 'uniform dosage' and 'random recruitment', the power (%) of simulated studies to show significant differences in (A) AUC, and (B) AUEC between the wild type and any single other *CYP2C9* genotype *vs* the number of subjects in the study population (n). Wild type was compared with: *1/*2 (◆), *1/*3 (●), *2/*2 (■), *2/*3 (△), *3/*3 (+). Dashed line represents 80% power, which is typically used in designing clinical studies.

Table 5.10 The outcomes of reported studies of the pharmacokinetics and pharmacodynamics of warfarin with respect to *CYP2C9* genotype. 'Comb' signifies the comparison between wild type *vs* the combination of all other genotypes. Cases where prediction matched observation are highlighted in green, those where it did not are highlighted in red. Crosses (✗) indicate failure of the study to show a statistically significant difference between the corresponding genotype and wild type Ticks (✓) indicate success of the study in showing a statistically significant difference between the corresponding genotype and wild type. The power (expressed as a percentage) of the study, estimated from the simulations, is recorded in the right hand side of the columns for the different genotypes.

Reference	Study Type	Outcome Measure	n	Significant relationship between *1/*1 *vs*											
				*1/*2		*2/*2		*1/*3		*2/*3		*3/*3		Comb	
Takahashi *et al.*, 2003	PK	Unbound oral clearance	47	✗	25	✗	0	✗	48	✗	20	✗	0		
Scordo *et al.*, 2002	PK	Unbound oral clearance	93	✗	20	✗	0	✗	82	✓	44	✓	10		
Kamali *et al.*, 2004	PK	Plasma *(S)*-warfarin concentration	121	✓	20	✗	0	✗	92	✗	48	✗	12		
Loebstein *et al.*, 2001	PK	Plasma clearance	156	✗	18	✗	0	✓	94	✗	52	✗	16		
Takahashi *et al.*, 2003	PD	Weight normalised maintenance dose	47	✗	15	✗	0	✗	48	✗	15	✗	0		
Khan *et al.*, 2004	PD	Maintenance dose	53	✗	16	✗	0	✓	50	✗	16	✗	0		
Joffe *et al.*, 2004	PD	Maintenance dose/bleeding rate	73	✗	12	✗	0	✗	64	✗	26	✗	4	✓	42
Scordo *et al.*, 2002	PD	Maintenance dose	93	✗	10	✗	0	✓	76	✓	45	✓	10		
Kamali *et al.*, 2004	PD	Maintenance dose	121	✗	10	✗	2	✓	82	✗	45	✗	12		
Siguret *et al.*, 2004	PD	Maintenance dose	126	✗	11	✗	2	✗	82	✗	45	✗	13		
Loebstein *et al.*, 2001	PD	Maintenance dose	156	✓	12	✗	3	✓	86	✗	45	✗	16		
Tabrizi *et al.*, 2002	PD	Maintenance dose	153	✓	12	✗	3	✓	86	✗	45	✗	17		
King *et al.*, 2004	PD	Maintenance dose	159	✗	12	✗	3	✓	88	✗	45	✗	18		
Peyvandi *et al.*, 2004	PD	Mean maintenance dose	175	✗	12	✗	3	✓	88	✗	45	✗	20	✓	60
Maragaglione *et al.*, 2002	PD	Mean maintenance dose	180	✓	12	✗	3	✓	88	✗	45	✗	20		
Higashi *et al.*, 2002	PD	Mainteneance dose/bleeding rate	185	✗	12	✗	3	✗	90	✗	45	✗	21	✓	62
Lindh *et al.*, 2005	PD	INR > 3	219	✗	14	✗	4	✗	92	✗	45	✗	26	✓	68
Sconce *et al.*, 2005	PD	Maintenance dose	297	✓	20	✓	8	✓	94	✗	50	✗	32	✓	78
(Aquilante *et al.*, 2006)	PD	Maintenance dose	350	✗	24	✗	12	✗	94	✗	54	✗	35	✓	80

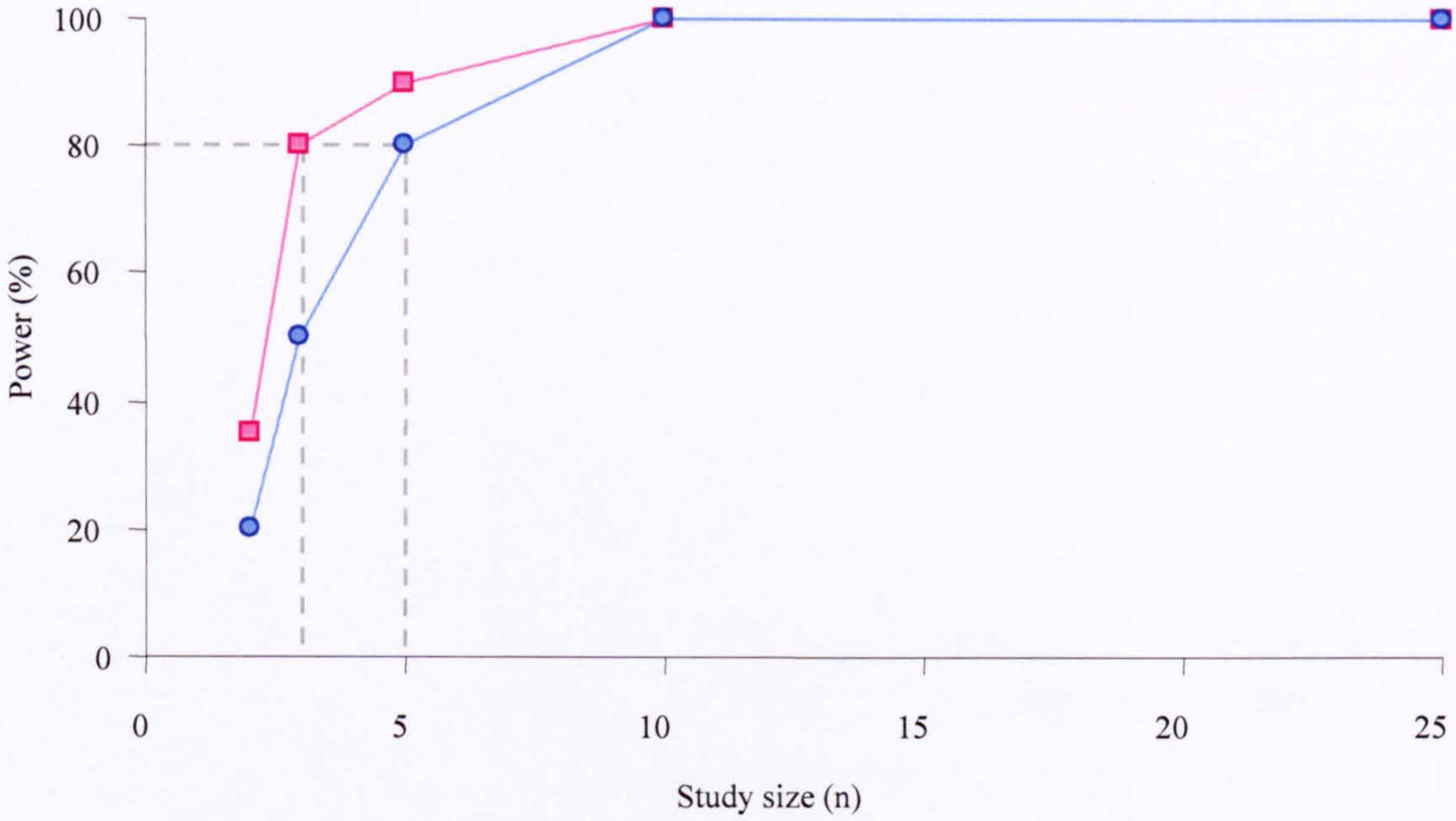


Figure 5.12 Under conditions of 'enriched recruitment', the power (%) of simulated studies to show significant differences in the AUC (■) and AUEC (●) between the wild type and the *3/*3 genotype *vs*. the number of subjects in each study (n).

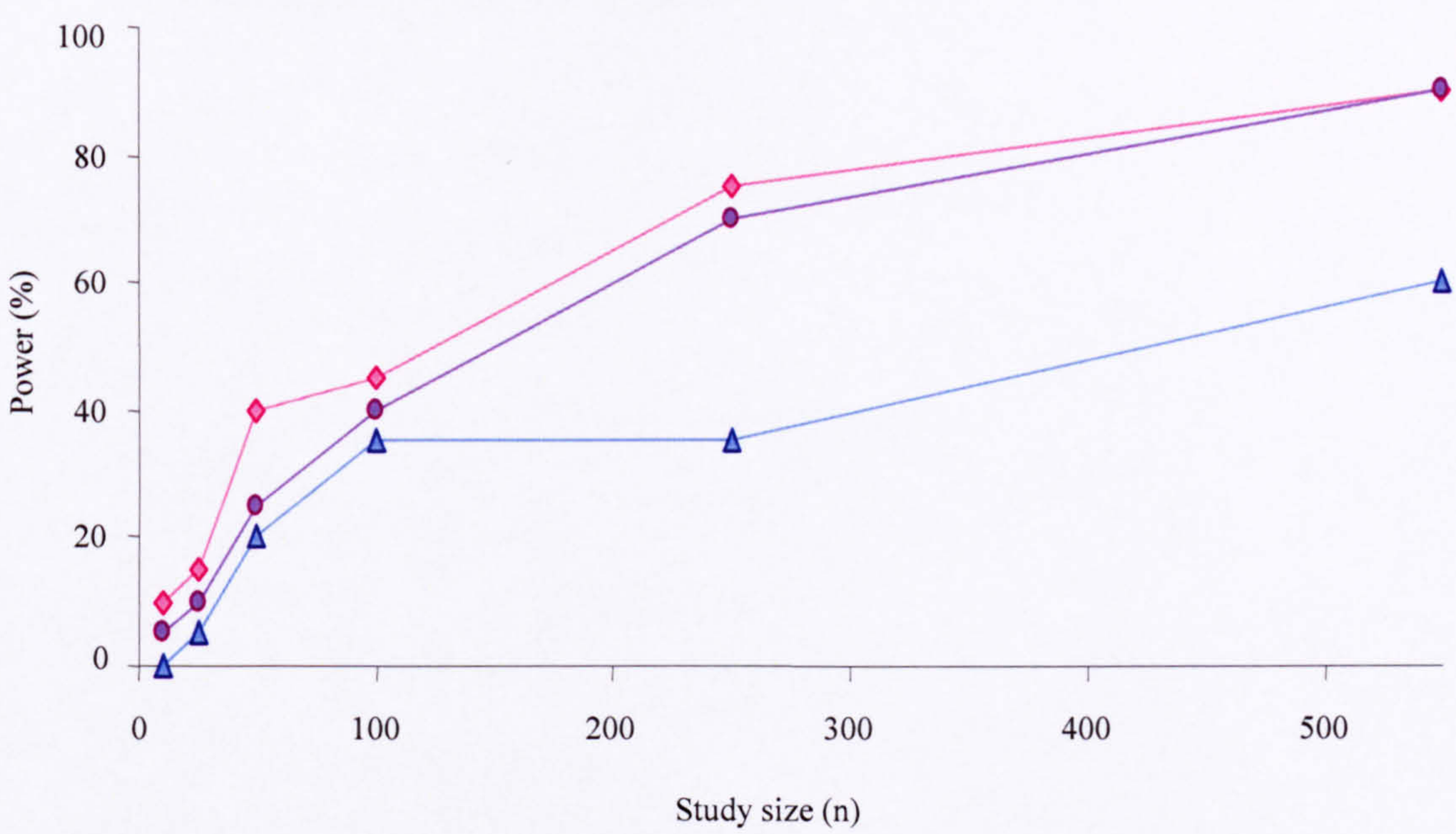


Figure 5.13 The influence of the proportional contribution of CYP2C9 to the overall clearance of *(S)*-warfarin on the power (%) of simulated studies to detect significant differences in AUEC (under uniform dosage conditions) between the wild type and a combination of any other *CYP2C9* genotypes *vs*. the number of subjects in the study population (n). ♦ = default condition; ● = 50% contribution of CYP2C9; ▲ = 25% contribution of CYP2C9.

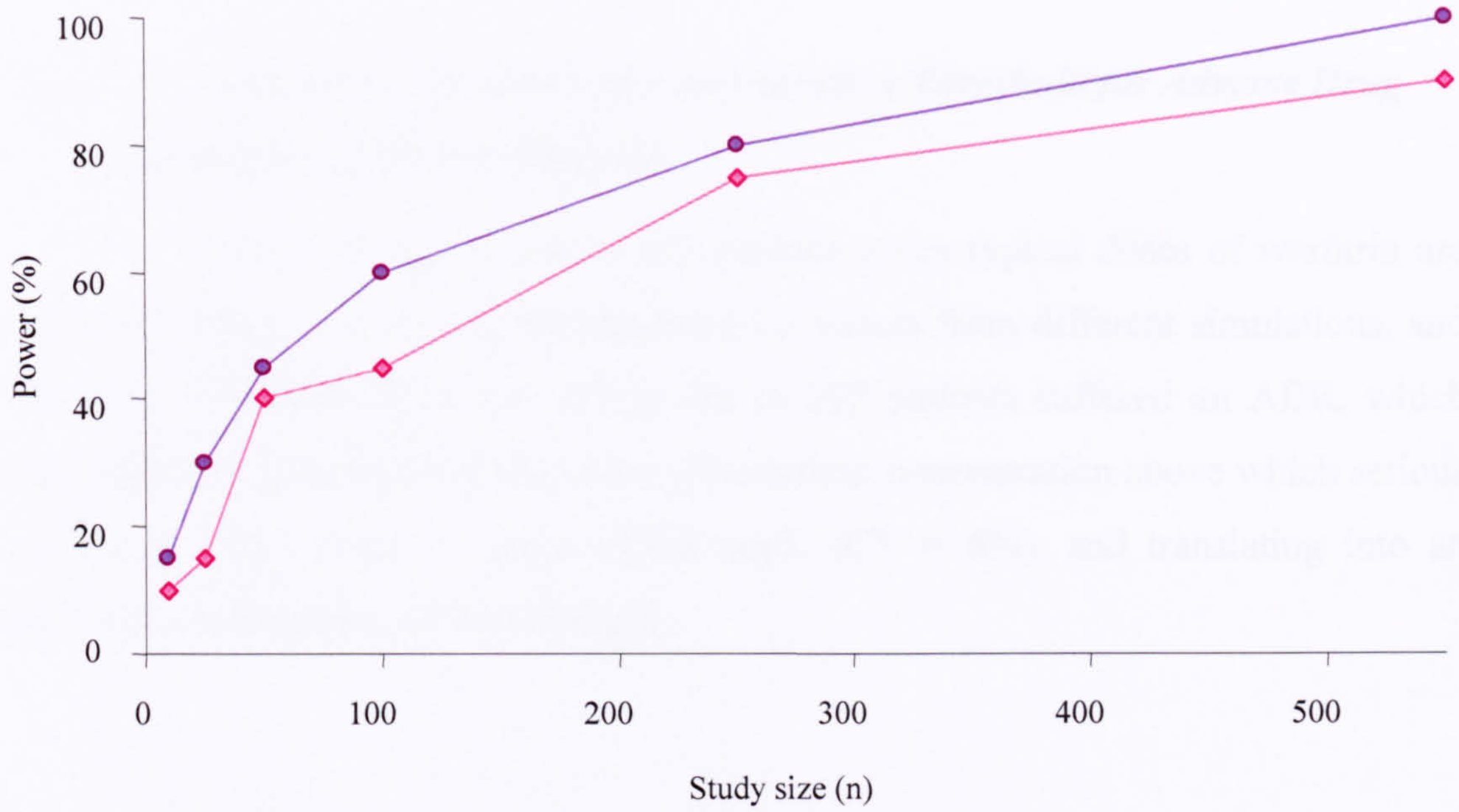


Figure 5.14 The influence of pharmacodynamic variability on the power (%) of studies to detect significant differences in the AUEC (under uniform dosage conditions) of *(S)*-warfarin between *CYP2C9* genotypes, not taking account of (◆), and taking account of (●)*VKORC1* genotype. The default value was 27%, decreasing to 14% when knowledge of *VKORC1* genotype was added to the model.

5.3.7 Defining a Hypothetical Concentration Threshold for Adverse Drug Reactions (ADRs) to Warfarin

Simulated C_{ss} values in 292 patients given typical doses of warfarin are shown in Figure 5.15, as are the threshold C_{ss} values from different simulations, and the mean threshold. Sixty two (21%) out of 292 patients suffered an ADR, which translated into a threshold total plasma *(S)*-warfarin concentration above which serious ADRs are more likely to occur of 0.8 mg/L (CV = 6%), and translating into an unbound concentration of 0.0104 mg/L.

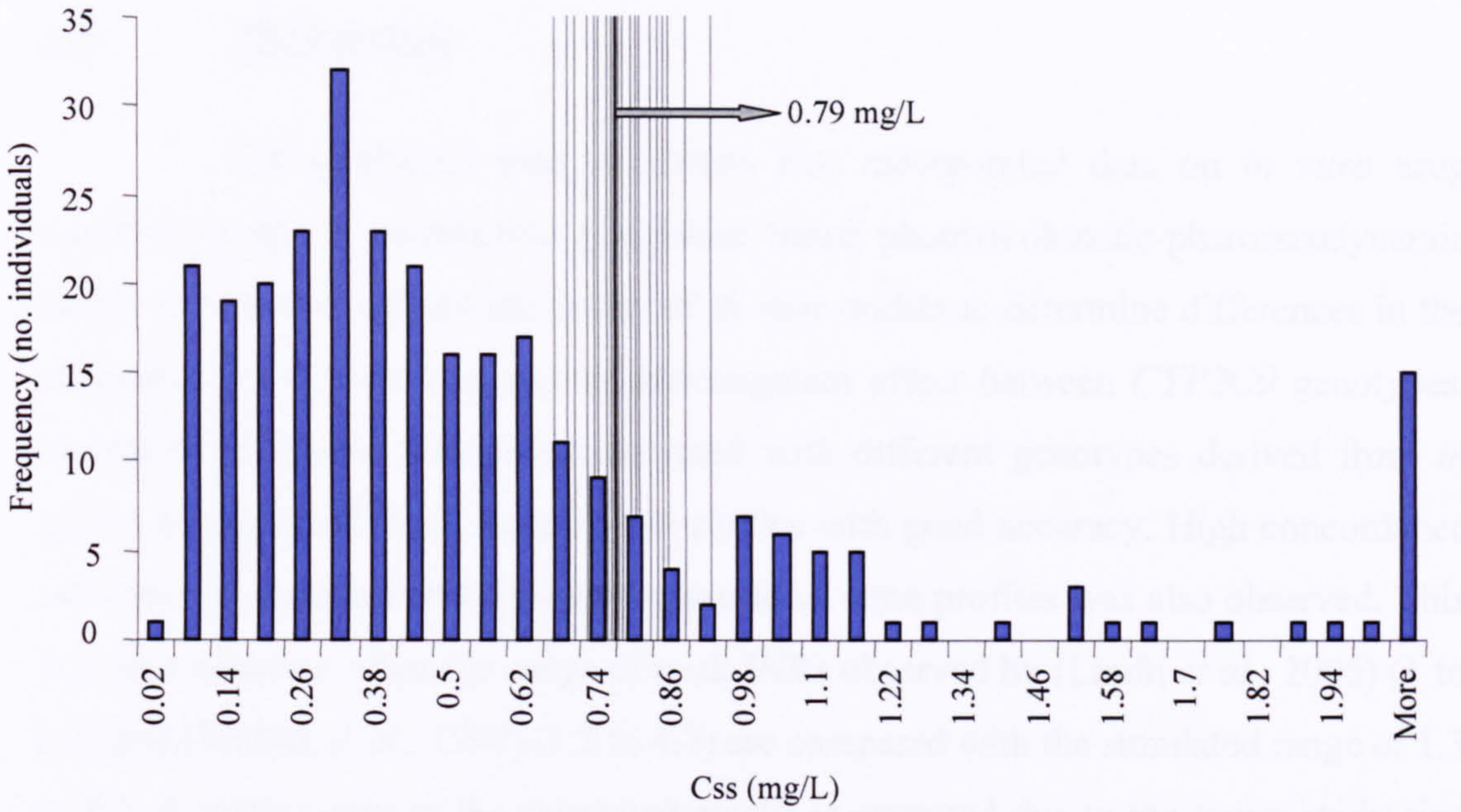


Figure 5.15 Simulated steady state total plasma *(S)*-warfarin concentrations (n = 292 representative individuals). The threshold values from each of the 20 simulations are shown (——) with the mean threshold value (——) above which ADRs are most likely to occur.

5.4 Discussion

Using clinical trial simulation that incorporated data on *in vitro* drug metabolism into a mechanistic population based pharmacokinetic-pharmacodynamic model, we have examined the power of *in vivo* studies to determine differences in the disposition of *(S)*-warfarin and its anticoagulant effect between *CYP2C9* genotypes. Values of metabolic clearance associated with different genotypes derived from *in vitro* data predicted those from *in vivo* studies with good accuracy. High concordance between the observed and expected response *vs*. time profiles was also observed. This was also apparent when the range of peak INRs observed by (Lindh *et al.*, 2005) (1 to 5.5) and (Vadher *et al.*, 1999) (1.2 to 4.7) are compared with the simulated range of 1.3 to 8.2. A wider range in the simulated results is expected due to the larger study size employed.

Previous experimental findings are fully consistent with the present power data. There were 4 and 7 false negative cases in pharmacokinetic and pharmacodynamic studies, respectively (indicated by the cells highlighted in red in Table 5.10). In these, the simulations suggested a lack of adequate power to detect differences between wild type and the respective non-wild-type genotype, but the *in vivo* study found a statistically significant difference. However, such an outcome may occur by chance, as the level of type 1 error is set at 5%, and hence every 1 out of 20 studies may indicate a difference that is not a true difference. On the other hand, the 11% rate for false negative cases *vs* the 8% rate for false positives (Table 5.10) may indicate some conservatism in our power calculations. These findings might reflect some overestimation of the variability in the model parameters. The variances in the parameters of the pharmacodynamic model used in our simulations were based on data from only 6 healthy subjects. Nevertheless, there was good overall concordance between the predicted and observed percentage of studies (20% *vs* 21%) successful in differentiating *(S)*-warfarin pharmacokinetics or pharmacodynamics between the wild type and any single other genotype.

The IVIVE of *(S)*-warfarin clearance in the different genotypes only took into account of *in vitro* differences in the intrinsic clearance, and possible differences in the expression of CYP2C9 between genotypes was not considered. Although some

authors have suggested that such a difference may exist (Coller *et al.*, 2002), the available data (Coller *et al.*, 2002; Tang *et al.*, 2001) was not complete enough for incorporation into the simulations. In addition, a meta-analysis of the data from these studies indicated little or no difference in the extent of CYP2C9 expression between the genotypes, thus having a minimal impact on the outcome of the simulations (Figure 5.16) (Coller *et al.*, 2002; Tang *et al.*, 2001).

The model prediction indicated that, under the assumptions of 'uniform dosage' and 'random recruitment' conditions, at least 90 subjects would be required to detect a difference (with 80% power) in the AUC of *(S)*-warfarin between wild genotype and the combination of all other genotypes (Figure 5.7). For the 'genotype related dosage' condition, the power is less (over 550 subjects required for a power of 80%). Also, comparisons between the wild type and the other genotypes would require much higher numbers of subjects (e.g. 420 subjects to achieve 80% power in discriminating pharmacokinetics between wild-type and *2/*3 under the 'uniform dosage' condition). Study sizes of this order are uncommon in traditionally designed studies, although they may be achieved in population pharmacokinetic studies using sparse data analysis. Four studies assessing the impact of genetic variation in CYP2C9 on *(S)*-warfarin pharmacokinetics using randomly selected subjects have been reported. One of these (Takahashi *et al.*, 2003) used 47 subjects and, as predicted by our model, failed to detect a difference in pharmacokinetics between wild type and all the other genotypes. The three other studies were successful in discerning a difference between the wild type and some, but not other, genotypes (Kamali *et al.*, 2004; Loebstein *et al.*, 2001; Scordo *et al.*, 2002). For example, each study had 0% power to establish differences in the pharmacokinetics between the wild type and the *2/*2 genotype, due to insufficient numbers of the latter (Table 5.10).

With regard to pharmacodynamic outcome, the model prediction indicated that, under the assumptions of 'uniform dosage' and 'random recruitment', about 250 subjects are required to achieve 80% power to detect a difference in AUEC between wild type and the combination of all other genotypes (Figure 5.7). Under the 'genotype related dosage' condition, the power decreased to 60% with 550 subjects. All reported studies assessing the impact of genetic variability in CYP2C9 on *(S)*-warfarin response and dosage requirements were sufficiently powered to detect the difference between wild type and the combination of all other genotypes (Table 5.10) However, like the

pharmacokinetic studies, the power of comparisons between specific genotypes was much lower. For example, with a study size of 121, Kamali *et al.* (2004) had a power of around 82% to differentiate the pharmacodynamics of *(S)*-warfarin between the wild type and the *1/*3 genotype. All other comparisons had powers of less than 80%. The experimental observations were fully consistent with these values of power (Table 5.10).

When 'genotype adjusted' conditions were considered, a power of only 60% to detect differences in the AUEC between the *CYP2C9* wild type and a combination of any other genotype was reached at the maximum study size of 550. Thus, the simulations suggest that if INR is used by prescribers to adjust the dose of warfarin, any differences between genotypes in respect to pharmacological effects are likely to disappear. Of the four studies that investigated differences in the INR of warfarin between *CYP2C9* genotypes, two were carried out during the initialization phase of therapy, before INR can be used to adjust dosage (Lindh *et al.*, 2005; Peyvandi *et al.*, 2004), and the other two studies find no significant difference in INR between the genotypes (Khan *et al.*, 2004; Loebstein *et al.*, 2001). Thus, when a good biomarker is available, as is the case for warfarin, emphasis should perhaps be on monitoring and adjustment the dose during maintenance therapy. However, the introduction of new methods to improve the prediction of initial dose requirements (*e.g.* genotyping) are likely to be useful.

It has recently been shown that although other sources of variability such as polymorphism in the vitamin K epoxide reductase (VKORC1) gene can explain some of the variability in the maintenance dose of acenocoumarol (21.4%), the time to achieve adequate and stable anticoagulation was associated with *CYP2C9* but not VKORC1 genotype (Schalekamp *et al.*, 2006). A similar outcome may apply to warfarin therapy. Our simulations have attempted to determine the increase in study power (with respect to the influence of CYP2C9 genotype) as a result of having prior knowledge of the *VKORC1* genotype (Figure 3).

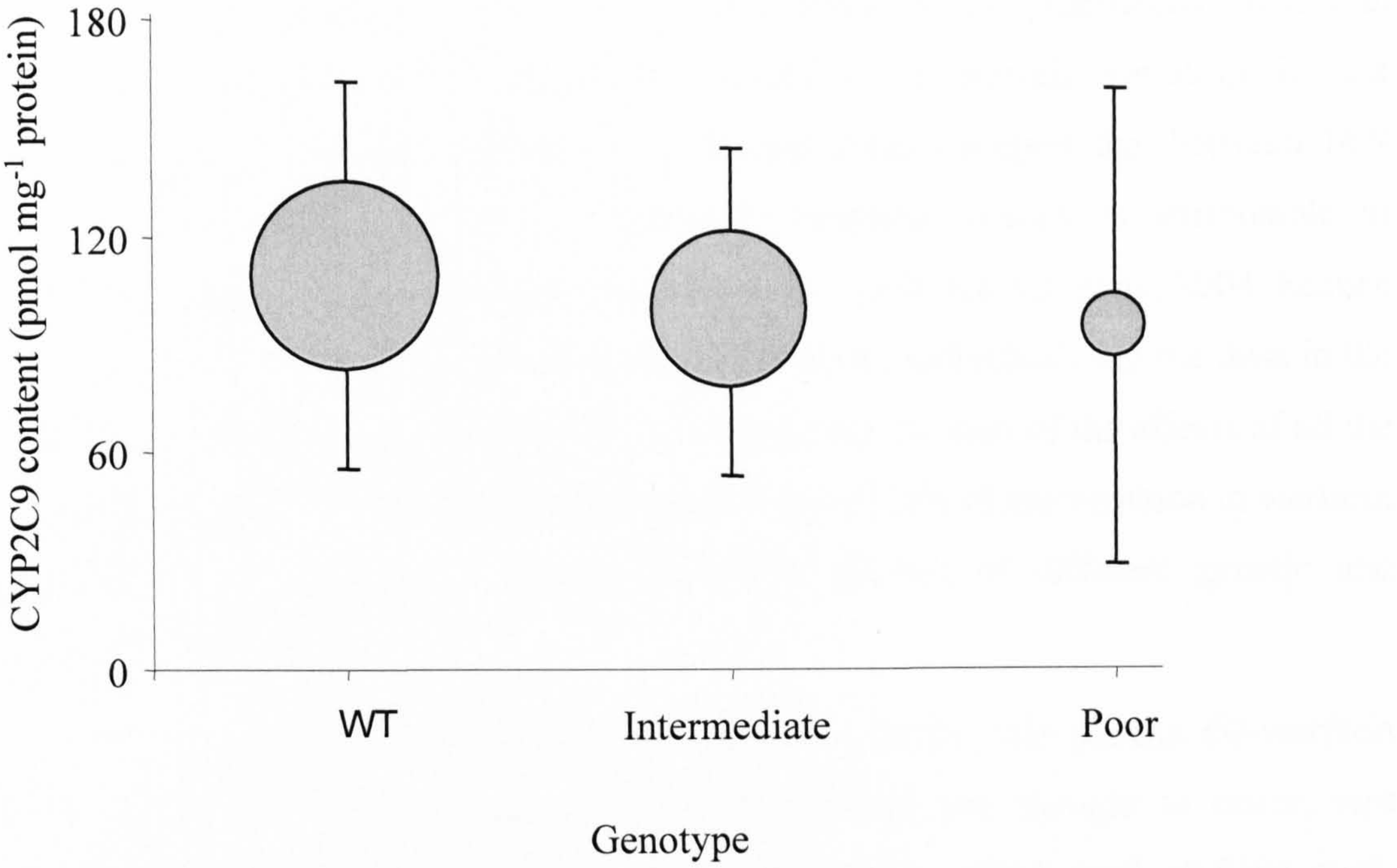


Figure 5.16 A meta-analysis of *CYP2C9* expression for wild type (*1*1), intermediate (*1*2 and *1*3) and poor (*2*2, *2*3 and *3*3) metaboliser genotypes. Data are expressed as mean ± SD, and the size of the circles indicate the number of observations.

Even when the effect of *CYP2C9* genotype on pharmacodynamics is known, the contribution of metabolic variability to overall variation in the pharmacological response could be small. Recent studies suggest that between 16.9 and 19.8% of the variability in response to warfarin therapy is attributable to polymorphisms of the *CYP2C9* gene (Hillman *et al.*, 2004; Kamali *et al.*, 2004; Sconce *et al.*, 2005). Although this proportion may seem small, individualising the dose in the absence of good biomarker may require accounting for the sum of the effects of all the covariates. Aquilante *et al.* (2006) have reported that 51.4% of the variation in warfarin dose can be attributed to a combination of a number of different genetic and environmental factors.

A simple threshold model, defining the steady state plasma *(S)*-warfarin concentration above which, serious ADRs to warfarin are thought to occur, was developed. The threshold total steady state concentration of 0.8 mg/L (0.0104 mg/L unbound) was obtained, and should be compared with the mean steady state concentration in patients of 0.347 ± 0.22 mg/L reported by (Henne *et al.*, 1998), and the unbound steady state concentration of 0.0036 ± 0.0019 mg/L reported by Scordo *et al.* (2002). The relatively small CV of 6% suggests that such a threshold value could prove useful in the clinic. It is perhaps surprising that, given the number of ADRs that occur as a result of warfarin treatment, a threshold concentration has not already been defined. This is possibly because a good biomarker is already available in the form of the INR. However, there is still a significant delay between the commencement of warfarin therapy and the availability of the first INR value. Thus, measurement of plasma concentrations may provide a faster approach to dosage adjustment. However, the threshold concentration calculated in this study and its potential use in preventing ADRs should be validated with further studies.

In conclusion, the results of this study have provided a new perspective for investigating the influence of CYP2C9 polymorphisms on the pharmacokinetics and pharmacodynamics of warfarin. The results on genotype adjusted dosage, demonstrate how the use of a biomarker will dampen any differences in response between *CYP2C9* genotypes and therefore calls into question the validity of genetic screening for dose maintenance. However, genotyping is undoubtedly of value for some individuals during the initiation of therapy with warfarin.

CHAPTER 6

The Propagation of Pharmacogenetic Differences in Cytochrome P450 into Pharmacokinetic & Pharmacodynamic Measures: The Example of CYP3A5 & Midazolam

6 MIDAZOLAM

6.1 Introduction

Midazolam (MDZ) is a benzodiazepine drug with several therapeutic properties including sedative, hypnotic, anticonvulsant, muscle-relaxant and anxiolytic effects (Pieri, *et al.*, 1981). The effects of MDZ on the human central nervous system can be quantified in a number of different ways as the drug affects performance on psychophysiologic tests such as reaction time (Mould, *et al.*, 1995) or the electroencephalographic (EEG) effect (Koopmans, *et al.*, 1988) by several different mechanisms.

The effect of MDZ on the EEG has proven useful as a measure of the central nervous system pharmacodynamic effects of the drug due to its accuracy, ease of quantification (Koopmans, *et al.*, 1988) and objective nature (Thakor, *et al.*, 2004). An EEG is a record of the electrical activity from the scalp, obtained with the aid of an array of electrodes (Thakor, *et al.*, 2004). Such electrical activity is generally the result of excitatory postsynaptic potentials which may be suppressed by the administration of MDZ (Thakor, *et al.*, 2004).

CYP3A4 and CYP3A5 are the only enzymes which contribute to the metabolism of MDZ. As such, the metabolism of MDZ to its primary metabolite is used as a probe reaction for the activity of these enzymes both *in vitro* (He, *et al.*, 2005; Masica, *et al.*, 2004; Tateishi, *et al.*, 2001) and *in vivo* (Tucker, *et al.*, 2001). CYP3A5, a polymorphic enzyme, has three common genetic variants in the Caucasian population which lead to reduced CYP3A5 expression and/or the expression of non-functional protein. If CYP3A5 is present in a human liver, it may contribute as much as 50% to hepatic CYP3A-mediated metabolism of MDZ (Kuehl, *et al.*, 2001).

MDZ is metabolised to two metabolites in humans (Figure 6.1), the main metabolite, α-hydroxy midazolam (α-OH MDZ) constitutes around 75 to 90% of the total metabolites with 4-hydroxy midazolam (4-OH MDZ) with the secondary metabolite 1',4-dihydroxymidazolam making up the rest (Desmeules, *et al.*, 1991; Mandema, *et al.*, 1992; von Moltke, *et al.*, 1996).

α-OH MDZ and 4-OH MDZ are both excreted in the urine, however, the main route of elimination is *via* glucuronidation of α-OH MDZ followed by excretion of the glucuronidate in urine (Fabre, *et al.*, 1988). The role of α-OH MDZ in eliciting the effects of MDZ is somewhat unclear and may vary depending on the method used to monitor outcome. However, while it is generally accepted that α-OH MDZ is likely to contribute to the nervous system effects of MDZ (Crevoisier, *et al.*, 1983; Ziegler, *et al.*, 1983), some authors have found evidence to the contrary (Greenblatt & Shader, 1986; Koopmans, *et al.*, 1988).

As discussed in the literature review (Chapter 2; Section 2.4.3.3), results from literature studies which describe attempts to characterise the relationship between the CYP3A5 polymorphism and the *in vivo* pharmacokinetics of MDZ are somewhat contradictory. While some studies have detected a significant influence of *CYP3A5* genotype on the pharmacokinetics of MDZ after an oral dose of the drug (Wong, *et al.*, 2004), others have been unable to replicate these results (Eap, *et al.*, 2004; Floyd, *et al.*, 2003).

A possible reason for the conflicting results with regard to the effect of *CYP3A5* genotype on the pharmacokinetics of MDZ is the differing size of the studies and therefore their possibly varying powers. Therefore, the aim of this section of the research project was to simulate the pharmacokinetics and pharmacodynamics of MDZ in virtual populations of human subjects. These simulations were used to mimic the *in vivo* studies and to estimate their power to differentiate the pharmacokinetics and pharmacodynamics of MDZ between *CYP3A5* genotypes. The effect of sample size on the power of such studies will be investigated.

Since the activity of α-OH MDZ compared to MDZ is an issue of some contention, the models described in this chapter were also used to investigate the outcome of studies should the proportional pharmacological activity of OH MDZ relative to MDZ be altered.

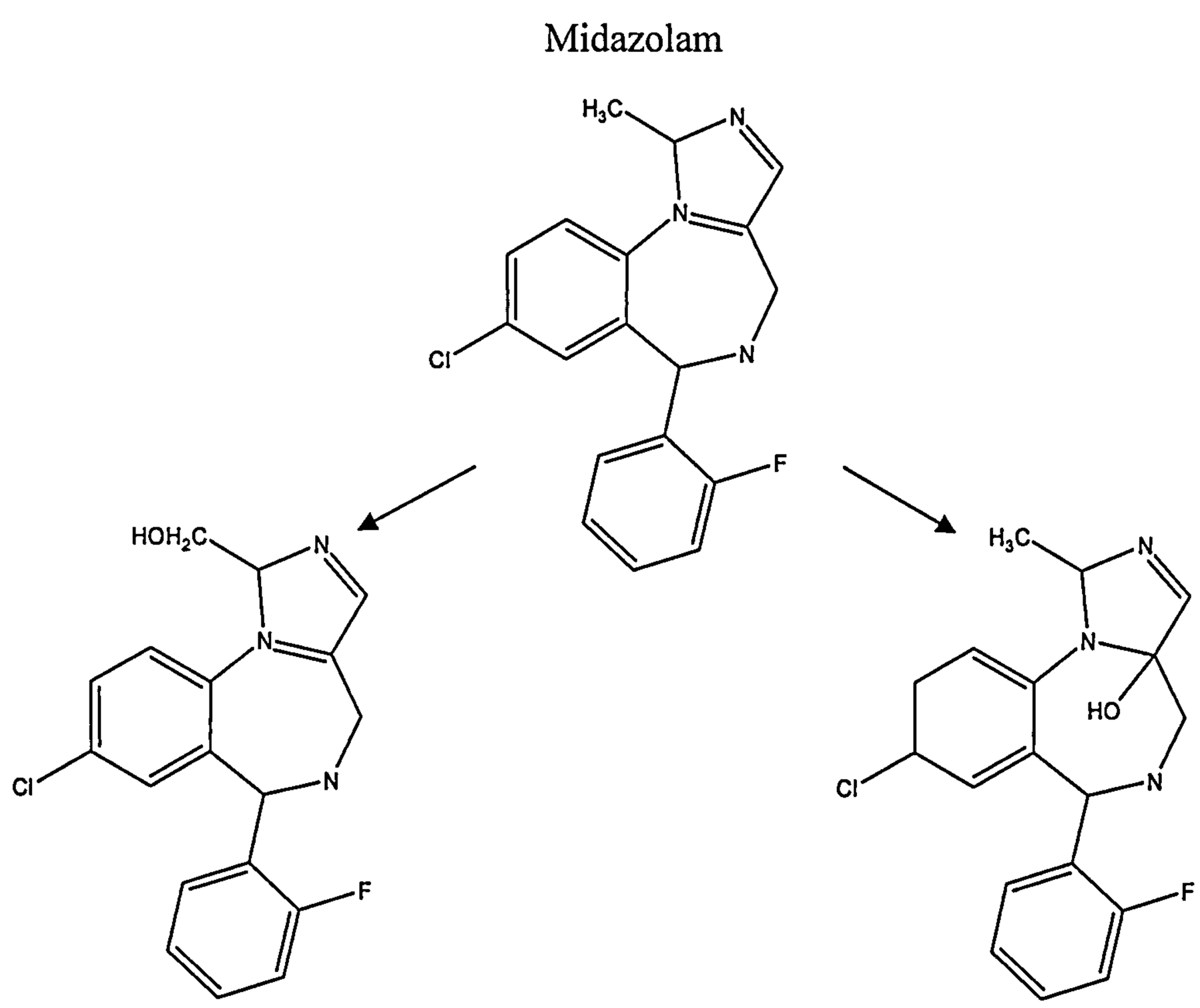


Figure 6.1 The chemical structure of MDZ and its two primary metabolites, α-OH MDZ and 4-OH MDZ.

6.2 Materials and Methods

6.2.1 The IVIVE Model Parameters

As described in Chapter 3, as part of the IVIVE process, several key parameters are required for input into the model. These include both *in vitro* parameters (such as K_m and V_{max}) and parameters gathered from *in vivo* experiments (such as CL_R). These were collected from the literature and either entered directly into the model or utilised as part of a meta-analysis. The following section describes the parameters and their uses.

6.2.1.1 *In Vitro* Data

Data on the *in vitro* metabolism of MDZ (in the form of K_m and V_{max}) were collected from published reports (Galetin, *et al.*, 2004; Huang, *et al.*, 2004; Williams, *et al.*, 2002) (Table 6.1).

Intersystem extrapolation factors (ISEFs), were applied as described by Proctor *et al.* (2004) to account for any differences between the intrinsic activity of recombinantly expressed CYP enzymes and human liver microsomes (Table 6.2). To account for the non-specific binding of MDZ in the experiments described in Table 6.1, a value of fu_{mic} was calculated (Table 6.2) and applied to the data as described in Chapter 3 (Sections 3.1.1.2 and 3.3). A meta-analysis was then conducted to find the overall V_{max} and K_m values for the metabolism of MDZ by each relevant CYP isoform.

6.2.1.2 Additional Parameters Required for IVIVE

The additional parameters listed in Table 6.3 were required for scaling the *in vitro* data to human whole body clearance and to the elimination rate constant (Chapter 3). These values were then entered into the pharmacokinetic-pharmacodynamic model described below (Sections 6.2.2 & 6.2.3).

Table 6.1 Parameters describing the *in vitro* metabolism of MDZ.

		α-OH-MDZ		4-OH	
Reference	**CYP**	**Vmax**	**Km**	**Vmax**	**Km**
Galetin *et al.*, 2004	3A4	1.96	2.69	2.52	29.0
	3A5	6.7	10.7	0.52	12.1
Williams *et al.*, 2002	3A4	35	5	40	59
	3A5	72	14	17	116
Huang *et al.*, 2004	3A4	5.18	2.82	1.35	8.44
	3A5	20	3.56	1.65	11.5

V_{max} = pmol min^{-1} $pmol^{-1}$ CYP; K_m = μM.

Table 6.2 ISEF and fu_{mic} values that were applied to the experimental data shown in Table 6.1 resulting in the revised V_{max} and K_m values shown here. The final values entered into the IVIVE algorithms are indicated in bold type at the bottom of the table.

						α-OH-MDZ formation		4-OH formation	
Reference	Expression System	CYP	Protein Conc. (mg/mL)	fu_{mic}	ISEF	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)
Galetin *et al.*, 2004	Baculovirus	3A4	0.05-0.2	0.983	4.92	9.6	2.64	12.4	28.5
		3A5				32.9	10.5	2.7	11.9
Williams *et al.*, 2002	Baculovirus	3A4	0.15	0.980	4.92	19.1	4.9	21.2	57.8
		3A5				39.4	13.7	9.3	113.7
Huang *et al.*, 2004	Baculovirus	3A4	0.0118	0.998	4.92	6.1	2.8	1.6	8.4
		3A5				23.5	3.6	1.9	11.5
Weighted Values		**3A4**				**11.6**	**3.5**	**11.7**	**31.6**
		3A5				**31.9**	**9.3**	**4.6**	**45.7**

Table 6.3 Mean pharmacokinetic parameter values of MDZ taken or calculated from the literature.

Parameter	Value	References
fu	0.034	Allonen, *et al.*, 1981; DJ Greenblatt, *et al.*, 1986; D. J. Greenblatt, *et al.*, 1984; Moschitto, *et al.*, 1983; Thummel, *et al.*, 1996; Section 3.1.1.3.
B:P	0.55	Allonen, *et al.*, 1981; Heizmann, *et al.*, 1983; Section 3.1.1.3.
fu_B	0.062	Section 3.1.1.3.
CL_R (L/h)	0.085	Allonen, *et al.*, 1981; D. J. Greenblatt, *et al.*, 1984; Heizmann, *et al.*, 1983; Ibrahim, *et al.*, 2002; Klotz, *et al.*, 1982; Thummel, *et al.*, 1996; Wandel, *et al.*, 2000; Section 3.3.3.
V (L/kg)	1.11 (0.25)*	Knoester, *et al.*, 2002; Section 3.3.4.

*Mean (SD); fu = fraction unbound in plasma; B:P = Blood to Plasma concentration drug ratio; fu_B = fraction unbound in blood; CL_R = renal clearance; V = initial volume of distribution.

Table 6.4 Additional literature values describing the pharmacokinetics of MDZ and α-OH-MDZ. All values are from Knoester *et al.* (2002) except for Q_{gut} which was from Yang *et al.* (2004).

Parameter	Value*
Q_{gut} (L/h)	20
V (L/kg)	1.11 (0.25)
k_a (h^{-1})	10.8 (3.18)
tlag (h)	0.014 (0.012)
k_{12} (min^{-1})	0.075 (0.018)
k_{21} (min^{-1})	0.206 (0.069)
$K_{OH\text{-}MDZ}$ (h^{-1})	0.216 (0.09)

*Mean (SD)

6.2.2 Pharmacokinetic Model

6.2.2.1 Concentration of MDZ in the Systemic Compartment

Plasma concentrations of MDZ ($C(t)_i$) in each individual were generated using a two compartment model with first order absorption and a lag time (Knoester, *et al.*, 2002):

$$C(t)_i = \frac{D \cdot k_{a,i} \cdot F_{H,i} \cdot f_a \cdot F_{G,i}}{V_i} \cdot (A1_i + A2_i + A3_i) \qquad \text{Equation 6.1}$$

where f_a is the oral bioavailability of drug (set to 1), D is the oral dose of MDZ (5mg), $k_{a,i}$, $F_{H,i}$ and V_i are the first order absorption rate constant (Table 6.4), fraction of the amount of drug reaching the liver escaping first pass hepatic metabolism (calculated using Simcyp algorithms; Chapter 3) and the central volume of distribution of drug (Table 6.4) respectively, in each individual and $A1_i$, $A2_i$ and $A3_i$ are given by:

$$A1_i = \frac{(k_{21,i} - \lambda_{1,i}) \cdot e^{-\lambda_{1,i} \cdot t\text{-}tlag_i}}{(\lambda_{2,i} - \lambda_{1,i}) \cdot (k_{a,i} - \lambda_{1,i})} \qquad \text{Equation 6.2}$$

$$A2_i = \frac{(k_{21,i} - \lambda_{2,i}) \cdot e^{-\lambda_{2,i} \cdot t\text{-}tlag_i}}{(\lambda_{1,i} - \lambda_{2,i}) \cdot (k_{a,i} - \lambda_{2,i})} \qquad \text{Equation 6.3}$$

$$A3_i = \frac{(k_{21,i} - k_{a,i}) \cdot e^{-k_{a,i} \cdot t\text{-}tlag_i}}{(\lambda_{1,i} - k_{a,i}) \cdot (\lambda_{2,i} - k_{a,i})} \qquad \text{Equation 6.4}$$

where $k_{21,i}$ is the transfer rate constant from the peripheral to the central compartment and α_i and $\beta_{,i}$ are the hybrid rate constants associated with the distribution and elimination phases, respectively, as defined in Chapter 4; Equations 4.5 and 4.6

6.2.2.2 Concentration of α-OH MDZ in the Systemic Compartment

Equation 6.5 describes the plasma concentration – time course of α-OH MDZ:

$$C(t)_{OH\text{-}MZ,i} = C(t)_{OH\text{-}MDZ(1st\,pass),i} + C(t)_{OH\text{-}MDZ(systemic),i} \qquad \text{Equation 6.5}$$

where $C(t)_{OH\text{-}MDZ(1st\ pass),i}$ and $C(t)_{OH\text{-}MDZ(systemic),i}$ are first-pass and systemic components. These components were described by Equations 6.6 and 6.11, respectively:

$$C(t)_{OH\text{-}MDZ(systemic),i} = \frac{Dm \cdot f_a \cdot F_{G,i} \cdot F_{H,i} \cdot k_{a,i} \cdot fm_i \cdot k_{10,i}}{V_{OH\text{-}MDZ,i}} \cdot (A1m_i + A2m_i + A3m_i + A4m_i) \qquad \text{E 6.6}$$

where $V_{OH\text{-}MDZ,i}$ is the volume of distribution of α-OH MDZ (Table 6.4). Assuming α-OH MDZ is the only metabolite of MDZ [accounting for around 75 - 95% of the total (Desmeules, *et al.*, 1991; Mandema, *et al.*, 1992; von Moltke, *et al.*, 1996)] and that no further metabolism of α-OH MDZ occurs (Gorski, *et al.*, 1994), the following equation was used to determine the fraction of MDZ converted to α-OH MDZ after an *iv* bolus dose in i^{th} individual (fm):

$$fm_i = \frac{CLm_{\alpha MZ,i}}{CL_i} \qquad \text{Equation 6.7}$$

where $CLm_{\alpha MZ,i}$ is the total metabolic clearance of MDZ to α-OH MDZ in the i^{th} individual, and CL_i is the total clearance of MDZ, including renal clearance in the i^{th} individual (both values were estimated using the Simcyp® algorithm). $A1m_i$, $A2m_i$, $A3m_i$ and $A4m_i$ are given by:

$$A1m_i = \frac{(k_{21,i} - \alpha_i) \cdot e^{-\alpha_i \cdot t\text{-}tlag_i}}{(\beta_i - \alpha_i) \cdot (k_{a,i} - \alpha_i) \cdot (k_{(OH\text{-}MDZ),i} - \alpha_i)} \qquad \text{Equation 6.8}$$

$$A2m_i = \frac{(k_{21,i} - \beta_i) \cdot e^{-\beta_i \cdot t\text{-}tlag_i}}{(\alpha_i - \beta_i) \cdot (k_{a,i} - \beta_i) \cdot (k_{(OH\text{-}MDZ),i} - \beta_i)} \qquad \text{Equation 6.9}$$

$$A3m_i = \frac{(k_{21,i} - k_{a,i}) \cdot e^{-k_{a,i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{a,i}) \cdot (\beta_i - k_{a,i}) \cdot (k_{(OH\text{-}MDZ),i} - k_{a,i})} \qquad \text{Equation 6.10}$$

$$A4m_i = \frac{(k_{21,i} - k_{(\alpha MZ),i}) \cdot e^{-k_{(OH\text{-}MDZ),i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{(OH\text{-}MDZ),i}) \cdot (\beta_i - k_{(OH\text{-}MDZ),i}) \cdot (k_{a,i} - k_{(OH\text{-}MDZ),i})} \qquad \text{Equation 6.11}$$

where $k_{(OH\text{-}MDZ),i}$ is the elimination rate constant of α-OH MDZ (Table 6.4).

The plasma concentration of α-OH MDZ contributed by its first-pass formation is given by:

$$C(t)_{OH\text{-}MDZ(1st\,pass),i} = \frac{Dm \cdot f_a \cdot (1 - F_{G,i} \cdot F_{H,i}) \cdot k_{a,i} \cdot F_{H,i}}{V_{OH\text{-}MDZ,i} \cdot (k_{OH\text{-}MDZ,i} - k_{a,i})} \cdot \left[e^{-k_{a,i} \cdot t\text{-}tlag_i} - e^{-k_{OH\text{-}MDZ,i} \cdot t\text{-}tlag_i} \right] \qquad \text{Equation 6.12}$$

6.2.2.3 Concentration of MDZ in the Effect Compartment

The following equation was used to calculate the concentration of MDZ in a hypothetical effect compartment:

$$Ce(t)_i = \frac{D \cdot k_{a,i} \cdot k_{e0,i} \cdot F_{H,i} \cdot f_a \cdot F_{G,i}}{V_i} \cdot (A1_i + A2_i + A3_i + A4_i) \qquad \text{Equation 6.13}$$

where $k_{e0,i}$ is the rate constant defining removal of drug from the effect compartment (Table 6.5). $A1_i$, $A2_i$, $A3_i$ and $A4_i$ are given by:

$$A1_i = \frac{(k_{21,i} - \alpha_i) \cdot e^{-\alpha_i \cdot t\text{-}tlag_i}}{(\beta_i - \alpha_i) \cdot (k_{a,i} - \alpha_i) \cdot (k_{e0,i} - \alpha_i)} \qquad \text{Equation 6.14}$$

$$A2_i = \frac{(k_{21,i} - \beta_i) \cdot e^{-\beta_i \cdot t\text{-}tlag_i}}{(\alpha_i - \beta_i) \cdot (k_{a,i} - \beta_i) \cdot (k_{e0,i} - \beta_i)} \qquad \text{Equation 6.15}$$

$$A3_i = \frac{(k_{21,i} - k_{a,i}) \cdot e^{-k_{a,i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{a,i}) \cdot (\beta_i - k_{a,i}) \cdot (k_{e0,i} - k_{a,i})} \qquad \text{Equation 6.16}$$

$$A4_i = \frac{(k_{21,i} - k_{e0,i}) \cdot e^{-k_{e0,i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{e0,i}) \cdot (\beta_i - k_{e0,i}) \cdot (k_{a,i} - k_{e0,i})} \qquad \text{Equation 6.17}$$

6.2.2.4 Concentration of α-OH MDZ in the Effect Compartment

Equation 6.18 was used to calculate the concentration of α-OH MDZ in the same hypothetical effect compartment as MDZ:

$$Ce(t)_{OH\text{-}MDZ,i} = Ce(t)_{OH\text{-}MDZ(1st\,pass),i} + Ce(t)_{OH\text{-}MDZ(systemic),i} \qquad \text{Equation 6.18}$$

where $Ce(t)_{OH\text{-}MDZ(1st\ pass),i}$ and $Ce(t)_{OH\text{-}MDZ(systemic),i}$ are first-pass and systemic components. These components were described by Equations 6.19 and 6.25, respectively:

$$Ce(t)_{OH\text{-}MDZ(systemic),i} = \frac{Dm \cdot f_a \cdot F_{G,i} \cdot F_{H,i} \cdot k_{a,i} \cdot fm_i \cdot k_{10,i}}{V_{OH\text{-}MDZ,i}} \cdot (A1m_i + A2m_i + A3m_i + A4m_i + A5m_i) \quad \text{E 6.19}$$

where the rate of removal of α-OH MDZ from the effect compartment was assumed to be the same as that for MDZ (k_{e0} - Table 6.5). $A1m_i$, $A2m_i$, $A3m_i$, $A4m_i$ and $A5m_i$ are given by:

$$A1m_i = \frac{(k_{21,i} - k_{(OH\text{-}MDZ),i}) \cdot e^{-k_{(OH\text{-}MDZ),i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{(OH\text{-}MDZ),i}) \cdot (\beta_i - k_{(OH\text{-}MDZ),i}) \cdot (k_{a,i} - k_{(OH\text{-}MDZ),i}) \cdot (k_{e0,i} - k_{(OH\text{-}MDZ),i})} \quad \text{Equation 6.20}$$

$$A2m_i = \frac{(k_{21,i} - \alpha_i) \cdot e^{-\alpha_i \cdot t\text{-}tlag_i}}{(\beta_i - \alpha_i) \cdot (k_{a,i} - \alpha_i) \cdot (k_{e0,i} - \alpha_i) \cdot (k_{(OH\text{-}MDZ),i} - \alpha_i)} \quad \text{Equation 6.21}$$

$$A3m_i = \frac{(k_{21,i} - \beta_i) \cdot e^{-\beta_i \cdot t\text{-}tlag_i}}{(\alpha_i - \beta_i) \cdot (k_{a,i} - \beta_i) \cdot (k_{e0,i} - \beta_i) \cdot (k_{(OH\text{-}MDZ),i} - \beta_i)} \quad \text{Equation 6.22}$$

$$A4m_i = \frac{(k_{21,i} - k_{a,i}) \cdot e^{-k_{a,i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{a,i}) \cdot (\beta_i - k_{a,i}) \cdot (k_{e0,i} - k_{a,i}) \cdot (k_{(OH\text{-}MDZ),i} - k_{a,i})} \quad \text{Equation 6.23}$$

$$A5m_i = \frac{(k_{21,i} - k_{e0,i}) \cdot e^{-k_{e0,i} \cdot t\text{-}tlag_i}}{(\alpha_i - k_{e0,i}) \cdot (\beta_i - k_{e0,i}) \cdot (k_{a,i} - k_{e0,i}) \cdot (k_{(OH\text{-}MDZ),i} - k_{e0,i})} \quad \text{Equation 6.24}$$

The contribution of the first-pass formation of α-OH MDZ to its effect compartment concentration was described by:

$$Ce(t)_{OH\text{-}MDZ(1st\,pass),i} = \frac{Dm \cdot f_a \cdot (1 - F_{G,i} \cdot F_{H,i}) \cdot k_{a,i} \cdot F_{H,i} \cdot k_{e0,i}}{V_{OH\text{-}MDZ,i}} \cdot (A1m_i + A2m_i + A3m_i) \quad \text{E 6.25}$$

where:

$$A1_i = \frac{e^{-k_{(\alpha MZ),i} \cdot t\text{-}tlag_i}}{(k_{e0,i} - k_{(\alpha MZ),i}) \cdot (k_{a,i} - k_{(\alpha MZ),i})} \quad \text{Equation 6.26}$$

$$A2_i = \frac{e^{-k_{e0,i} \cdot t\text{-}tlag_i}}{\left(k_{(\alpha MZ),i} - k_{e0,i}\right) \cdot \left(k_{a,i} - k_{e0,i}\right)}$$ Equation 6.27

$$A3_i = \frac{e^{-k_{a,i} \cdot t\text{-}tlag_i}}{\left(k_{e0,i} - k_{a,i}\right) \cdot \left(k_{(\alpha MZ),i} - k_{a,i}\right)}$$ Equation 6.28

6.2.3 Pharmacodynamic Model

Concentrations of MDZ and α-OH MDZ were calculated in a hypothetical effect compartment (Equations 6.13 & 6.18). The pharmacokinetic and pharmacodynamic data were linked assuming an excitatory sigmoidal E_{max} (EEG activity) model such that the combined effects of MDZ and α-OH MDZ on the human central nervous system ($E(t)_i$) were modelled assuming an additive interaction between the two chemical moieties (Mandema, *et al.*, 1992) (Equation 6.29):

$$E(t)_i = E_0 + \left[\frac{\left(E_{max.1} - E_0\right) \cdot \left(Ce(t)_i\right)^{\gamma_i}}{\left(EC_{50.1,i}\right)^{\gamma_i} + \left(Ce(t)_i\right)^{\gamma_i}}\right] + \left[\frac{\left(E_{max.2} - E_0\right) \cdot \left(Ce(t)_{OH\text{-}MDZ,i}\right)^{\gamma_i}}{\left(EC_{50.2,i}\right)^{\gamma_i} + \left(Ce(t)_{OH\text{-}MDZi}\right)^{\gamma_i}}\right]$$ Equation 6.29

where E_0 is the baseline EEG effect (%) in the absence of drug (Table 6.5), E_{max} is the maximum drug effect (Table 6.5) and $Ce(t)_i$, and $Ce(t)_{OH\text{-}MDZ,i}$ are the concentration of active moiety (MDZ) and metabolite (α-OH MDZ), respectively, $EC_{501,i}$, and $EC_{502,i}$ are the concentrations of parent and metabolite, respectively, in the effect compartment which are associated with half the E_{max} (Table 6.5) and γ_i is the Hill-coefficient (Table 6.5), in each individual.

6.2.4 Study Design

Individual plasma MDZ and α-OH MDZ concentration and response *vs.* time profiles following administration of oral MDZ were simulated for all time points between 0 and 5h. For the purposes of data-analysis, samples were taken every 15 minutes (20 samples). Simulated plasma drug concentration- and response-time profiles in expressors and non-expressors of CYP3A5 were compared using a range of population sizes (n). For all the simulations n was set at 5, 10, 20, 50, 100, 200 or 400. Twenty simulations were run for each study size.

Table 6.5 Literature values describing the pharmacodynamics of MDZ. All parameters were from Mandema *et al.* (1988), except for E_0 and E_{max} which were from Koopmans *et al.* (1988).

Parameter	**Value***
E_0 (%)	100
E_{max1} (%)	36
E_{max2} (%)	42
EC_{501} (ng/ml)	77 (15)
EC_{502} (ng/ml)	98 (17)
n_1	3.1 (0.3)
n_2	3.1 (0.5)
k_{e01} (min^{-1})	0.77 (0.23)
k_{e02} (min^{-1})	0.56 (0.08)

*Mean (SD)

6.2.5 Sensitivity of Study Power to the Activity of α-OH MDZ

In addition to the simulations and analysis described above (which will now be referred to as the 'default setting'); further simulations were carried out to assess the sensitivity of study power to the activity of α-OH MDZ.

Simulations were carried out under two separate conditions: (i) α-OH MDZ was assumed to be pharmacologically inactive and have no effect on the EEG measurement and, (ii) α-OH MDZ was assumed to be active and have a different EC_{50} value to MDZ. Based on the analysis of Mandema *et al.* (1992) the activity of α-OH MDZ was assumed to be 78% that of MDZ.

Overall, 140 simulations were carried out using up to 400 subjects in each study set (a total of 15, 700 virtual subjects).

6.2.6 Data Analysis

Values of AUC and AUEC (decrease in amplitude of the EEG measure) up to 5 hours were calculated using the trapezoidal rule. For the purposes of this study we assumed that no measurement error occurred. The probability of detecting statistically significant differences in pharmacokinetics and pharmacodynamics between phenotypes was assessed using ANOVA (SPSS v 12; SPSS Inc. 2003). The number of studies out of the 20 simulations that led to a statistically significant difference in AUC (or AUEC) between expressors and non-expressors of CYP3A5 was recorded as the power of that particular trial.

Clearance values and other pharmacokinetic parameters obtained from the IVIVE model were compared with those values observed *in vivo*. The accuracy of the resulting concentration- and effect-time profiles were assessed by comparing them with those observed *in vivo* by Mandema *et al.* (1992) and Wandel *et al.* (2000). The EEG effect of MDZ *vs* time was validated by comparison with that observed *in vivo* by Koopmans *et al.* (1988). In this case, the metabolite was assumed to be inactive to match the assumptions of Koopmans *et al.* (1988) in their study.

6.3 Results

6.3.1 Model Validation

Comparisons of observed (Mandema, *et al.*, 1992; Wandel, *et al.*, 2000) and simulated plasma concentration - time curves in a Caucasian population of MDZ are shown in Figure 6.2 and Figure 6.3, respectively.

A comparison of observed (Koopmans, *et al.*, 1988) and simulated effect - time curves (in randomly selected individuals) is shown in Figure 6.4.

A summary of some mean (± SD) simulated pharmacokinetic parameters that describe the data in Caucasian populations of subjects compared with the weighted means of those reported in the literature are given in Table 6.6.

6.3.2 Power of Studies

The power of studies to detect a statistically significant difference in the AUC of MDZ between CYP3A5 expressors and non-expressors was around 80% with 240 subjects (Figure 6.5). 100% power was reached when 400 subjects were used. Around 320 subjects were needed to differentiate a difference in the pharmacokinetics of OH-MDZ between expressors and non-expressors of CYP3A5 (Figure 6.5).

6.3.2.1 Midazolam Only Active

When MDZ itself was assumed to be the only active moiety, around 360 subjects were required to achieve 80% power to observe a difference in the pharmacodynamic effect (AUEC) of MDZ between expressors and non-expressors of CYP3A5 (Figure 6.6).

6.3.2.2 Midzolam and α-OH MDZ Contribute to EEG Effect

When α-OH MDZ was assumed have 78% the activity of MDZ (Mandema, *et al.*, 1992), the power of studies to differentiate a difference in the pharmacodynamics of MDZ between expressors and non-expressors of CYP3A5 was reduced. Power was 55% with the maximum number of subjects studied, 400 (Figure 6.6).

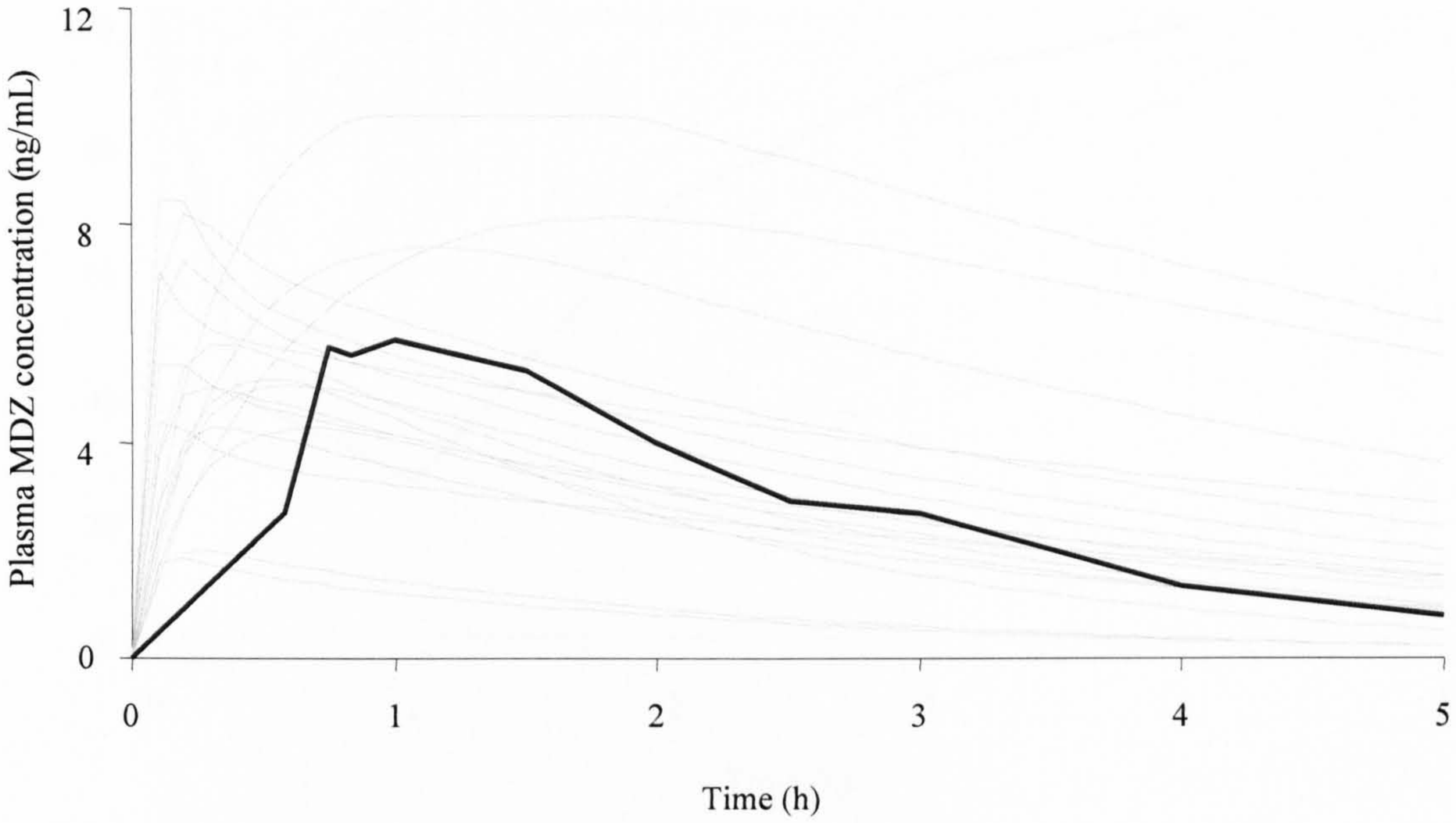


Figure 6.2 Observed (Mandema, *et al.*, 1992); (in 8 real subjects; ▬) and predicted (in 20 virtual subjects; ▬) plasma concentration-time profiles of MDZ over 5 hours in Caucasian individuals of unspecified phenotype.

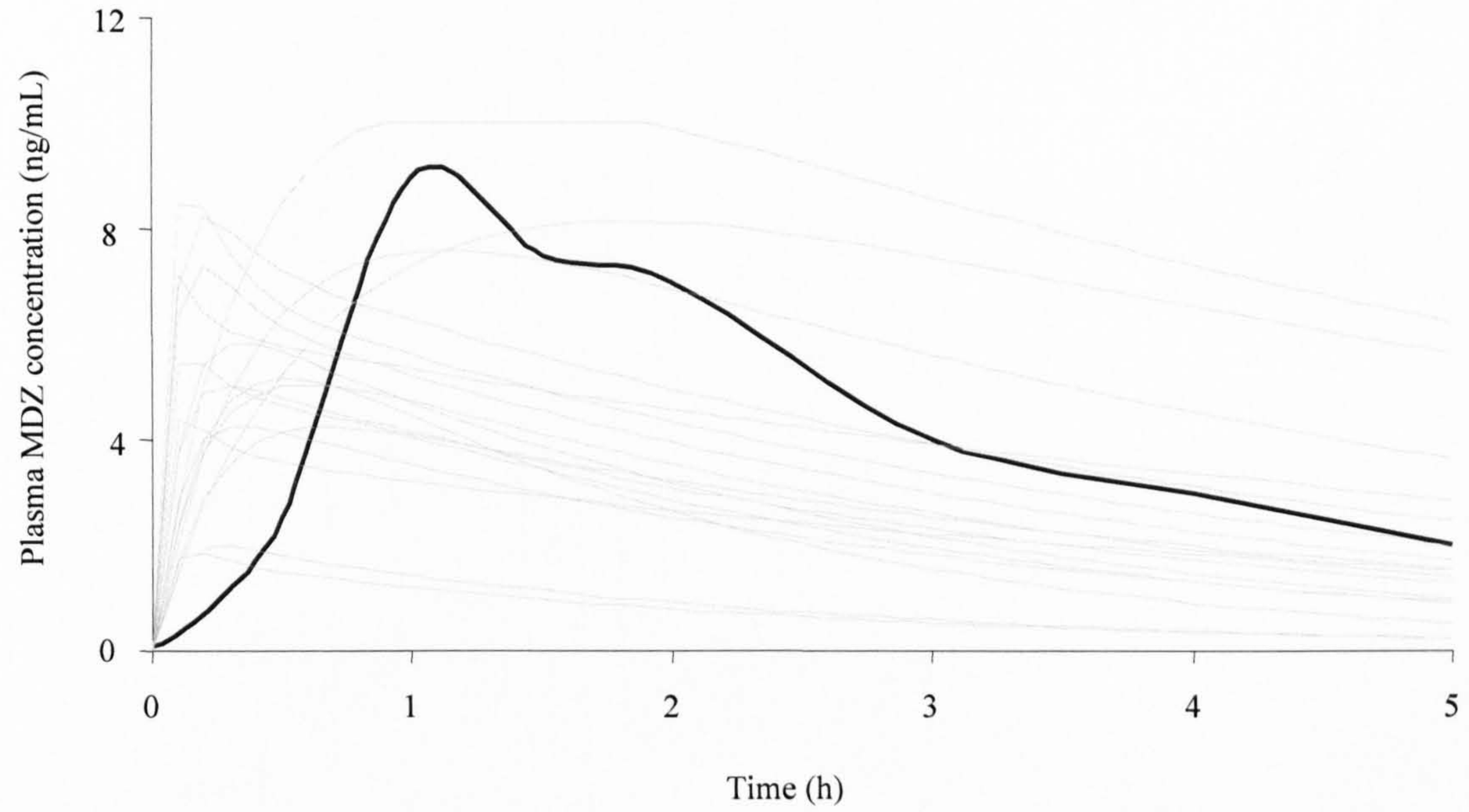


Figure 6.3 Observed (Wandel, *et al.*, 2000); (in 15 real subjects; ▬) and predicted (in 20 virtual subjects; ▬) plasma concentration-time profiles of MDZ over 5 hours in Caucasian individuals of unspecified phenotype.

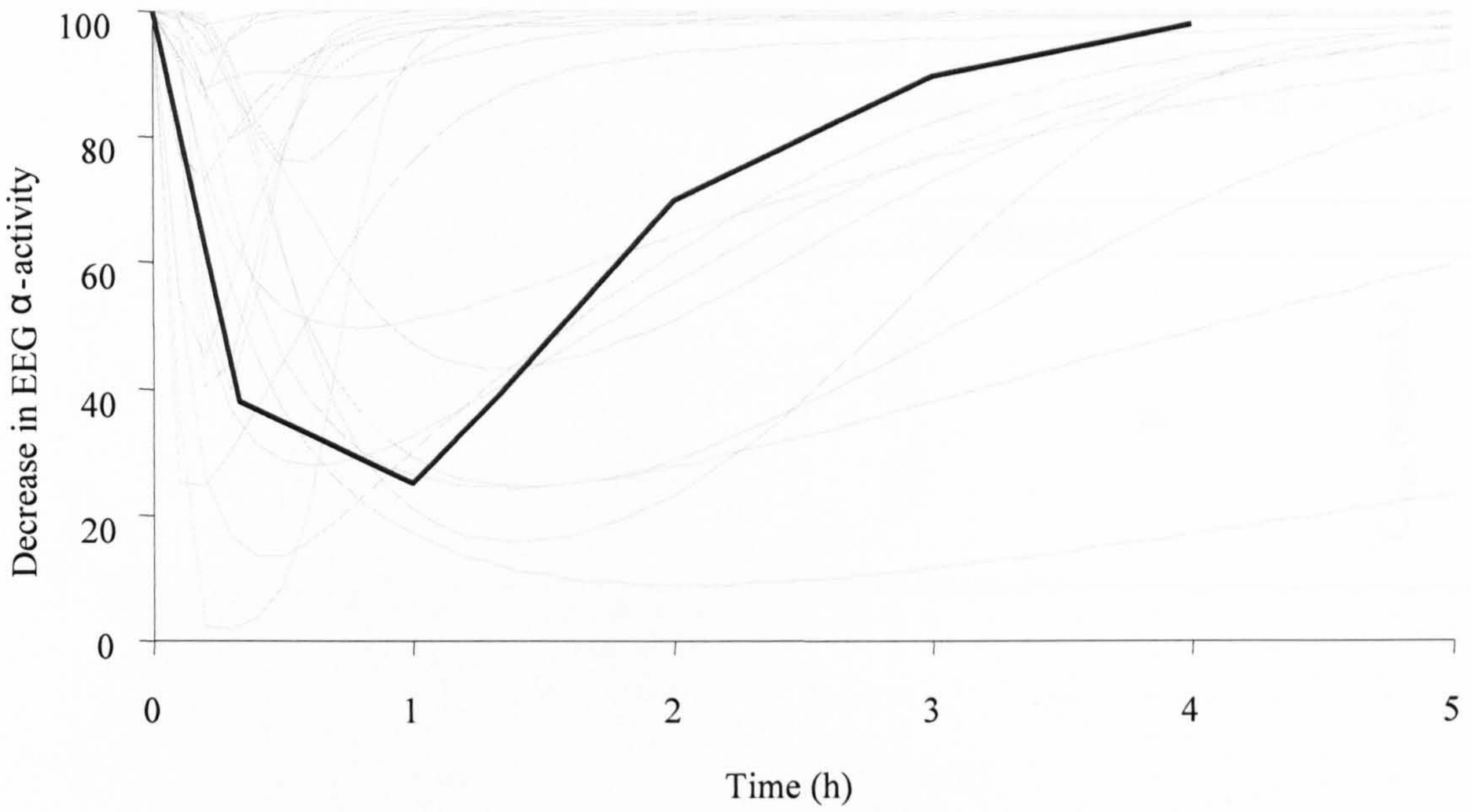


Figure 6.4 Observed (Koopmans, *et al.*, 1988); (in 6 real subjects; ▬) and predicted (in 20 virtual subjects; ▬) effect (EEG inhibition)-time profiles of MDZ over 5 hours in Caucasian individuals of unspecified phenotype.

Table 6.6 A summary of observed and predicted pharmacokinetic parameter values of MDZ. Predicted values are the average of the mean values from 10 clinical trial simulations.

		Parameter			
CYP3A5 Phenotype	**Reference**	**$CL_{p.o.}$ (L/h)**	**$CL_{i.v.}$ (L/h)**	**F**	**C_{max} (ng/mL)**
Non-expressor/ unknown	Allonen *et al.*, 1981	16.98 (2.58)	-	-	-
	Greenblatt *et al.*, 1984	33.06 (9.54)	-	-	-
		31.8 (8.892)	-	-	-
	He *et al.*, 2005	88.2	-	-	-
	Heizmann *et al.*, 1983	-	19.38 (5.16)	-	-
	Ibrahim *et al.*, 2002	-	29 (6)	-	-
	Klotz *et al.*, 1982	-	27.92 (10.29)	-	-
	Koopmans *et al.*, 1988	88.9 (38.2)	-	-	-
	Lepper *et al.*,2005	-	22.3	-	-
	Mandema *et al.*, 1992	165.7 (93.67)	31.38 (5.29)	0.24 (0.108)	25 (14.7)
	Masica *et al.*, 2004	91.98 (45.54)	22.98 (4.38)	0.301 (0.135)	14.5 (7.4)
	Tateishi *et al.*, 2001	103.7 (60.3)	24.72 (7.44)	0.28 (0.094)	10.3 (5.4)
	Thummel *et al.*, 1996	84.78 (48.42)	22.2 (6.84)	0.32 (0.10)	-
	Wandel *et al.*, 2000	68.58 (24.78)	18.6 (3.36)	0.30 (0.12)	
	Wong *et al.*, 2004	71.28 (36.6)	26.04 (13.02)	-	-
	Yu *et al.*, 2004	-	28.4 (12.5)	-	-
	WEIGHTED MEAN	**77.4 (33.5)**	**24.1 (6.1)**	**0.29 (0.11)**	**14.5 (7.8)**
	SIMULATED MEAN	**100.3 (96.0)**	**25.6 (9.0)**	**0.22 (0.11)**	**8.6 (5.2)**
Expressors	He *et al.*, 2005	115.5	-	-	-
	Lepper *et al.*,2005	-	26.8	-	-
	Wong *et al.*, 2004	115.1 (41.88)	36.12 (4.92)	-	-
	Yu *et al.*, 2004	-	32.3 (12.4)	-	-
	WEIGHTED MEAN	**115.3 (41.9)**	**30.8 (10.5)**	**-**	**-**
	SIMULATED MEAN	**146.7 (123.5)**	**30.4 (7.6)**	**0.17 (0.09)**	**6.6 (4.5)**

Table 6.7 Powers of the reported studies to detect difference in the pharmacokinetics and pharmacodynamics of MDZ compared with the reported outcomes (only pharmacokinetics is reported by the *in vivo* studies). Cases where prediction matched observation are highlighted in green, those where it did not are highlighted in red. Crosses (✗) indicate failure of the study to show a statistically significant difference between phenotypes (✓) indicate success of the study in showing a statistically significant difference between the phenotypes.

				Power PD	
Reference	n	PK difference?	Power - PK	without active metabolite	with active metabolite
Lepper *et al.*, 2005	58	✗	10	7	6
Wong *et al.*, 2004	67	✓	20	12	7
Shih *et al.*, 2002	42	✗	5	5	5
Eap *et al.*, 2004	21	✗	0	0	5
Floyd *et al.*, 2003	57	✗	10	7	8
Yu *et al.*, 2004	19	✗	0	0	5

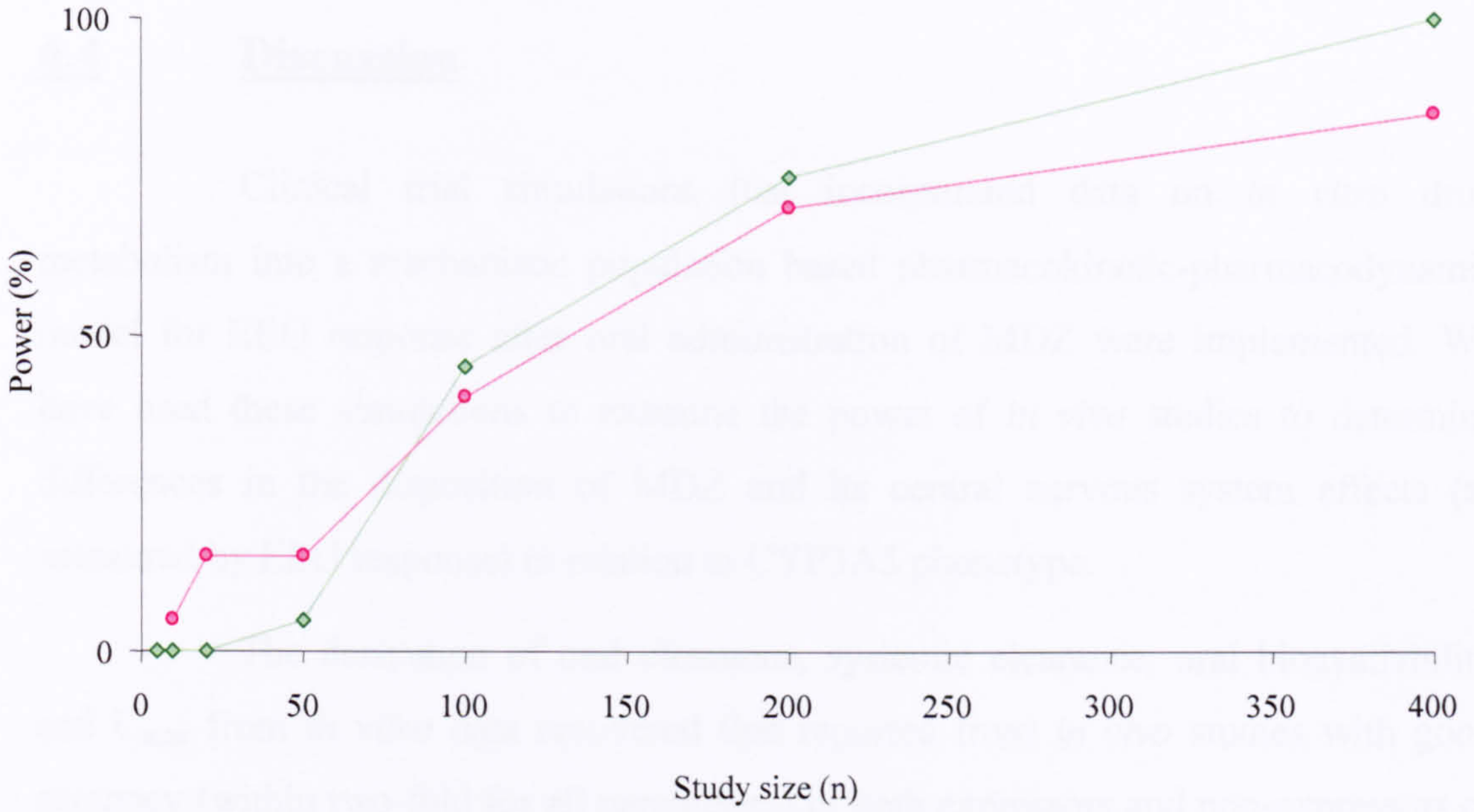


Figure 6.5 The power (%) of simulated studies to show significant differences in the AUC of parent drug (MDZ; ◇) and its metabolite (OH-MDZ; ●) between different CYP3A5 phenotypes *vs* the number of subjects in each study (n).

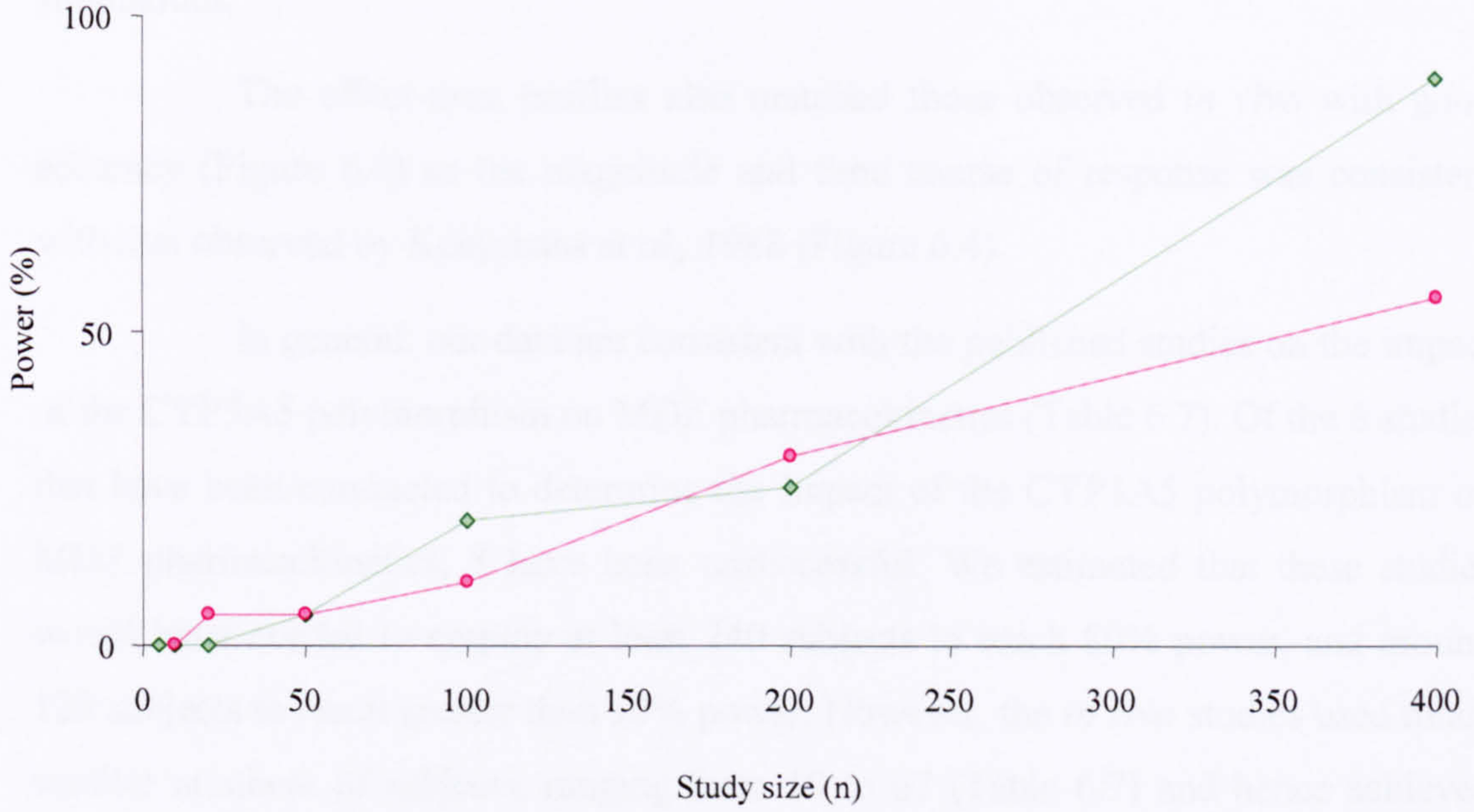


Figure 6.6 The power (%) of simulated studies to show significant differences in the AUEC of parent drug between different CYP3A5 phenotypes, assuming (i) no activity of OH-MDZ (◇) and, (ii) 78% activity (Mandema, *et al.*, 1992) of MDZ (●) *vs* the number of subjects in each study (n).

6.4 Discussion

Clinical trial simulations that incorporated data on *in vitro* drug metabolism into a mechanistic population based pharmacokinetic-pharmacodynamic model for EEG response after oral administration of MDZ were implemented. We have used these simulations to examine the power of *in vivo* studies to determine differences in the disposition of MDZ and its central nervous system effects (as measured by EEG response) in relation to CYP3A5 phenotype.

The derivation of oral clearance, systemic clearance, oral bioavailability and C_{max} from *in vitro* data recovered that reported from *in vivo* studies with good accuracy (within two-fold for all parameters) in both expressors and non-expressors of CYP3A5 (Table 6.6). Figure 6.2 and Figure 6.3 demonstrate good concordance between the mean observed (Mandema, *et al.*, 1992; Wandel, *et al.*, 2000) plasma MDZ concentration-time profiles and the expected range of profiles predicted by our simulations.

The effect-time profiles also matched those observed *in vivo* with good accuracy (Figure 6.4) as the magnitude and time course of response was consistent with that observed by Koopmans *et al.*, 1988 (Figure 6.4).

In general, our data are consistent with the published studies on the impact of the CYP3A5 polymorphism on MDZ pharmacokinetics (Table 6.7). Of the 6 studies that have been conducted to determine the impact of the CYP3A5 polymorphism on MDZ pharmacokinetics, 5 have been unsuccessful. We estimated that these studies would have needed to employ at least 240 subjects to reach 80% power, and around 120 subjects to reach greater than 50% power. However, the *in vivo* studies used much smaller numbers of subjects, ranging from 19 to 67 (Table 6.7) and hence achieved powers of only 0 – 10% (Table 6.7). The single study that was successful in determining a difference in pharmacokinetics between genotypes used the largest sample size of 67 (Wong, *et al.*, 2004), achieving the highest power of all the studies of 20% (Table 6.7). Nevertheless, this outcome is a false negative and suggests that either the model underpredicts study power or that the study in question produced a false positive result. The latter explanation is the most likely, given the consistency of the simulated outcomes and predicted powers with what is observed *in vivo*.

Recently published data have described polymorphic CYP3A5 expression in the gut (Paine, *et al.*, 2006). However, at the time of model construction, the latter information was not available, neither was any data on the relative abundance of CYP3A5 in the gut published in the literature. As a result, the CYP3A5 content of the gut is incorporated into a general 'CYP3A' content (Chapter 3; Section 3.3.1.2) and no consideration is given to the polymorphic expression of CYP3A5 within the gut. Given the low bioavailability (F) of MDZ (0.29 in non-expressors of CYP3A5; Table 6.6) it is clear that, had such enteric, polymorphic expression been incorporated into the model, the relationship between CYP3A5 phenotype and MDZ pharmacokinetics may have been more apparent in the simulations and the power to determine such differences would have increased. However, given the consistency of the predicted powers with the outcomes observed *in vivo*, it may be postulated that such an increase may not have affected the results to any great degree. Furthermore, using a recent version of the Simcyp® software (version 6; www.simcyp.com) it was possible to carry out a simulation to predict the change in F_G when CYP3A5 is assumed to be absent from the gut as it is in a non-expressor of the enzyme. The simulation demonstrated an increase in the average value of F_G from 0.41 to 0.54. This is further evidence to suggest that had such polymorphisms been incorporated into the model, the impact on the results is likely to have been minimal.

In conclusion, the results of this study have provided some explanation as to why there is an apparent discrepancy in the results of published *in vivo* studies to discern a difference in the pharmacokinetics of MDZ between CYP3A5 phenotypes. Where some studies are successful, others fail. This may be due to the fact that study sizes are generally low compared to what would be required to achieve 80% power to discern such differences (240 subjects). The powers, therefore all fall short of 80%, or even 50% (0 to 20%). Any reported success (e.g. Wong *et al.*, 2004) may be the result of type II statistical errors or simply luck. It is interesting to note the lack of pharmacodynamic studies of MDZ in the literature. Given the general abundance of pharmacokinetic-related studies of MDZ this is unusual and may perhaps be due to underreporting of negative results by prominent journals in the literature (See Discussion; Section 9.3).

CHAPTER 7

The Propagation of Pharmacogenetic Differences in Cytochrome P450 into Pharmacokinetic & Pharmacodynamic Measures: The Example of CYP2C19 & Omeprazole

7 OMEPRAZOLE

7.1 Introduction

7.1.1 H^+, K^+ ATPase

The pH of gastric acid is approximately between 2 and 3 in the stomach lumen when in the fasting state (Neymour, 1993). This acidity is maintained by the enzyme H^+, K^+ ATPase, also known as the proton pump. H^+, K^+ ATPase is situated in the parietal cells of the gastric mucosa, the enzyme transports one hydrogen ion into the stomach lumen against a concentration gradient of up to 3 million to 1 in exchange for one potassium ion (Sachs et al., 1995).

The reduction of acid secretion by H^+, K^+ ATPase inhibition is a target for two different categories of drugs. H_2-receptor antagonists inhibit the signalling pathway that leads to activation of the enzyme whilst proton pump inhibitors directly inhibit the enzyme (Klotz et al., 2004).

7.1.2 Proton Pump Inhibitors

Proton pump inhibitors are a group of drugs that, when activated, form a disulphide bond with, and irreversably deactivate, H^+, K^+ ATPase (Klotz et al., 2004). In doing so they may lower acid secretion by as much as 99% (Junghard *et al.*, 2002; Lind *et al.*, 1983). This mechanism is utilised in the treatment of a number of diseases which are associated, either directly or indirectly, with an excess of acid secretion in the stomach (e.g. peptic ulcer disease and gastroesophageal reflux disease) (Klotz et al., 2004). The lack of acid also helps to ease the pain associated with indigestion and heartburn when they occur as a result of excess acid secretion (Klotz et al., 2004). A member of the proton pump inhibitor family of drugs, omeprazole (OMZ) is also used in combination with antibiotics (e.g. amoxicillin with clarithromycin or metronidazole) to eradicate Helicobacter pylori (H. pylori), which is the causative factor in the majority of peptic and duodenal ulcers (Beil et al., 2001).

7.1.3 Omeprazole

Omeprazole (OMZ) was the first proton pump inhibitor to be successfully developed and marketed as such (Kendall, 2003). It is acid-transformed in the parietal cells of the stomach to its active form, a sulphenamide. The latter compound binds tightly to H^+,K^+-ATPase, deactivating it, thus increasing stomach pH in gastric acid disorders (Klotz et al., 2004). This reaction is reversible, although dissociation occurs very slowly.

There is wide interindividual variability both in the pharmacokinetics of OMZ and in response to the drug. CYP2C19 is responsible for between 71 and 99% of the metabolism of the drug (Abelo *et al.*, 2000; Karam *et al.*, 1996; Yamazaki *et al.*, 1997), and its 5-Hydroxylation pathway is often used as a probe reaction for CYP2C19 both *in vitro* (Tucker et al., 2001) and *in vivo* (Wedlund & Wilkinson, 1996).

Many studies have attempted to define the relationship between CYP2C19 phenotype and OMZ pharmacokinetics and/or pharmacodynamics. All of the studies which report attempts to discern the relationship between OMZ pharmacokinetics and CYP2C19 genotype have been successful (Fu *et al.*, 2004; Hu *et al.*, 2005; Kita *et al.*, 2001; Qiao *et al.*, 2006; Yin *et al.*, 2004). In contrast, reports attempting to explain the wide interindividual variability in response to OMZ based on CYP2C19 phenotype have been conflicting. However, this inconsistency may be due to the different endpoints used in the various studies, while successful studies have generally utilised stomach acid secretion as a measure of response (Egan *et al.*, 2003; Hu *et al.*, 2005; Kita *et al.*, 2001; Shimatani *et al.*, 2003), others have investigated cure rates of helicobacter pylori infection, gastric acid reflux disease or the occurrence of adverse events as the endpoint (Egan et al., 2003; Ohkusa et al., 2005).

The aim of this study was to simulate the pharmacokinetics of OMZ and acid inhibitory response to the drug in virtual populations of human subjects. These simulations were used to mimic the *in vivo* studies and to estimate their power to differentiate the pharmacokinetics and pharmacodynamics of OMZ between CYP2C19 phenotypes. The ultimate aim was to help explain the failure of *in vivo* studies to detect such differences in pharmacodynamics, by assessing the effect of sample size and other aspects of study design, on the power of such studies.

7.2 Materials and Methods

7.2.1 The IVIVE Model Parameters

As described in Chapter 3, as part of the IVIVE process, several key parameters are required for input into the model. These include both *in vitro* parameters (such as K_m and V_{max}) and parameters gathered from *in vivo* experiments (such as CL_R). These were collected from the literature and either entered directly into the model or utilised as part of a meta-analysis. The following section describes the parameters and their uses.

7.2.1.1 *In Vitro Data*

Data on the *in vitro* metabolism of OMZ (in the form of K_m and V_{max}) were collected from published reports (Abelo *et al.*, 2000; Karam *et al.*, 1996; Yamazaki *et al.*, 1997) (Table 7.1).

Intersystem extrapolation factors (ISEFs), were applied as described by Proctor *et al.* (2004) to account for any differences between the intrinsic activity of recombinantly expressed CYP enzymes and human liver microsomes (Table 7.2). To account for the non-specific binding of OMZ in the experiments described in Table 7.1, a value of fu_{mic} was calculated and applied to the data as described in Chapter 3; Sections 3.1.1.2 and 3.3. ISEF values and values of fu_{mic} are shown in Table 7.2. A meta-analysis was then conducted to find the overall V_{max} and K_m values for the metabolism of OMZ by each relevant CYP isoform.

7.2.1.2 Additional Parameters Required for IVIVE

The additional parameters listed in Table 7.3 were required for scaling the *in vitro* data to human whole body clearance and to the elimination rate constant (Chapter 3). These values were then entered into the pharmacokinetic-pharmacodynamic model described below (Sections 7.2.2 & 7.2.3).

Table 7.1 Parameters describing the *in vitro* metabolism of OMZ.

		5-Hydroxylation		*O*-Demethylation		Sulfoxidation	
Reference	CYP	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
Abelo *et al.*, 2000	2C9	1.71	209	0.77	107	-	-
	2C19	6.95	5	1.48	3.04	-	-
	3A4	4.04	342	0.74	262	7.3	82.9
Yamazaki *et al.*, 1997	2C19	8.3	6.6	-	-	-	-
	3A4	2.4	60	-	-	13	140
Karam *et al.*, 1996	2C8	3.3	300	-	-	-	-
	2C19	10.2	12.2	-	-	-	-
	3A4	10.9	50	-	-	18.2	71

Vmax - pmol/min/pmol P450; Km – μM.

Table 7.2 ISEF and fu_{mic} values that were applied to the data shown in Table 7.1 resulting in the revised V_{max} and K_m values shown here. The final values entered into the IVIVE algorithms are indicated in bold type at the bottom of the table.

						5-Hydroxylation		**O-Demethylation**		**Sulfoxidation**	
Reference	Expression System	CYP	Protein Conc (mg/mL)	fu_{mic}	ISEF	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)
Abelo *et al.*, 2000	Lymphoblastoid	2C9	1	0.951	6.82	11.7	198.8	5.25	101.8	-	-
		2C19			7.69	53.4	4.8	11.4	2.9	-	-
		3A4			3.83	15.5	325.2	2.8	249.2	28.0	78.8
Yamazaki *et al.*, 1997	Baculovirus	2C19	0.5	0.975	18.3	151.9	6.4	-	-	-	-
		3A4			0.34	0.8	57.1	-	-	4.4	136.5
Karam *et al.*, 1996	Yeast	2C8	0.25*	0.987	0.75	13	296.1	-	-	-	-
		2C19			20.2	206.0	12.0	-	-	-	-
		3A4			3.95	43.1	49.4	-	-	71.9	70.1
Weighted Mean		**2C8**				**13.0**	**296.1**	**-**	**-**	**-**	**-**
		2C19				**133**	**8.11**	**11.4**	**2.9**	**-**	**-**
		2C9				**11.7**	**198.8**	**5.25**	**101.8**	**-**	**-**
		3A4				**25.2**	**169**	**2.8**	**249.2**	**12.8**	**83.8**

*A default value of 0.25 mg/mL was assumed as no specific value was given within the report (Chapter 3; Section 3.1.1.2).

Table 7.3 Mean pharmacokinetic parameter values of OMZ taken or calculated from the literature.

Parameter	Value	References
fu	0.064	Regardh *et al.*, 1985; Section 3.1.1.3.
B:P	0.6	Regardh *et al.*, 1985; Section 3.1.1.3.
fu_B	0.11	Section 3.1.1.3.
CL_R (L/h)	0.034	Andersson *et al.*, 1990a; Andersson *et al.*, 1990b; Regardh *et al.*, 1990; Section 3.3.3.
V (L/kg)	0.236 (0.708)*	Regardh *et al.*, 1990; Section 3.3.4.

*Mean (SD); fu = fraction unbound in plasma; B:P = Blood to Plasma concentration drug ratio; fu_B = fraction unbound in blood; CL_R = renal clearance; V = initial volume of distribution.

Table 7.4 Additional literature values describing the pharmacokinetics of OMZ.

Parameter	Value*	Reference
V_{OMZ} (L/kg)	0.236 (0.708)	Regardh *et al.*, 1990
k_a (h^{-1})	6 (1.8)	Andersson *et al.*, 1990

*Mean (SD)

7.2.2 Pharmacokinetic Model

Values for $k_{10,i}$, $F_{H,i}$ and $F_{G,i}$ (Calculated using Simcyp® algorithms – Chapter 3) were entered into a pharmacokinetic-pharmacodynamic model, adapted from Katashima *et al.* (1998). In the latter model, plasma concentrations of OMZ in each individual ($C(t)_{(OMZ),i}$) were described using a one-compartment model with first order absorption:

$$C(t)_{(OMZ),i} = \frac{D \cdot k_{a,i} \cdot F_{H,i} \cdot f_a \cdot F_{G,i}}{V_{OMZ,i} \cdot (k_{a,i} - k_{10,i})} \times \left[e^{-k_{10,i} \cdot t} - e^{-k_{a,i} \cdot t}\right] \qquad \text{Equation 7.1}$$

where f_a is the oral bioavailability of OMZ (set to 1), D is the oral dose of OMZ (20mg), $k_{a,i}$, and $V_{OMZ,i}$ are the absorption rate constant and the steady state volume of distribution in the i^{th} individual, respectively (Table 7.4).

7.2.3 Pharmacodynamic Model

The pharmacokinetic and pharmacodynamic data were linked assuming a pharmacokinetic-pharmacodynamic model including the apparent turnover process of H^+K^+-ATPase (Katashima et al., 1998). In this model, the drug in the plasma is transformed in the parietal cells to the active form, which reacts with active H^+K^+-ATPase with a second rate constant, K, to inactivate the H^+K^+-ATPase (Figure 7.1). The total amount of H^+K^+-ATPase (E_t) is kept at a constant level, assuming that active H^+K^+-ATPase (E_a) is biosynthesized at a constant rate (K_S), and that H^+K^+-ATPase is eliminated with a first order rate constant (k_1) (Figure 7.1).

Assuming that the inhibitory effect on gastric acid secretion is proportional to the ratio of E_i to E_t, the response to OMZ in each individual could be described by Equation 7.2 (Katashima *et al.,* 1998):

$$\frac{d\varepsilon c}{dt} = \left(K \cdot C(t)_{(OMZ),i} \cdot \varepsilon\right) - \left[(k_1 + k_2) \cdot \varepsilon c\right] \qquad \text{Equation 7.2}$$

where K, ε, k_1, k_2 and εc are a constant for the reaction of omeprazole with H^+K^+-ATPase (Table 7.5), the gastric acid secretion ratio (E_a/E_t), the apparent elimination rate constant of H^+K^+-ATPase, the apparent recovery rate constant of

H^+K^+-ATPase and the gastric acid inhibition ratio (E_i/E_t), respectively. The apparent turnover rate constant of H^+K^+-ATPase (k; Table 7.5) is equal to the sum of k_1 and k_2.

By definition:

$$\varepsilon c = 1 - \varepsilon \qquad \text{Equation 7.3}$$

therefore:

$$\frac{d\varepsilon c}{dt} = \left(K \cdot C(t)_{(OMZ),i} \cdot [1 - \varepsilon c]\right) - (k \cdot \varepsilon c) \qquad \text{Equation 7.4}$$

Since the initial value for *εc* can be assumed to be 0 (no inhibition of *εc* H^+K^+-ATPase prior to administration of OMZ), the above equation was solved for a series of time points.

7.2.4 Study Design

Individual plasma OMZ concentration and response *vs.* time profiles following administration of oral OMZ were simulated between 0 and 8h. For the purposes of data-analysis, samples were taken every 15 minutes (32 samples). Simulated plasma drug concentration- and response-time profiles in EMs and PMs (with equal numbers of each phenotype in each study arm) were compared using a range of population sizes (n). For all simulations n was set at 25, 50, 100, 200, 300 or 400. Twenty simulations were run for each study size.

Study size and the number of subjects of a given phenotype/genotype in the study population where chosen using two different techniques:

(i) Study sizes were chosen arbitrarily and the proportion of PM subjects within the study population was set according to their natural occurrence in the Caucasian population, and

(ii) To our knowledge, there are 17 literature studies which investigate the relationship between CYP2C19 phenotype and the pharmacokinetics and/or pharmacodynamic of OMZ (Table 7.6). Since the majority of these studies are carried out in oriental populations, the abundance of poor metaboliser phenotypes in these studies tends to be different to that seen in the Caucasian population, study sizes and the proportion of each phenotype/genotype were also chosen in order to mimic those seen in the literature studies.

Overall, 580 simulations were carried out using up to 400 subjects in each study set (a total of 57,200 virtual subjects).

7.2.5 Data Analysis

Values for (i) the area under the concentration-time curve (AUC) of OMZ, and (ii) the area under the effect-time curve for OMZ (AUEC; defined as the area associated with the decrease in acid secretion with time), were calculated using the trapezoidal rule. For the purposes of this study we assumed that no measurement error occurred. The probability of detecting a statistically significant difference in pharmacokinetics and pharmacodynamics between phenotypes was assessed by comparing values for the AUC and AUEC, respectively, using ANOVA (SPSS v 12; SPSS Inc. 2003). The number of studies out of the 20 simulations that led to a statistically significant difference in AUC (or AUEC) between phenotype groups was recorded as the power of that particular trial.

Clearance values and other pharmacokinetic parameters resulting from the IVIVE model were compared with those values observed *in vivo* (Andersson et al., 1990a; Andersson et al., 1990b; Regardh et al., 1990). It should be noted that for the purposes of this study it was assumed that H^+K^+-ATPase inhibition is comparable to stomach pH. However, due to the nature of the pharmacodynamic outcome of the simulations (percentage H^+K^+-ATPase inhibition), and the lack of adequate conversion information in the literature, it was not possible to validate the simulations by comparison with what is observed *in vivo* (commonly, stomach pH rather than % acid inhibition).

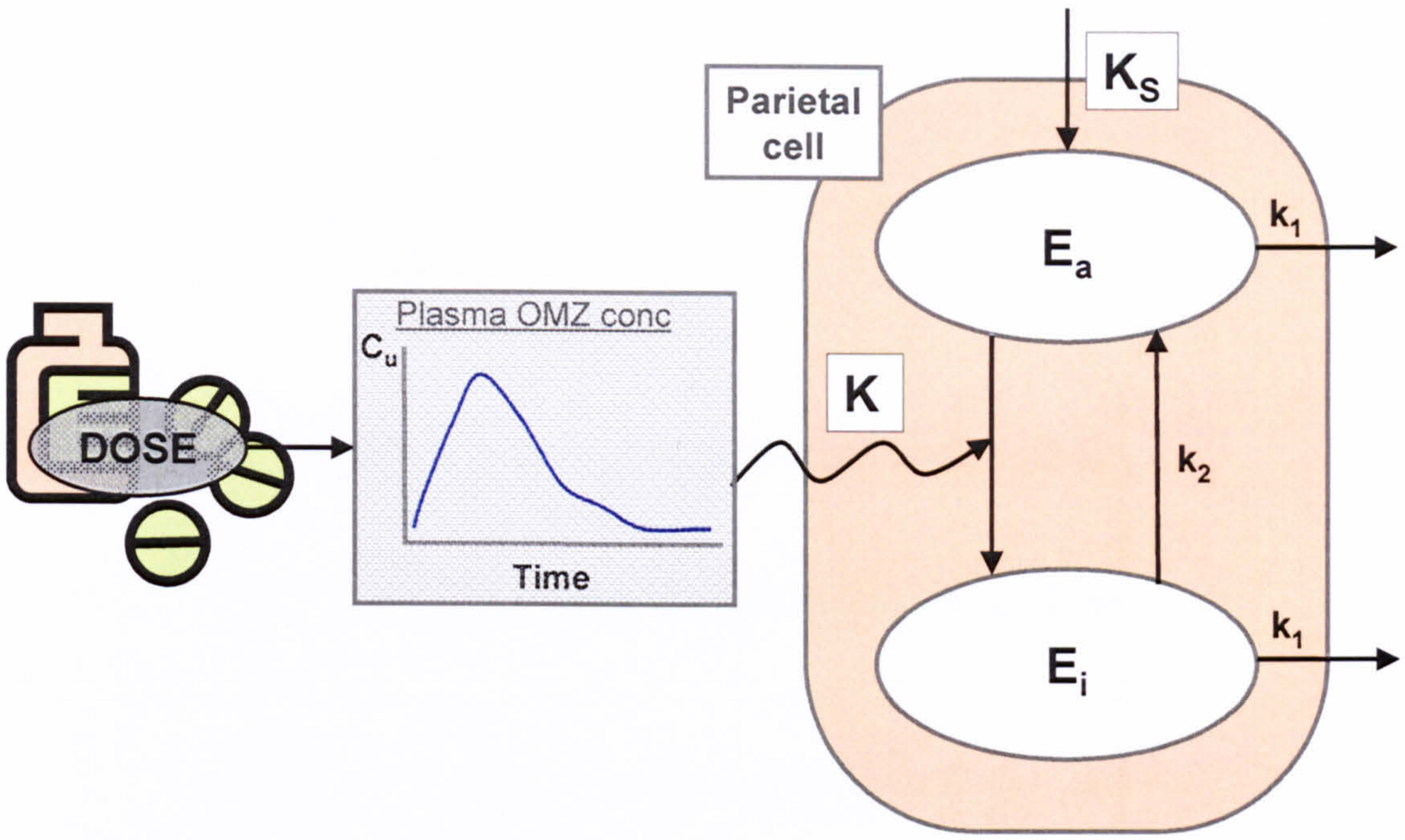


Figure 7.1 A schematic representation of the pharmacokinetic-pharmacodynamic model for the action of OMZ on H^+K^+-ATPase in parietal cells. K_S = biosynthesis rate of active H^+K^+-ATPase; E_a = amount of active H^+K^+-ATPase; E_i = amount of inactive H^+K^+-ATPase; K = apparent reaction rate constant of omeprazole with active H^+K^+-ATPase; k_1 = elimination rate constant of both active and inactive H^+K^+-ATPase; k_2 = apparent recovery rate constant of inactive to active H^+K^+-ATPase.

Table 7.5 Literature values describing the pharmacodynamics (inhibition of acid secretion) of OMZ (Katashima *et al.,* 1998).

Parameter	Value*
K ($\mu M^{-1}.h^{-1}$)	1.343 (0.172)
k (h^{-1})	0.0252 (0.0019)

*Mean (SD).

Table 7.6 Results of the published studies which have attempted to identify a significant relationship between CYP2C19 phenotype and the pharmacokinetics and/or pharmacodynamics of OMZ. The powers of these studies estimated by the current study are shown. Results highlighted in green indicate that the observed result matched the calculated power, red indicates the opposite.

Study	N			% PMs	Difference in PK?	Difference in PD?	Power - PK	Power - PD
	EMs	PMs	total					
Miyoshi *et al.*, 2001	164	35	199	18	NA	×	100	100
Ohkusa *et al.*, 2005	99	20	119	17	NA	×	100	100
Tanigawara *et al.*, 1999	87	21	108	19	NA	✓	100	100
Yin *et al.*, 2004	19	7	26	27	✓	NA	95	95
Fu *et al.*, 2004	19	6	25	24	✓	NA	95	95
Furuta *et al.*, 1999b	10	6	16	38	✓	✓	90	90
Hu *et al.*, 2005	12	6	18	33	✓	✓	90	90
Qiao *et al.*, 2006	12	6	18	33	✓	NA	90	90
Shimatani *et al.*, 2003	12	6	18	33	NA	✓	90	90
Sakai *et al.*, 2001	12	6	18	33	✓	NA	90	90
Yasuda *et al.*, 1995	9	6	15	40	✓	NA	85	85
Tybring *et al.*, 1997	5	5	10	50	✓	NA	80	80
Shirai *et al.*, 2001	11	4	15	27	✓	✓	75	75
Furuta *et al.*, 1999a	17	4	21	19	✓	NA	70	70
Ohnishi *et al.*, 2005	14	3	17	18	✓	NA	55	55
Kita *et al.*, 2001	3	3	6	50	✓	✓	45	45
Egan *et al.*, 2003	59	2	61	3	NA	×	40	40

NA = not assessed.

7.3 Results

7.3.1 Model Validation

Table 7.7 shows a comparison of simulated *vs* observed pharmacokinetic parameters of OMZ in both extensive (Table 7.7 (A)) and poor metabolisers (Table 7.7 (B)). All simulated parameters are within two-fold of the observed data (Table 7.7).

Mean simulated concentration *vs.* time profiles in EMs and PMs are shown in Figure 7.2. Unfortunately, no observed concentration-time data were available in PM subjects. Andersson *et al.* (1990a) provided concentration-time data but did not specify the phenotype of the subjects. Accordingly, the data from Andersson *et al.* (1990a) is compared with that simulated from both EMs and PMs, respectively in Figure 7.3 (A) and (B).

7.3.2 Power of Studies Using Natural Caucasian Abundance of PM Phenotypes

Using natural abundances of genotypes, around 250 subjects were required to detect a difference in the pharmacokinetics (AUC) of OMZ between CYP2C19 EMs and PMs (Figure 7.4). The number of subjects required to detect similar differences in the pharmacodynamics of OMZ was 300 (Figure 7.4). 100% power for detecting differences in both the pharmacokinetics and the pharmacodynamics of OMZ was reached with 400 subjects.

Table 7.7 Observed *vs.* predicted pharmacokinetic parameters of OMZ in (A) Extensive, and (B) Poor metabolisers of CYP2C19. Predicted results are the means (± SD) of 10 trials of 10 subjects.

(A) Source	n	F*	C_{max}* (mg/L)	t_{max}* (mins)	AUC* (mg/L/h)	$CL_{i.v.}$* (L/h)	$CL_{p.o.}$* (L/h)
Predicted	50	0.34 (0.15)	0.31 (0.18)	20.3 (1.9)	1.0 (0.9)	29.3 (10.0)	80.9 (107.4)
Observed (weighted mean)		0.40 (0.23)	0.41 (0.14)	12.6 (3.6)	0.68 (0.39)	34.0 (14.2)	86.0 (71.8)
Andersson *et al.*, 1990a	10	0.40 (0.23)	-	12.6 (3.6)	0.62 (0.40)	38.0 (8.0)	65.5 (13.8)
Andersson *et al.*, 1990b	10	0.40	-	-	-	31.8 (12.8)	103.0 (70.3)
Kita *et al.*, 2001	3	-	0.41 (0.14)	-	0.90 (0.31)	-	-
Qiao *et al.*, 2006	7	-	0.29 (0.15)	-	1.01 (0.93)	-	-
Regardh *et al.*, 1990	8	-	-	-	-	31.8 (17.4)	90.2 (63.6)

(B) Source	N	F*	C_{max}* (mg/L)	t_{max}* (mins)	AUC* (mg/L/h)	$CL_{i.v.}$* (L/h)	$CL_{p.o.}$* (L/h)
Predicted		0.59 (0.12)	0.63 (0.28)	24.7 (8.4)	3.8 (2.5)	14.62 (7.65)	18.1 (17.8)
Observed (weighted mean)			1.14 (3.24)		4.9 (1.7)		
Kita *et al.*, 2001	3	-	0.89 (0.31)	-	3.1 (0.72)	-	-
Qiao *et al.*, 2006	11	-	1.21 (0.21)	-	5.6 (1.93)	-	-

*Mean (S D).

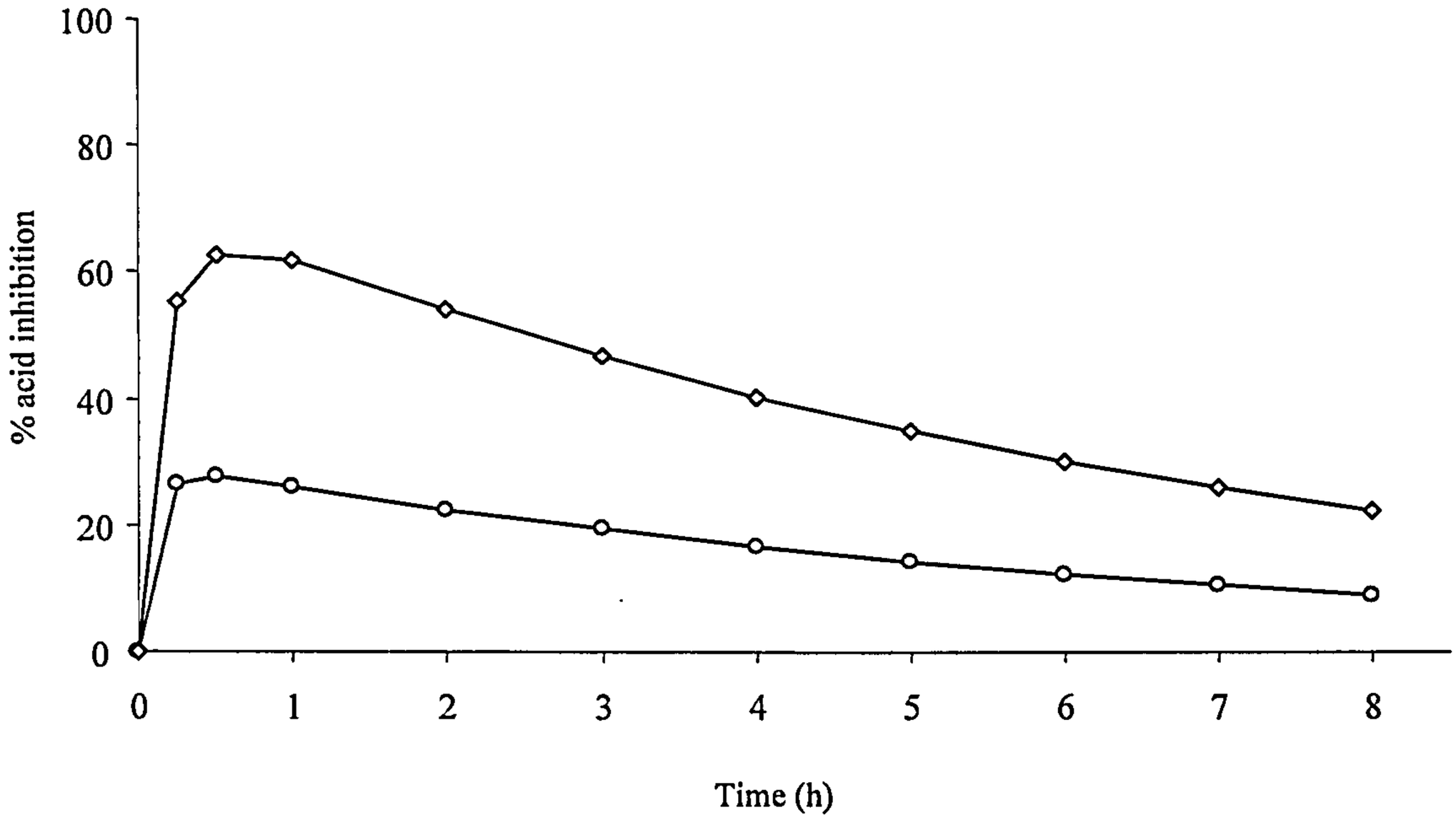
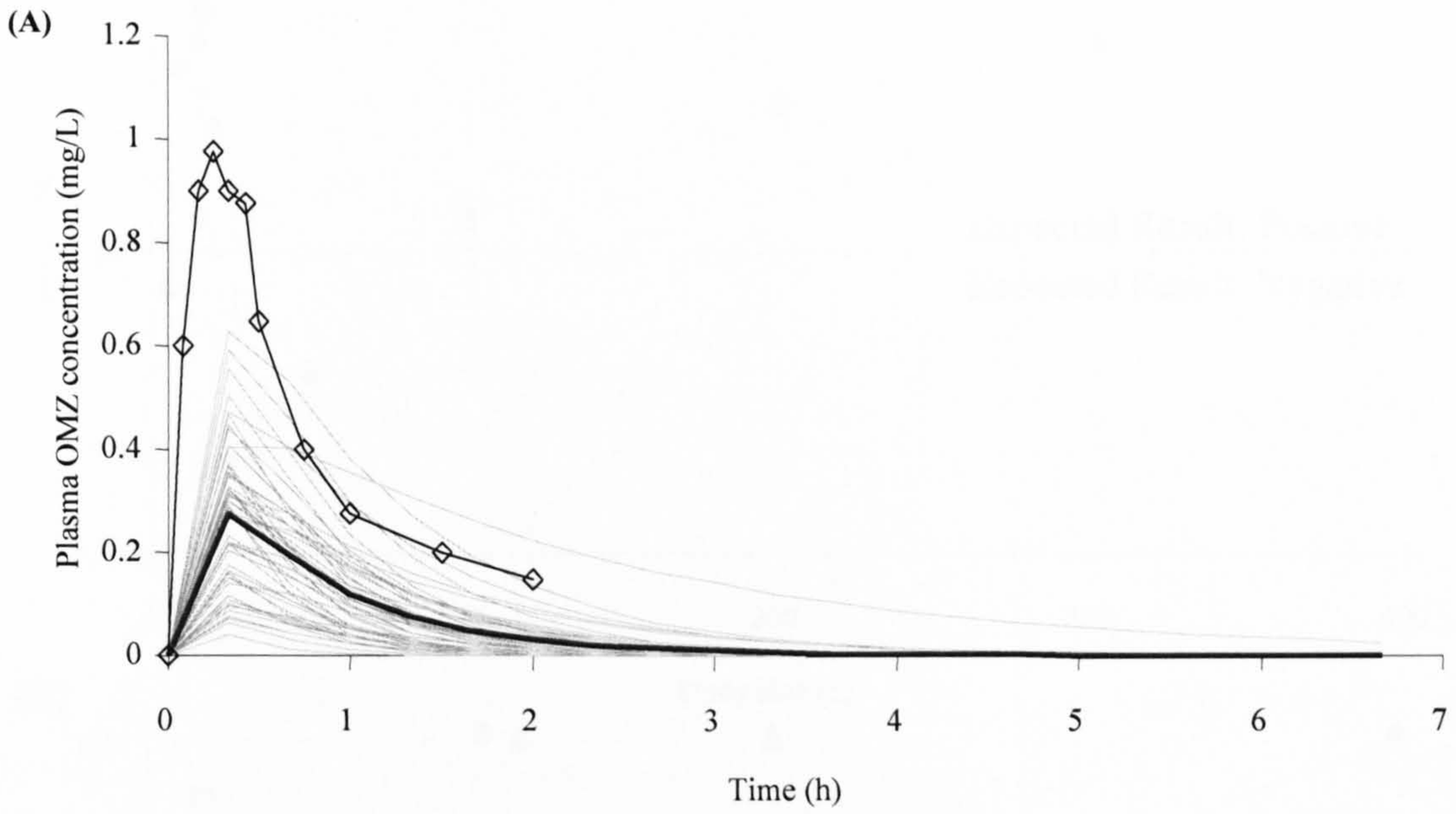


Figure 7.2 Mean simulated response-time profiles in 100 poor (◇) and 100 extensive (○) metabolisers.



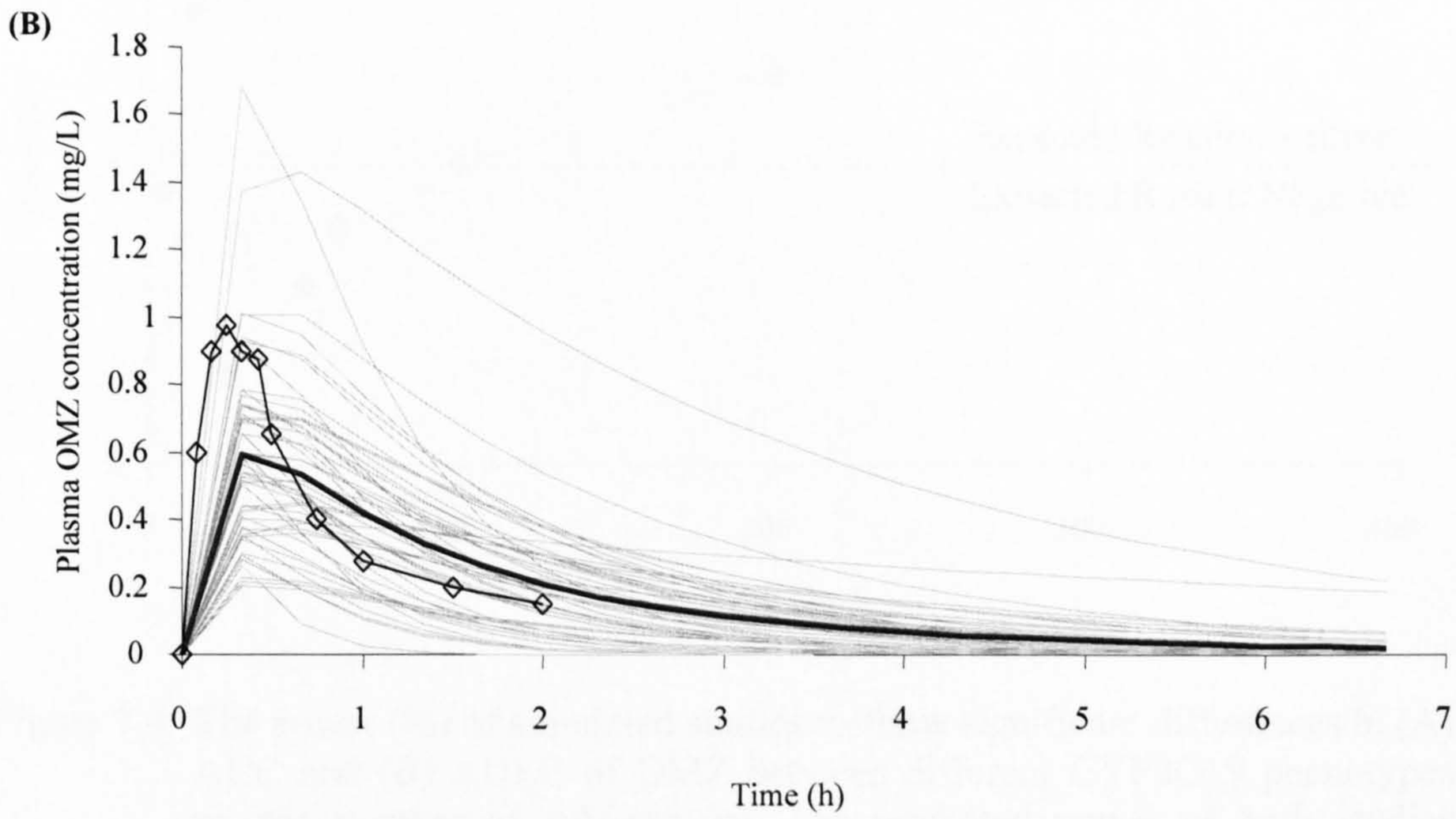


Figure 7.3 Observed (Andersson *et al.*, 1990a) (◇; phenotype not specified) and predicted plasma concentration-time profiles of OMZ over 6.75 hours in (A) 50 virtual EMs (——) and (B) 50 virtual PMs (——). The mean of the simulated data is shown (——).

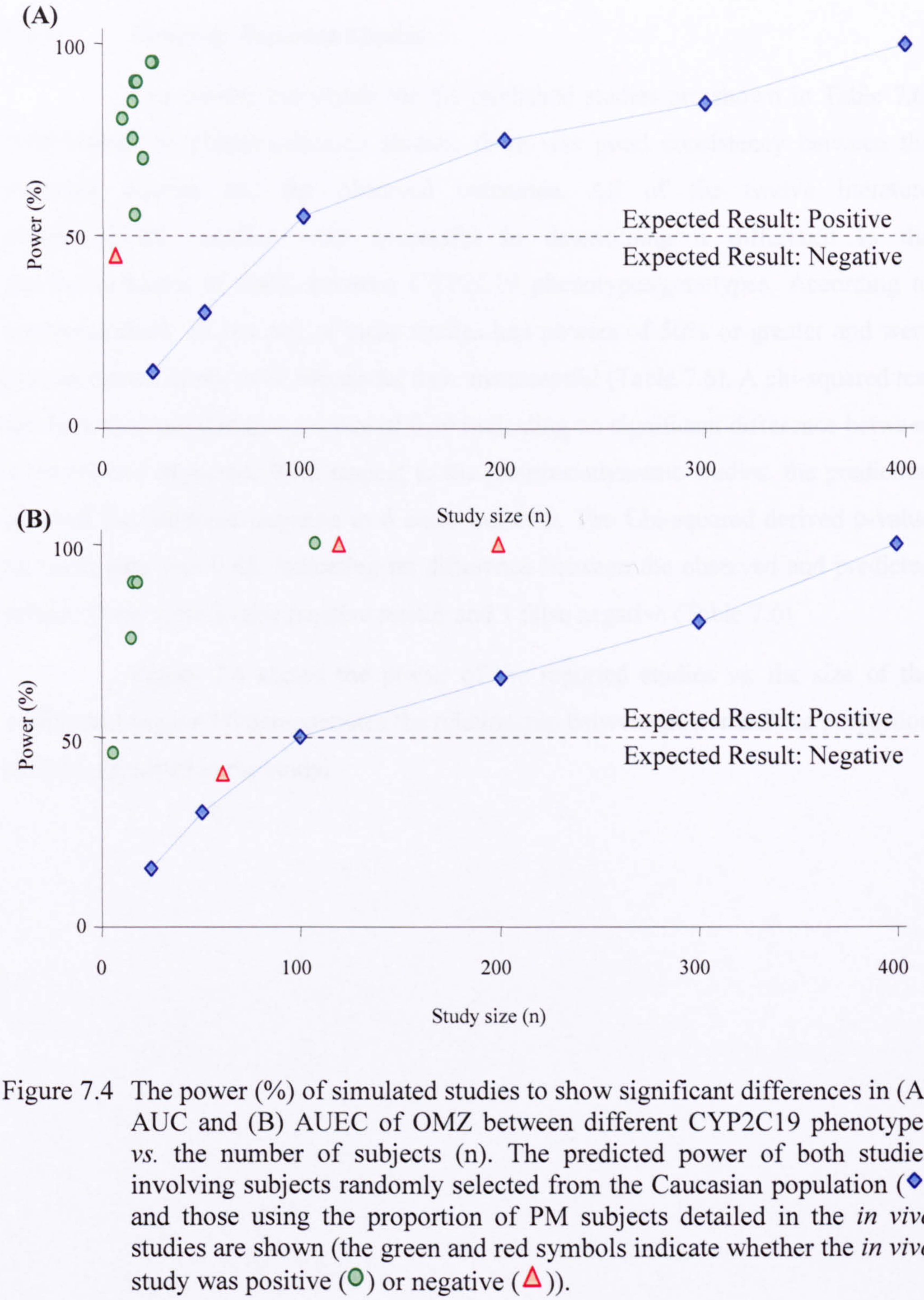


Figure 7.4 The power (%) of simulated studies to show significant differences in (A) AUC and (B) AUEC of OMZ between different CYP2C19 phenotypes *vs.* the number of subjects (n). The predicted power of both studies involving subjects randomly selected from the Caucasian population (◆) and those using the proportion of PM subjects detailed in the *in vivo* studies are shown (the green and red symbols indicate whether the *in vivo* study was positive (○) or negative (△)).

7.3.3 Power of Reported Studies

The powers calculated for the published studies are shown in Table 7.6. With respect to pharmacokinetic studies, there was good consistency between the predicted powers and the observed outcomes. All of the twelve literature pharmacokinetic studies were successful in determining a difference in the pharmacokinetics of OMZ between CYP2C19 phenotypes/genotypes. According to our simulations, all but one of these studies had powers of 50% or greater and were therefore more likely to be successful than unsuccessful (Table 7.6). A chi-squared test for these data resulted in a p-value of 0.30 indicating no significant difference between observed and expected. With respect to the pharmacodynamic studies, the prediction matched the observed outcome in 6 cases out of 9. The Chi-squared derived p-value for these data was 0.42, indicating no difference between the observed and predicted values. There were 2 false positive results and 1 false negative (Table 7.6).

Figure 7.4 shows the power of the reported studies *vs.* the size of the studies and Figure 7.6 demonstrates the relationship between power and the proportion of PMs employed in the study.

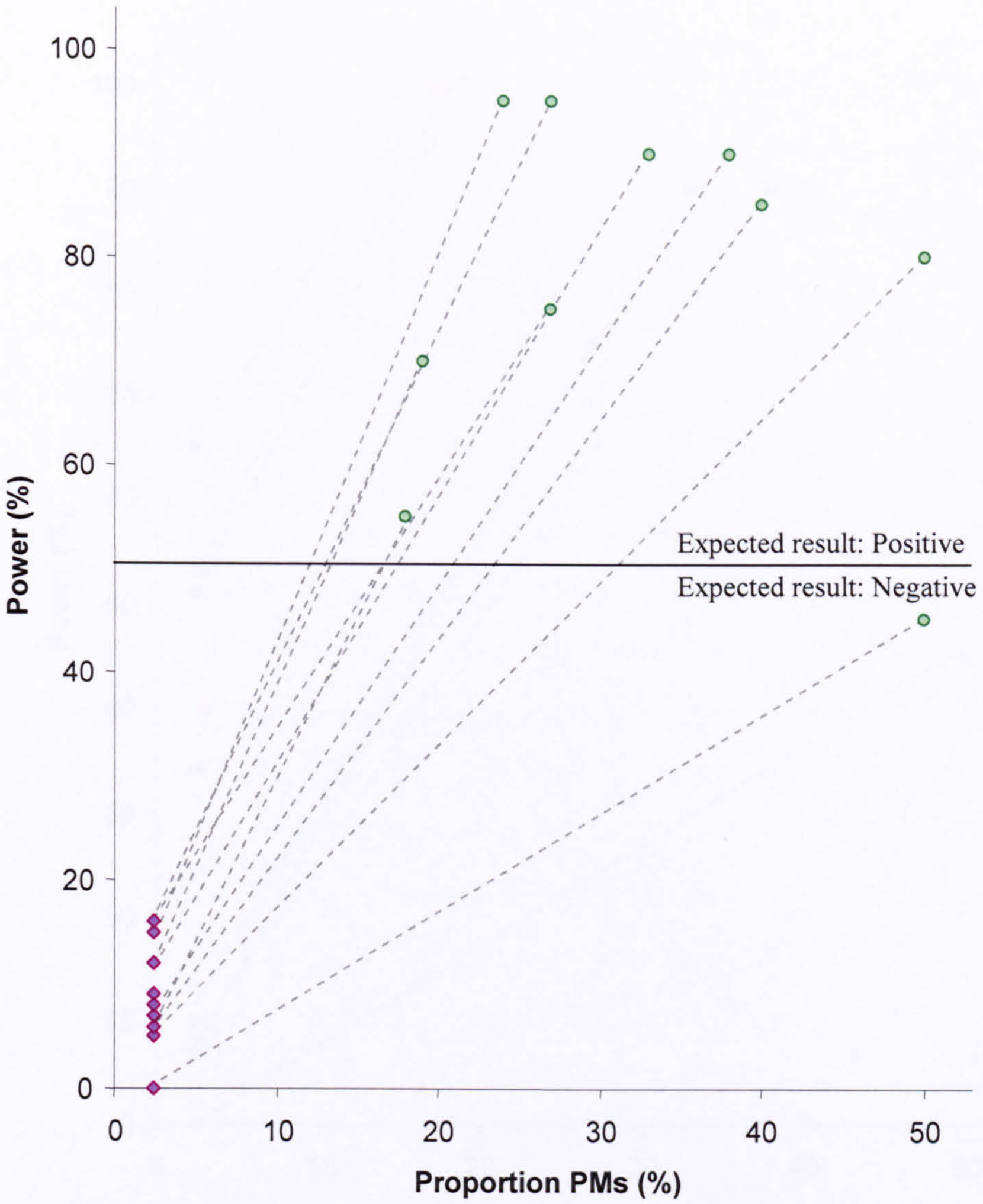


Figure 7.5 The power (%) of (i) of simulated studies that use subjects randomly selected from a Caucasian population (◆), and (ii) of simulated studies that use proportions of PM subjects comparable to those reported in the *in vivo* studies (○) to detect significant differences in the AUC of OMZ *vs.* the proportion of CYP2C19 PMs employed in the study (%).

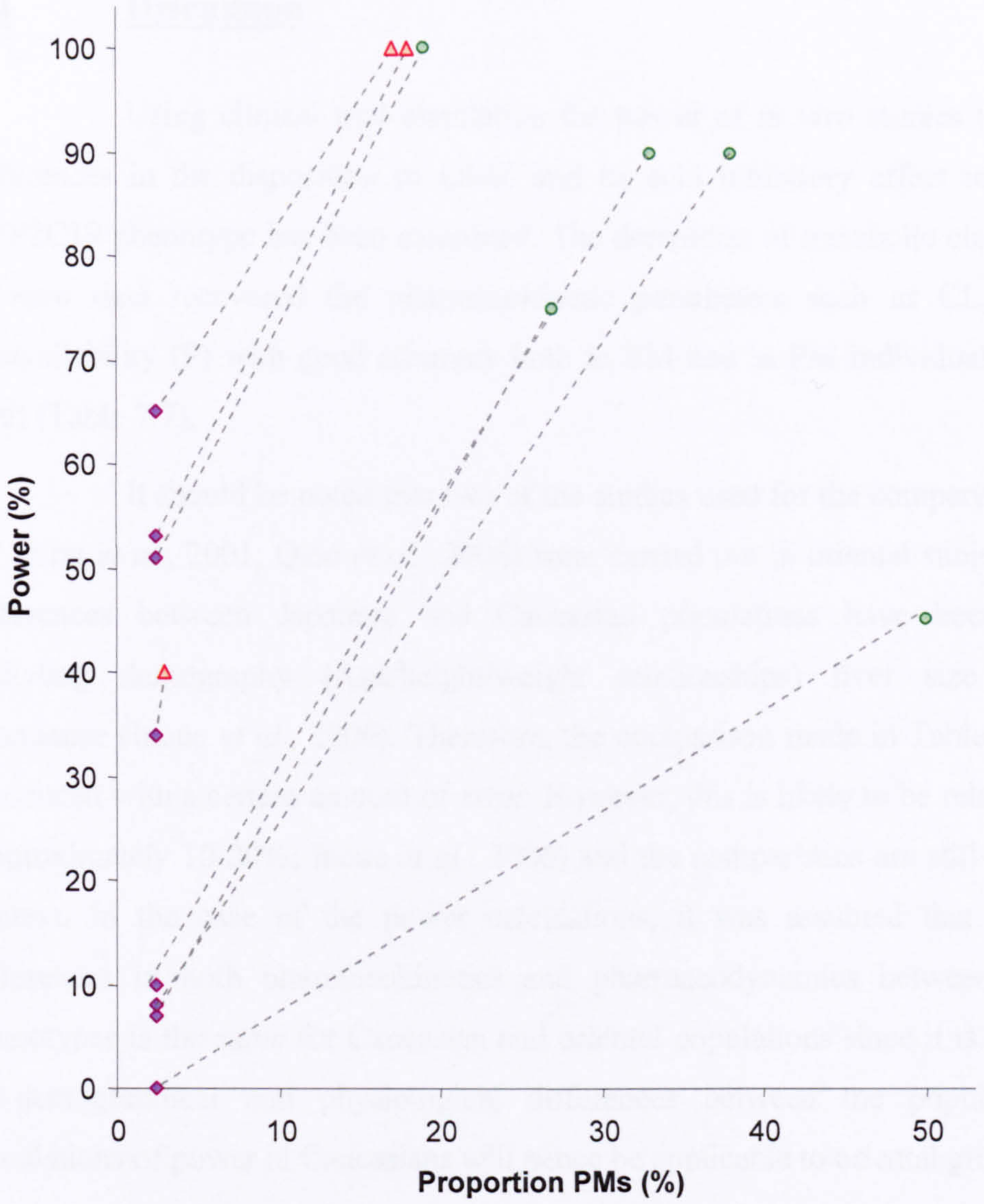


Figure 7.6 The power (%) of (i) of simulated studies that use subjects randomly selected from a Caucasian population (◆), and (ii) of simulated studies that use proportions of PM subjects comparable to those reported in the *in vivo* studies (symbol indicates whether the outcome of the *in vivo* study was positive (○); or negative (△)) to detect significant differences in the AUEC of OMZ *vs.* the proportion of CYP2C19 PMs employed in the study (%).

7.4 Discussion

Using clinical trial simulation the power of *in vivo* studies to determine differences in the disposition of OMZ and its acid inhibitory effect in relation to CYP2C19 phenotype has been examined. The derivation of metabolic clearance from *in vitro* data recovered the pharmacokinetic parameters such as CL, AUC and bioavailability (F) with good accuracy both in EM and in PM individuals (within 2-fold) (Table 7.7).

It should be noted that two of the studies used for the comparison in Table 7.7 (Kita *et al.*, 2001; Qiao *et al.*, 2006) were carried out in oriental subjects. Several differences between Japanese and Caucasian populations have been identified including demography (Age/height/weight relationships) liver size and CYP abundance (Inoue *et al.*, 2006). Therefore, the comparison made in Table 7.7 may be associated with a certain amount of error. However, this is likely to be relatively small (approximately 10-20%; Inoue *et al.,* 2006) and the comparisons are still valid in this context. In the case of the power calculations, it was assumed that the relative differences in both pharmacokinetics and pharmacodynamics between CYP2C19 phenotypes is the same for Caucasian and oriental populations since it is independent of demographical and physiological differences between the populations. The calculations of power in Caucasians will hence be applicable to oriental groups.

The plasma concentration-time profiles were comparable with those observed by Andersson *et al.* (1990a), although the CYP2C19 phenotype/ genotype of the individuals included in the study was not specified. If it is assumed that the simulated plasma concentration-time profiles are representative of the *in vivo* situation then it may be inferred that one or more PM individuals were present in the study (Figure 7.3).

Had natural abundances of CYP2C19 PMs in the Caucasian population been relied upon, lower powers than those calculated for the reported studies would have resulted (Figure 7.4). For example, 250 and 300 subjects would have been required to detect a difference in the pharmacokinetics and pharmacodynamics of OMZ, respectively if such populations had been used. Perhaps this explains the absence of such reports in the literature while those carried out in oriental populations

are abundant since Japanese populations have much higher frequencies of CYP2C19 PMs than Caucasian populations (2.8 *vs* 21.3%; Wedlund *et al.*, 2000).

Figure 7.4 and Figure 7.6 demonstrate the usefulness of study enrichment. The higher proportions of CYP2C19 PMs present in the reported studies have much higher powers than those simulated using a Caucasian population of subjects (Figure 7.4). However, Figure 7.6 is a reminder of the importance of study size, given that studies with similar proportions of PMs can have widely varying powers.

The estimates of study power are consistent with the findings of published reports. At least 12 literature studies have been successful in linking OMZ pharmacokinetics to CYP2C19 phenotype. We calculated their powers to be between 40% and 90% (Fu *et al.*, 2004; Furuta *et al.*, 1999a; Furuta *et al.*, 1999b; Hu *et al.*, 2005; Kita *et al.*, 2001; Ohnishi *et al.*, 2005; Qiao *et al.*, 2006; Sakai *et al.*, 2001; Shirai *et al.*, 2001; Tybring *et al.*, 1997; Yasuda *et al.*, 1995; Yin *et al.*, 2004). Of the 12 studies, 11 were predicted to have a power greater than 50% and therefore be more likely to succeed than fail to determine a difference in OMZ pharmacokinetics between CYP2C19 phenotypes. Despite the above consistency between the predicted and observed powers, it is not possible to make inferences about the ability of the model to predict the failure of an *in vivo* study effectively. This is because only 3 negative studies have been reported in the literature. While the model is clearly capable of predicting study success, the same cannot be said for the converse situation.

Of the 6 studies which were able to determine a difference in the pharmacodynamics of OMZ between CYP2C19 pheno/genotypes (Furuta *et al.*, 1999b; Hu *et al.*, 2005; Kita *et al.*, 2001; Shimatani *et al.*, 2003; Shirai *et al.*, 2001; Tanigawara *et al.*, 1999), we predicted that 5 had a power greater than 50% and were therefore likely to succeed. Three pharmacodynamic studies were unsuccessful in differentiating OMZ pharmacokinetics between CYP2C19 pheno/genotype (Egan *et al.*, 2003; Miyoshi *et al.*, 2001; Ohkusa *et al.*, 2005), we predicted that these studies would have powers of 45 to 100%. In the two cases where a false positive outcome was predicted, this could be due to the use of endpoints such as cure rates of oesophageal reflux disease (Miyoshi *et al.*, 2001) and adverse reactions to OMZ (Ohkusa *et al.*, 2005) in the reported studies. Since the endpoint used in the simulation studies was gastric acid secretion (assumed to be comparable to gastric pH), the

variability may be lower in the simulated PD than in *in vivo.* This would hence lead to a lower power *in vivo* than was indicated by the simulations.

The estimated powers were similar for pharmacokinetic studies and pharmacodynamic studies because stomach pH is highly dependent on plasma OMZ concentration due to the low variability in PD parameters. The absence of any non-linearity in the effect should also be noted. According to the model, concentration increases approaching infinity, lead to an infinite increase in gastric acid secretion. This lack of a maximum effect is somewhat unrealistic since in reality, feedback mechanisms within the body would prevent the effect increasing indefinitely. In addition to the latter, a further limitation of the model is the lack of time delay between drug concentration in the systemic compartment and acid secretory response in the stomach. This is unrealistic given the indirect nature of the pharmacodynamic response mechanisms (enzyme inhibition). In addition to the above limitations, the model does not allow for the possibility of acid inhibition occurring in the parietal cells of the stomach *before* the drug passes through the gut wall and enters the systemic circulation.

In the case of OMZ, much higher powers to detect differences in both the pharmacokinetics and the pharmacodynamics of the drug between CYP phenotypes are in evidence than for the other model drugs that have been researched within this thesis. In addition to the limitations discussed above, further reasons for the latter effect may include, the large difference in the abundance and hence activity of CYP2C19 between EMs and PMs, and the lack of any significant involvement from other enzymes in the overall metabolism of OMZ.

As mentioned in the methods, the majority of the reported studies of the impact of CYP2C19 genotype on OMZ pharmacokinetics and pharmacodynamics are carried out in oriental populations. As discussed above, several differences exist between Japanese and Caucasian populations. However, for the purposes of this study, it was assumed that the relative differences between CYP2C19 phenotypes in Japanese populations would be similar to that for Caucasians and hence, the calculated powers would still be applicable. The only difference incorporated into these simulations was the abundance of CYP2C19 PM subjects in the population which was altered to correspond with that seen in each individual study. Future work could involve the

incorporation of ethnic differences between Japanese and Caucasian populations into the Simcyp® algorithms.

In conclusion, the results of this study have demonstrated the value of study enrichment with respect to rarer CYP phenotypes by the wide difference in power between studies that are enriched, and those that are not. The study has also investigated some possible explanations for the apparent discrepancy between the success of published *in vivo* studies to discern a difference in the pharmacokinetics of OMZ between CYP2C19 phenotypes and the apparent failure of studies to consistently replicate the corresponding differences in the pharmacodynamics of OMZ response.

CHAPTER 8

The Propagation of Pharmacogenetic Differences in Cytochrome P450 into Pharmacokinetic & Pharmacodynamic Measures: The Example of CYP2C9 & Tolbutamide

8 TOLBUTAMIDE

8.1 Introduction

Blood glucose levels are maintained in the body by negative feedback mechanisms involving pancreatic hormones (Figure 8.1). Insulin is the hormone responsible for stimulating the liver to take up glucose and store it as glucagons, thereby reducing blood sugar levels (Figure 8.1).

Tolbutamide (TLB) is a member of the sulfonylurea family of drugs, which stimulate insulin secretion from pancreatic β-cells and are often used in the treatment of type II diabetes mellitus (Kirchheiner *et al.*, 2002). It is mostly metabolised by cytochrome P450 2C9 (CYP2C9) [around 64% - (Lasker *et al.*, 1998)] to hydroxytolbutamide. This pathway is widely used as a probe reaction for measuring CYP2C9 activity both *in vitro* (Tucker *et al.*, 2001) and *in vivo* (Jetter *et al.*, 2004). CYP2C19 has also been shown to contribute to the metabolism of TLB (Lasker *et al.*, 1998).

As discussed in the Literature Review (Chapter 2), it is clear that the CYP2C9 polymorphism is important for determining differences in the pharmacokinetics of TLB between subjects (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Lee *et al.*, 2003; Lee *et al.*, 2002b; Shon *et al.*, 2002; Wang *et al.*, 2005). However, it is uncertain whether these differences in pharmacokinetics between genotypes translate into differences in response to the drug (Holstein *et al.*, 2005; Kirchheiner *et al.*, 2002; Shon *et al.*, 2002) where response measures included plasma insulin and glucose concentration (Kirchheiner *et al.*, 2002) and glucose tolerance and serum glucose concentration (Shon *et al.*, 2002). A possible reason for this disparity is the size of the study populations involved in the studies. In both cases, the same study size has been employed for both the pharmacokinetic and the pharmacodynamic study. Although the sample size may be sufficient to observe differences in pharmacokinetics, it may result in a much lower power for observing significant difference in pharmacodynamics and hence a lower chance of the study being successful in that respect.

Two studies have investigated the relationship between TLB pharmacokinetics and pharmacodynamics and CYP2C19 phenotype, however, no correlation has been observed (Kirchheiner *et al.*, 2002; Shon *et al.*, 2002).

The aim of the study represented in this chapter was to simulate the pharmacokinetics of TLB and insulin secretory response to the drug in virtual populations of human subjects. These simulations were used to mimic the *in vivo* studies and to estimate their power to differentiate the pharmacokinetics and pharmacodynamics of TLB between *CYP2C9* genotypes. These simulations were used to mimic the *in vivo* studies and to estimate their power to identify differences in the pharmacokinetics and pharmacodynamics of TLB between *CYP2C9* genotypes. The ultimate aims of the work were to facilitate a more comprehensive understanding of the relationship between drug pharmacokinetics/pharmacodynamics and *CYP2C9* genotypes, using TLB as a model drug, and to investigate the impact of study size on the likelihood of success in defining differences in the pharmacokinetics and response of TLB between *CYP2C9* genotypes.

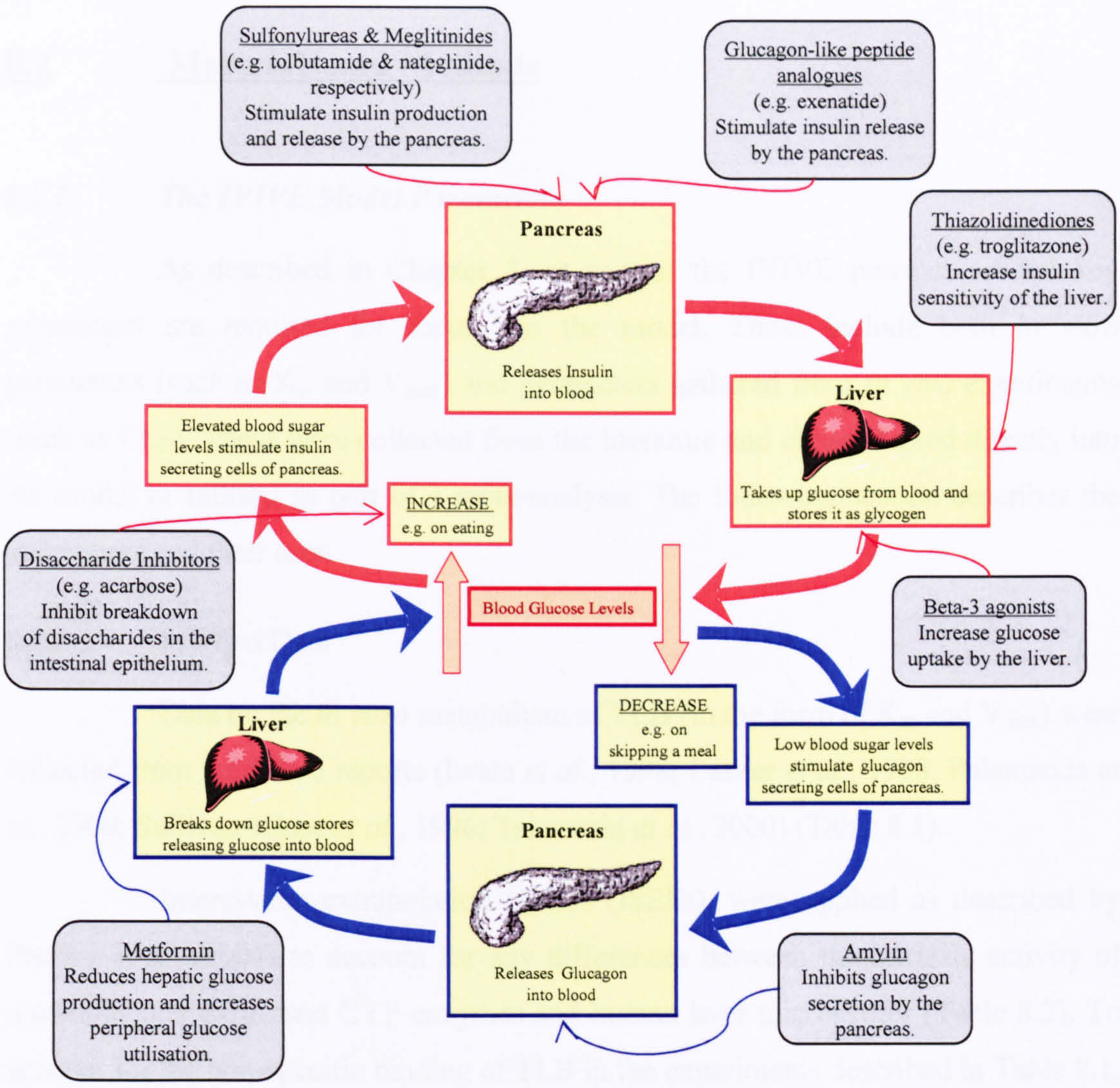


Figure 8.1 Regulation of blood glucose levels by negative feedback mechanisms involving the hormones insulin and glucagon. The drugs that act on these processes are shown. Adapted from Marieb (1997) with additional elements from Rendell (2004).

8.2 Materials and Methods

8.2.1 The IVIVE Model Parameters

As described in Chapter 3, as part of the IVIVE process, several key parameters are required for input into the model. These include both *in vitro* parameters (such as K_m and V_{max}) and parameters gathered from *in vivo* experiments (such as CL_R). These were collected from the literature and either entered directly into the model or utilised as part of a meta-analysis. The following section describes the parameters and their uses.

8.2.1.1 *In Vitro* Data

Data on the *in vitro* metabolism of TLB (in the form of K_m and V_{max}) were collected from published reports (Iwata *et al.*, 1998; Lasker *et al.*, 1998; Palamanda *et al.*, 2000; Sullivan-Klose *et al.*, 1996; Takanashi *et al.*, 2000) (Table 8.1).

Intersystem extrapolation factors (ISEFs), were applied as described by Proctor *et al.* (2004) to account for any differences between the intrinsic activity of recombinantly expressed CYP enzymes and human liver microsomes (Table 8.2). To account for the non-specific binding of TLB in the experiments described in Table 8.1, a value of fu_{mic} was calculated and applied to the data as described in Chapter 3; Sections 3.1.1.2 and 3.3. ISEF values and values of fu_{mic} are shown in Table 8.2. A meta-analysis was then conducted to find the overall V_{max} and K_m values for the metabolism of TLB by CYP2C9 and CYP2C19.

8.2.1.2 Additional Parameters Required for IVIVE

The additional parameters listed in Table 8.3 were required for scaling the *in vitro* data to human whole body clearance and to the elimination rate constant (Chapter 3). These values were then entered into the pharmacokinetic-pharmacodynamic model described below (Sections 8.2.3 & 8.2.4).

Table 8.1 Parameters describing the *in vitro* metabolism of TLB.

Reference	CYP	V_{max}	K_m
Iwata *et al.*, 1998	2C9	4.59	192
Palamanda *et al.*, 2000	2C9	4.7	115
Lasker *et al.*, 1998	2C9	8.36	523
	2C19	12.51	395
Takanashi *et al.*, 2000	2C9	9.2	151
Sullivan-Klose *et al.*, 1996	2C9	0.61	145

Vmax - pmol/min/pmol P450; Km – μM.

Table 8.2 ISEF and fu_{mic} values that were applied to the data shown in Table 5.2, resulting in the revised V_{max} and K_m values shown here. The final values entered into the IVIVE algorithms are indicated in bold type at the bottom of the table.

Reference	System	CYP	Protein Conc (mg/mL)	fu_{mic}	ISEF	New V_{max} (pmol/min/ pmol P450)	New K_m (μM)
Iwata *et al.*, 1998	E. coli	2C9	0.25*	0.996	3.16	14.5	191
Palamanda *et al.*, 2000	E. coli	2C9	0.25*	0.996	3.16	14.9	115
Lasker *et al.*, 1998	E. coli	2C9	0.25*	0.996	3.16	26.4	521
		2C19	0.25*	0.996	2.76	35	393
Takanashi *et al.*, 2000	Yeast	2C9	0.1	0.998	2.66	24.5	151
Sullivan-Klose *et al.*, 1996	Yeast	2C9	0.25*	0.996	2.66	1.62	144
Weighted Mean		**2C9**				**16.1**	**187**
		2C19				**35**	**393**

*A default value of 0.25 mg/mL was assumed if no specific value was available (Chapter 3; Section 3.1.1.2).

Table 8.3 Mean pharmacokinetic parameter values of TLB taken or calculated from the literature.

Parameter	Value	References
fu	0.102	Miners *et al.*, 1984; Rostami-Hodjegan *et al.*, 1998; Tremaine *et al.*, 1997; Wilner *et al.*, 1995; Section 3.1.1.3.
B:P	0.55	Obach, 1999; Section 3.1.1.3.
fu_B	0.056	Section 3.1.1.3.
CL_R (L/h)	0.0015	Lee *et al.*, 2002; Miners *et al.*, 1984; Tremaine *et al.*, 1997; Section 3.3.3.
V (L/kg)	0.112 (0.034)*	Kirchheiner *et al.*, 2002; Peart *et al.*, 1987; Section 3.3.4.

*Mean (SD); fu = fraction unbound in plasma; B:P = Blood to Plasma concentration drug ratio; fu_B = fraction unbound in blood; CL_R = renal clearance; V = initial volume of distribution.

Table 8.4 Results of a meta-analysis to calculate the relative activity of each of the *CYP2C9* genotypes. All studies are carried out in yeast microsomes except for Guo *et al.* (2005) and Veronese *et al.* (1993) which were carried out in Cos cells and Rettie *et al.* (1994) which used HepG2 cells. The overall decrease in CL_{int} for the variant alleles is shown in bold.

Allele	No. Observations	Vmax (pmol/min/ pmol P450)	Km (μM)	Vmax (pmol/min/ pmol P450)	CL_{int} (μL/min/ pmol P450)	Reduction in CL_{int} (%)	References
*1	3	0.611	145	1.63	0.0112		Sullivan-Klose *et al.*, 1996
	3	9.2	151	24.5	0.162		Takanashi *et al.*, 2000
	2	73.2	286	195	0.682		Hanatani *et al.*, 2003
	3	7.96	105		0.0758		Guo *et al.*, 2005
	2	620	90.5		6.85		Veronese *et al.*, 1993
	1	1.93	106		0.0182		Rettie *et al.*, 1994
*2	3	0.442	122		0.000344		Sullivan-Klose *et al.*, 1996
	2	189	94.4		2.00	**↓ 32.7**	Veronese *et al.*, 1993
	1	1.15	72		0.0160		Rettie *et al.*, 1994
*3	3	0.373	745	0.992	0.00133		Sullivan-Klose *et al.*, 1996
	3	10	1730	26.6	0.0154		Takanashi *et al.*, 2000
	2	3.40	431	9.04	0.0210	**↓ 80.5**	Hanatani *et al.*, 2003
	3	8.04	397		0.0203		Guo *et al.*, 2005
	2	467	132		3.54		Veronese *et al.*, 1993

8.2.2 Genotype Frequencies and Activities

A meta-analysis of the published literature was carried out to determine the relative activity of the enzyme expressed by each *CYP2C9* allele with respect to the *in vitro* metabolism of TLB. Mean values of the activity of each allelic form were weighted for study size. Percentage decreases in intrinsic clearance (CLu_{int}) with respect to wild type (*1/*1) enzyme were calculated as described for *(S)*-warfarin in Chapter 5; Section 5.2.2 by assuming that the *in vitro* activities of heterologously expressed variant enzymes represented those in the respective homozygous genotype. Values of CLu_{int} in heterozygous genotypes were assumed to be the mean of those for homozygotes. A summary of the studies included in the meta-analysis is given in Table 8.4 (Guo *et al.*, 2005; Hanatani *et al.*, 2003; Rettie *et al.*, 1994; Sullivan-Klose *et al.*, 1996; Takanashi *et al.*, 2000; Veronese *et al.*, 1993). The results of this meta-analysis are shown in Table 8.5. The prevalence of each of the 6 established, common *CYP2C9* genotypes in Caucasians were taken from a review by Lee *et al.* (2002) (Table 8.5).

Table 8.5 Frequency and relative activity of each of the common *CYP2C9* genotypes. Frequencies taken from (Lee *et al.*, 2002a) relative activities calculated from a meta-analysis of the literature (Guo *et al.*, 2005; Hanatani *et al.*, 2003; Rettie *et al.*, 1994; Sullivan-Klose *et al.*, 1996; Takanashi *et al.*, 2000; Veronese *et al.*, 1993).

Genotype	Frequency (%)	Relative Activity (%)
*1*1	65.3	100
*1*2	20.4	84
*1*3	11.6	60
*2*2	0.9	68
*2*3	1.4	44
*3*3	0.4	20

Table 8.6 Literature values describing the pharmacokinetics of TLB.

Parameter	Value*	References
V_{ss} (L)	6.9 (1.59)	Rostami-Hodjegan *et al.*, 1998
k_a (h^{-1})	0.52 (0.30)	Kivisto & Neuvonen, 1992; Peart *et al.*, 1987
tlag (h)	0.08 (0.1)	Nishimura *et al.*, 1998
k_{12} (min^{-1})	0.071 (0.080)	Rostami-Hodjegan *et al.*, 1998
k_{21} (min^{-1})	0.087 (0.064)	Rostami-Hodjegan *et al.*, 1998

*Mean (SD)

Table 8.7 Mean pharmacodynamic parameter values of TLB taken from the literature. All parameters are taken from Rostami-Hodjegan *et al.* (1998) except E_0 which was from Polonsky *et al.* (1986).

Parameter	Tolbutamide*
E_0 (pmol/min)	89.1 (13.4)
$E_{max,1}$ (pmol/min)	1641 (279)
$E_{max,2}$ (pmol/min)	2480 (595)
$EC_{50.1}$ (ng/ml)	1.78×10^8 (1.96×10^7)
$EC_{50.2}$ (ng/ml)	1.49×10^8 (7.45×10^6)
n_1	10 (2.3)
n_2	43 (35)
k_{e01} (min^{-1})	0.045 (0.0085)

*Mean (SD)

8.2.3 Pharmacokinetic Model

Plasma concentrations after oral administration of TLB were generated using a two compartment model with first order absorption after a lag time (tlag – Table I) (adapted from the model given for the intravenous administration of TLB reported by Rostami-Hodjegan *et al.*, (1998) (Figure 8.2). Equation 8.1 describes the plasma concentration of TLB (C(t)i).

$$C(t)_{TLB,i} = \frac{D \cdot k_{a,i} \cdot F_{H,i} \cdot f_a}{V_i} \cdot (A1_i + A2_i + A3_i) \qquad \text{Equation 8.1}$$

where f_a is the oral bioavailability of TLB (set to 1), D is the dose of TLB administered (set to a 500mg oral dosage), $k_{a,i}$, $F_{H,i}$ and V_i are the first order absorption rate constant (Table 8.6), fraction of TLB escaping first pass metabolism (calculated using the Simcyp® model) and the steady state volume of distribution of TLB (Table 8.3) respectively, in each individual and $A1_i$, $A2_i$ and $A3_i$ are given by:

$$A1_i = \frac{(k_{21,i} - \alpha_i) \cdot e^{-\alpha_i \cdot t}}{(\beta_i - \alpha_i) \cdot (k_{a,i} - \alpha_i)} \qquad \text{Equation 8.2}$$

$$A2_i = \frac{(k_{21,i} - \beta_i) \cdot e^{-\beta_i \cdot t}}{(\alpha_i - \beta_i) \cdot (k_{a,i} - \beta_i)} \qquad \text{Equation 8.3}$$

$$A3_i = \frac{(k_{21,i} - k_{a,i}) \cdot e^{-k_{a,i} \cdot t}}{(\alpha_i - k_{a,i}) \cdot (\beta_i - k_{a,i})} \qquad \text{Equation 8.4}$$

where $k_{21,i}$ is the transfer rate constant from the peripheral to the central compartment (Table 8.6) and α_i and β_i are the hybrid rate constants associated with the distribution and elimination phases, respectively, defined as shown in Chapter 4; Equations 4.5 & 4.6.

8.2.4 Pharmacodynamic Model

The following equation was used to calculate the concentration of TLB in a hypothetical effect compartment:

$$Ce(t)_{TLB,i} = \frac{D \cdot k_{a,i} \cdot k_{e0,i} \cdot F_{H,i} \cdot f_a}{V_i} \cdot (A1_i + A2_i + A3_i + A4_i) \qquad \text{Equation 8.5}$$

where $k_{e0,i}$ is the rate constant defining removal of TLB from the effect compartment (Table 8.7). $A1_i$, $A2_i$, $A3_i$ and $A4_i$ are given by:

$$A1_i = \frac{(k_{21,i} - \alpha_i) \cdot e^{-\alpha_i \cdot t}}{(\beta_i - \alpha_i) \cdot (k_{a,i} - \alpha_i) \cdot (k_{e0,i} - \alpha_i)} \qquad \text{Equation 8.6}$$

$$A2_i = \frac{(k_{21,i} - \beta_i) \cdot e^{-\beta_i \cdot t}}{(\alpha_i - \beta_i) \cdot (k_{a,i} - \beta_i) \cdot (k_{e0,i} - \beta_i)} \qquad \text{Equation 8.7}$$

$$A3_i = \frac{(k_{21,i} - k_{a,i}) \cdot e^{-k_{a,i} \cdot t}}{(\alpha_i - k_{a,i}) \cdot (\beta_i - k_{a,i}) \cdot (k_{e0,i} - k_{a,i})} \qquad \text{Equation 8.8}$$

$$A4_i = \frac{(k_{21,i} - k_{e0,i}) \cdot e^{-k_{e0,i} \cdot t}}{(\alpha_i - k_{e0,i}) \cdot (\beta_i - k_{e0,i}) \cdot (k_{a,i} - k_{e0,i})} \qquad \text{Equation 8.9}$$

The pharmacokinetic data were linked with the pharmacodynamic data by assuming that TLB in both the systemic and effect compartments can cause insulin secretion (Figure 8.2), resulting in a biphasic response-time profile. Response was modelled using a sigmoidal E_{max} model for the effect of TLB on insulin secretion (E) (Rostami-Hodjegan *et al.*, 1998):

$$E_i = E_0 + \frac{E_{max,1,i} \cdot (C(t)_{TLB,i})^{n_{1,i}}}{(EC_{50,1,i})^{n_{1,i}} + (C(t)_{TLB,i})^{n_{1,i}}} + \frac{E_{max,2,i} \cdot (Ce(t)_{TLB,i})^{n_{2,i}}}{(EC_{50,2,i})^{n_{2,i}} + (Ce(t)_{TLB,i})^{n_{2,i}}} \qquad \text{Equation 8.10}$$

where E_0, is the average baseline level of insulin secretion (Polonsky *et al.*, 1986) (Table 8.7), the numbers '1' and '2' refer to TLB contained within the systemic and effect compartments, respectively.

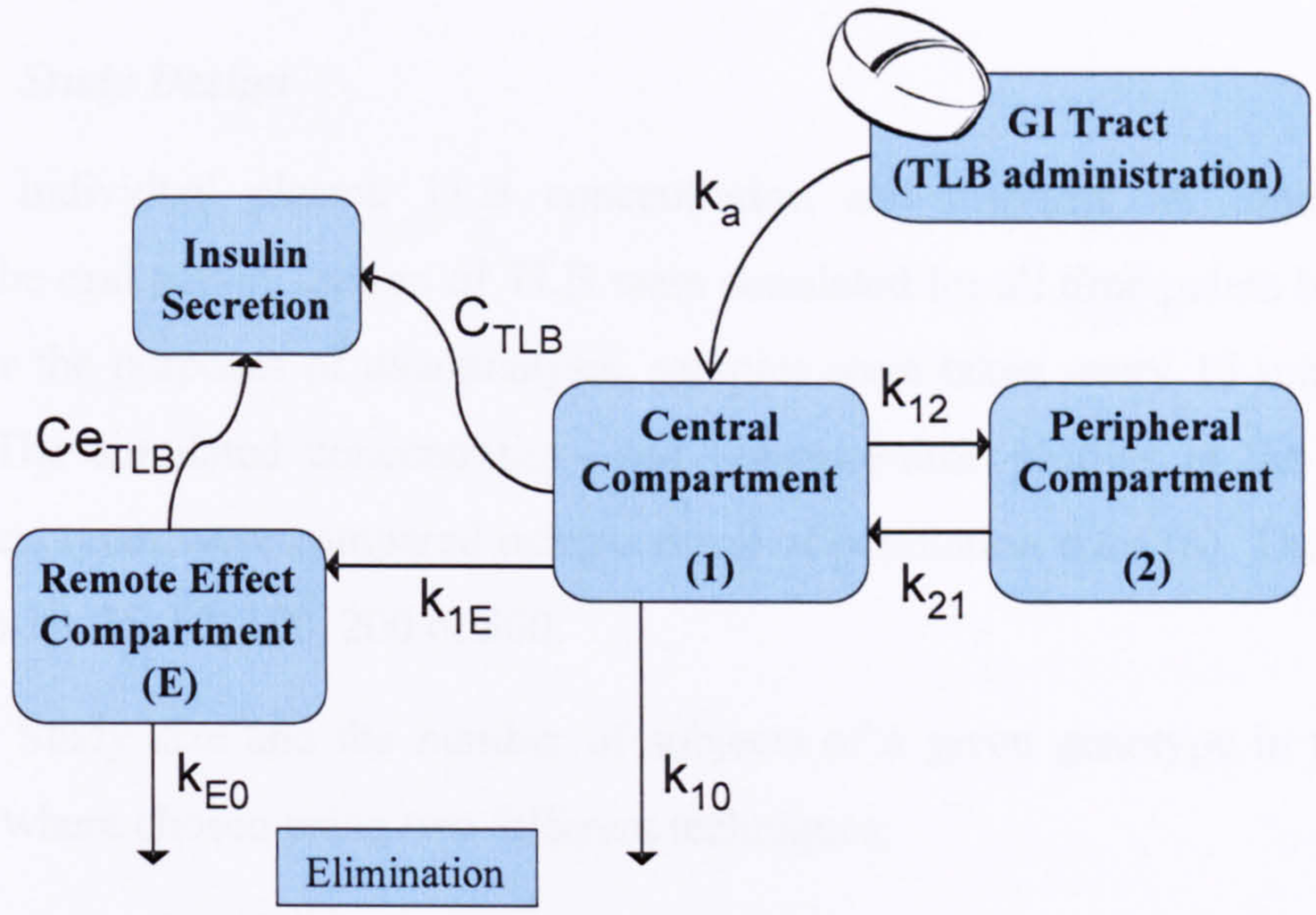


Figure 8.2 Schematic representation of the pharmacokinetic-pharmacodynamic model used to describe the biphasic insulinergic effect of tolbutamide.

8.2.5 Study Design

Individual plasma TLB concentration and response *vs.* time profiles following the oral administration of TLB were simulated for all time points between 0 and 3h. For the purposes of data-analysis, samples were taken every 15 minutes (12 samples). The simulated concentration- and response-time profiles in the different *CYP2C9* genotypes were compared using a range of population sizes (n). The value of n was set to 10, 25, 50, 100, 200 or 300.

Study size and the number of subjects of a given genotype in the study population where chosen using two different techniques:

(i) Study sizes were chosen arbitrarily and the proportion of subjects of each genotype was chosen according to that of the general Caucasian population, this will be referred to as 'random selection' of subjects, and

(ii) To our knowledge, there are 8 literature studies which investigate the relationship between *CYP2C9* genotype and the pharmacokinetics and/or pharmacodynamic of TLB. The size of these studies ranged from 15 to 63 with varying proportions of the different non-wild type genotypes within the study populations. Details of these are reported later in Table 8.10. As the majority of these studies are carried out using populations of subjects with a higher than the Caucasian average frequency of rarer *CYP2C9* genotypes, they will be referred to as 'enriched'. Study sizes and the proportion of each phenotype/genotype were also chosen in order to mimic those seen in the literature studies and their powers were, hence, calculated.

8.2.6 Data Analysis

Twenty simulations were run for each study size involving 120 simulations and a total of 13,700 virtual subjects. Values of the areas under the plasma drug concentration-time curves (AUC) and effect (insulin secretion)-time curves (AUEC) were calculated up to 3h (trapezoidal rule) for consistency with the reported, *in vivo* studies. For the purposes of this study we assumed that no measurement error occurred. The probability of detecting statistically significant differences in pharmacokinetics and pharmacodynamics between the wild type (*1/*1) and the combination of the other *CYP2C9* genotypes was assessed by comparing values of

AUC and AUEC, respectively, using ANOVA (SPSS v 12; SPSS Inc, Chicago 2003). The corresponding probabilities of detecting differences in AUC or AUEC between the wild type and any other single genotype were calculated using Tukey's post hoc test for multiple comparisons. The percentage of studies out of the 20 simulations that led to a statistically significant difference in AUC (or AUEC) between *CYPC9* genotypes was recorded as the power of that comparison.

Clearance values and other pharmacokinetic parameters obtained from the IVIVE model were compared with those values observed *in vivo* (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Lee *et al.*, 2002b; Shon *et al.*, 2002). The accuracy of the resulting plasma TLB concentration-time profiles was assessed by comparison with the profiles reported in the literature by Shon *et al.* (2002) and Lee *et al.* (2002).

8.3 Results

8.3.1 Model Validation

The meta-analysis of the enzyme activities associated with the variant alleles of CYP2C9 indicated mean decreases in CLu_{int} of 16% and 40% compared to wild-type activity, respectively, for every *2 and *3 allele present in an individual. The results for the different *CYP2C9* genotypes are shown in Table 8.5. Propagation of these values through the Simcyp® algorithm resulted in mean values of unbound oral clearance for TLB from 100 individuals of 0.72 (*1*1), 0.63 (*1*2), 0.48 (*1*3), 0.52 (*2*2), 0.39 (*2*3) and 0.26 (*3*3) l/h which were all within 2 fold of the *in vivo* values available in the literature (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Lee *et al.*, 2002b) as shown in Figure 8.3.

Table 8.8 is a summary of simulated pharmacokinetic parameters for TLB in all *CYP2C9* genotypes compared with those observed by Jetter *et al.* (2004); Kirchheiner *et al.* (2002); Lee *et al.* (2002b) and Shon *et al.* (2002).

Figure 8.4 shows concentration-time profiles in each of the *CYP2C9* genotypes. The profiles for wild-type, *1*2 and *1*3 individuals were compared with those observed by Shon *et al.* (2002) and Lee *et al.* (2002) (Figure 8.5).

Representative response (insulin secretion)-time profiles are shown in Figure 8.6. The biphasic nature of the response-time curve can be observed, particularly in the *2/*3 and *3/*3 genotypes. No literature reports were available to compare the simulated insulin secretion-time profiles with those observed *in vivo*.

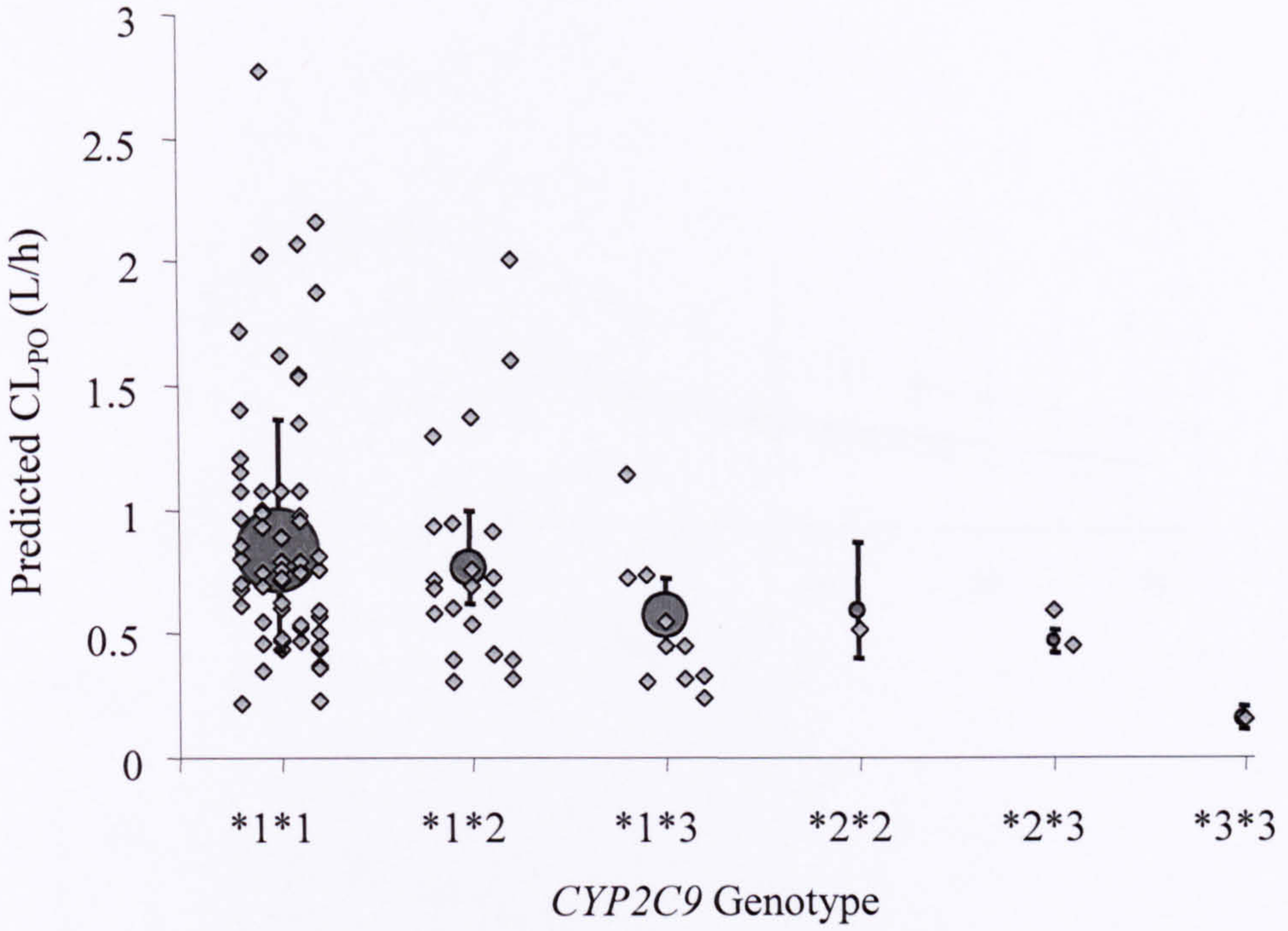


Figure 8.3 Observed (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Lee *et al.*, 2002b; Shon *et al.*, 2002; Wang *et al.*, 2005) and predicted (n = 100) oral clearances for TLB. The bubble size indicates the number of subjects included in the studies.

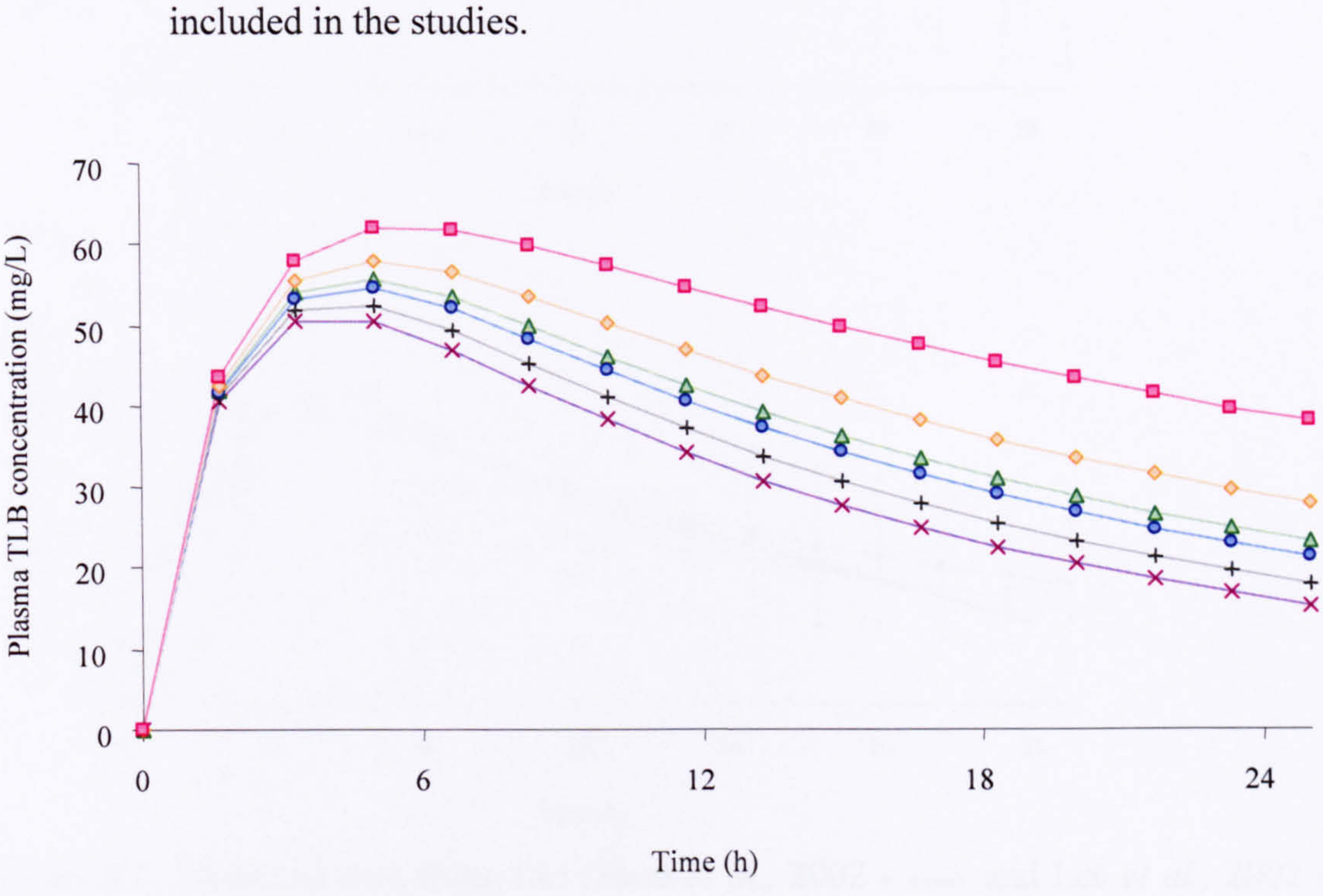


Figure 8.4 Representative simulated concentration-time profiles over 25 hours in *CYP2C9* *1*1 (×), *1*2 (+), *1*3 (△), *2*2 (●), *2*3 (◇) and *3*3 (■) genotypes.

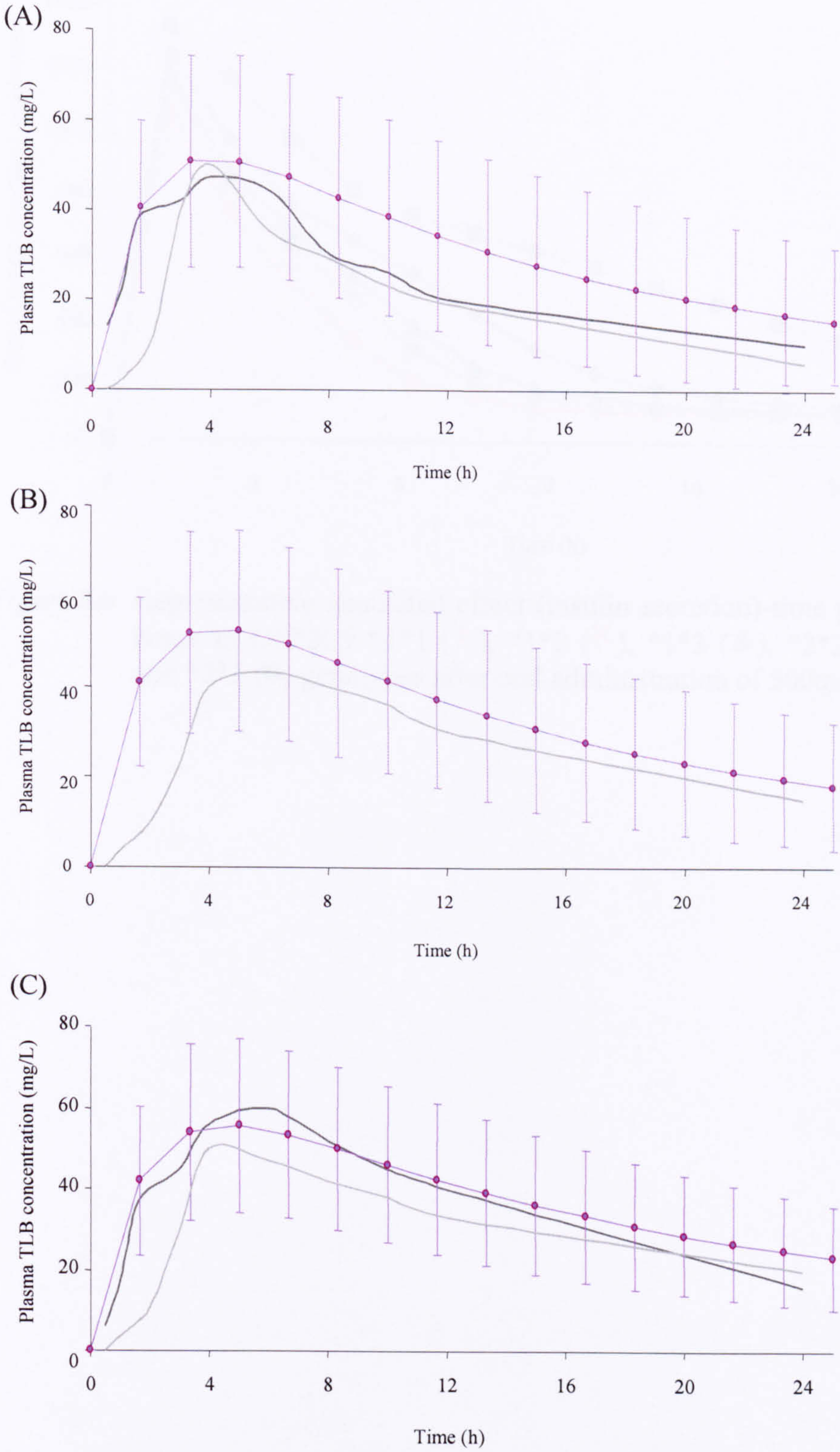


Figure 8.5 Observed data from, (A) (Shon *et al.,* 2002 - ▬ and Lee *et al.,* 2002 - ▬), (B) (Lee *et al.,* 2002), and (C) (Shon *et al.,* 2002 - ▬ and Lee *et al.,* 2002 - ▬) and predicted plasma concentration-time profiles of TLB over 25 hours in 12 real and 50 virtual (●; mean ± 95% CI) (A) wild type, (B) *1*2 and, (C) *1*3 individuals.

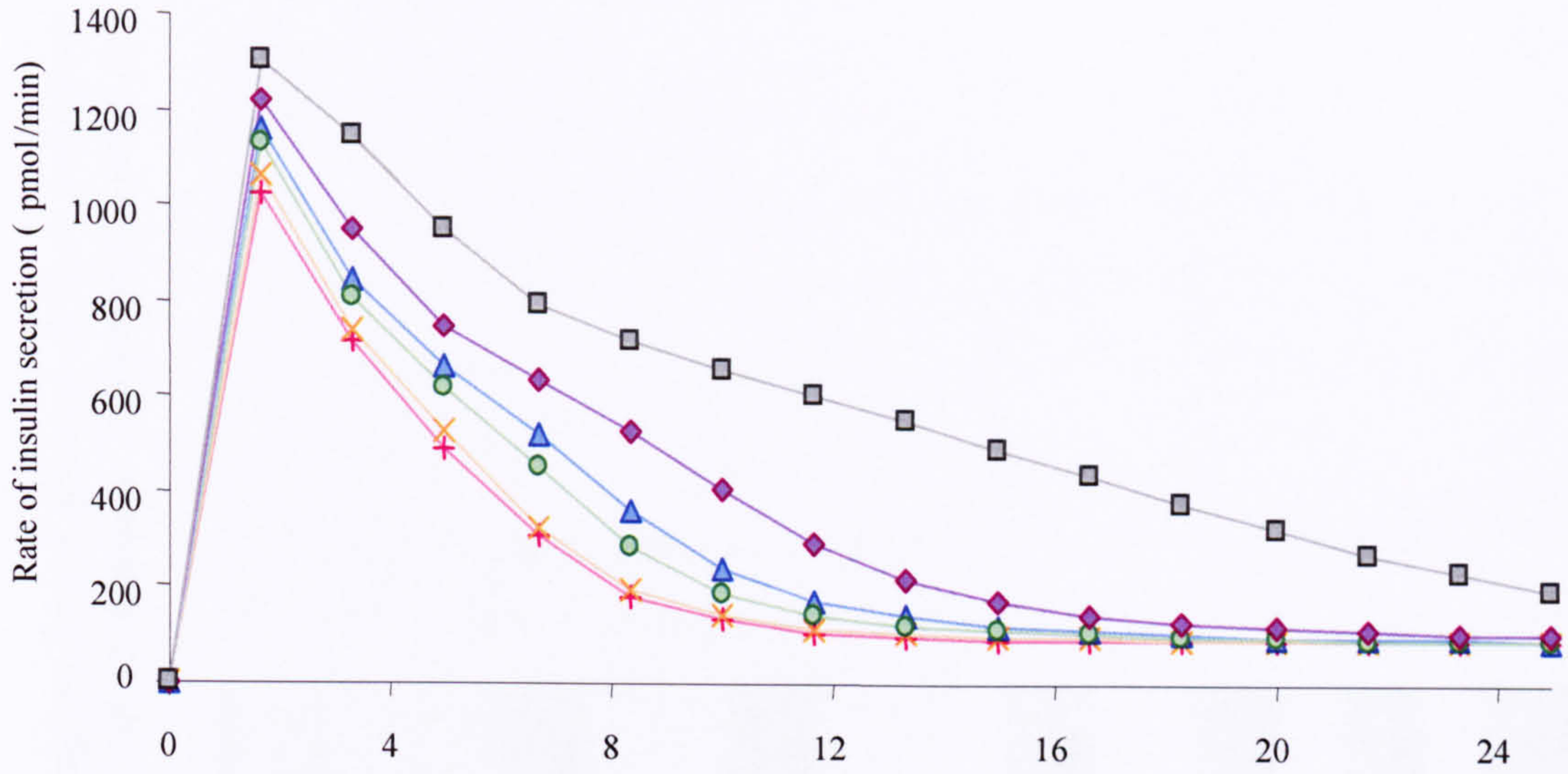


Figure 8.6 Representative simulated effect (insulin secretion)-time profiles over 25 hours in *CYP2C9* *1*1 (+), *1*2 (×), *1*3 (△), *2*2 (○), *2*3 (◆) and *3*3 (□) genotypes after oral administration of 500mg TLB

Table 8.8 Observed (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Lee *et al.*, 2002b; Shon *et al.*, 2002) and predicted pharmacokinetic parameter values of TLB. Predicted values are the mean (± SD) of the average values from 10 clinical trial simulations. The predicted value not within 2-fold of the observed weighted mean is highlighted in bold type.

Genotype	Reference	$CL_{p.o.}$ (L/h)	$AUC_{0-\infty}$ (mg/L/h)	$t_{1/2}$ (h)	C_{max} (mg/L)
*1*1	Shon *et al.*, 2002	0.79	662	7.1	53
	Kirchheiner *et al.*, 2002	0.97		6.6	
	Jetter *et al.*, 2004	0.85		7.5	
	Lee *et al.*, 2002	0.9	561 (58)	7.1 (0.7)	
	WEIGHTED MEAN	0.86	632	7.2	53
	SIMULATED	0.73 (0.5)	536 (341)	5.4 (3.8)	50.6 (16.4)
*1*2	Kirchheiner *et al.*, 2002	0.86		7.5	
	Jetter *et al.*, 2004	0.77		8.5	
	Lee *et al.*, 2002	0.64	815 (177)	8.9 (1.7)	
	WEIGHTED MEAN	0.75	815	8.4	
	SIMULATED	0.62 (0.4)	675 (442)	6.9 (4.6)	52.4 (17.3)
*1*3	Shon *et al.*, 2002	0.54	1241	11.6	67.8
	Kirchheiner *et al.*, 2002	0.56		11.5	
	Jetter *et al.*, 2004	0.6		12.2	
	Lee *et al.*, 2002	0.47	1079 (86)	13.2 (1.8)	
	WEIGHTED MEAN	0.54	1167	12.1	67.8
	SIMULATED	0.50 (0.3)	866 (587)	8.8 (6.2)	55.6 (18.9)
*2*2	Kirchheiner *et al.*, 2002	0.75		8.6	
	Jetter *et al.*, 2004	0.78		10.7	
	WEIGHTED MEAN	0.76		9.1	
	SIMULATED	0.54 (0.4)	791 (528)	8.0 (5.5)	54.5 (18.3)
*2*3	Kirchheiner *et al.*, 2002	0.45		14.3	
	WEIGHTED MEAN	0.45		14.3	
	SIMULATED	0.48 (0.3)	834 (465)	8.7 (5.6)	58.0 (20.1)
*3*3	Kirchheiner *et al.*, 2002	0.15		42.8	
	WEIGHTED MEAN	0.15		**42.8**	
	SIMULATED	0.2 (0.2)	1423 (613)	13.1 (9.6)	61.9 (22.8)

8.3.2 Power of Studies

The power of studies to detect a statistically significant difference in AUC of TLB between the wild type and a combination of the other *CYP2C9* genotypes was around 80% with about 50 subjects (Figure 8.1). 100% power was reached when 200 subjects were used (Figure 8.7).

The corresponding power to observe a difference in the AUEC of TLB between the wild type and a combination of any other genotype did not increase above 45% with the maximum sample size investigated of 300 subjects (Figure 8.7).

The power to differentiate between wild type and any other single genotype was less than that for the comparison between wild type and a combination of all non-wild type genotypes, to a degree depending on the genotype (Figure 8.8 (A)). For example, about 300 subjects were required to detect a difference in AUC between the *2/*3 genotype and the wild type with a power of 80% (Figure 8.8 (A)) while, with the same number of subjects, a power of only about 30% was achieved when comparing the *2/*2 genotype with the wild type (Figure 8.8 (A)).

Corresponding powers to detect differences in AUEC between *CYP2C9* genotypes are shown in Figure 8.8 (B). The power to detect differences in AUEC between specific genotypes was low (Figure 8.8 (B)). For example only around 20% was reached to detect a difference between the *2/*3 genotype and the wild type subjects with the maximum study size investigated of 300. By comparison, the same number of subjects gave a power of 5% when comparing the *3/*3 genotype with the wild type (Figure 8.8 (B)).

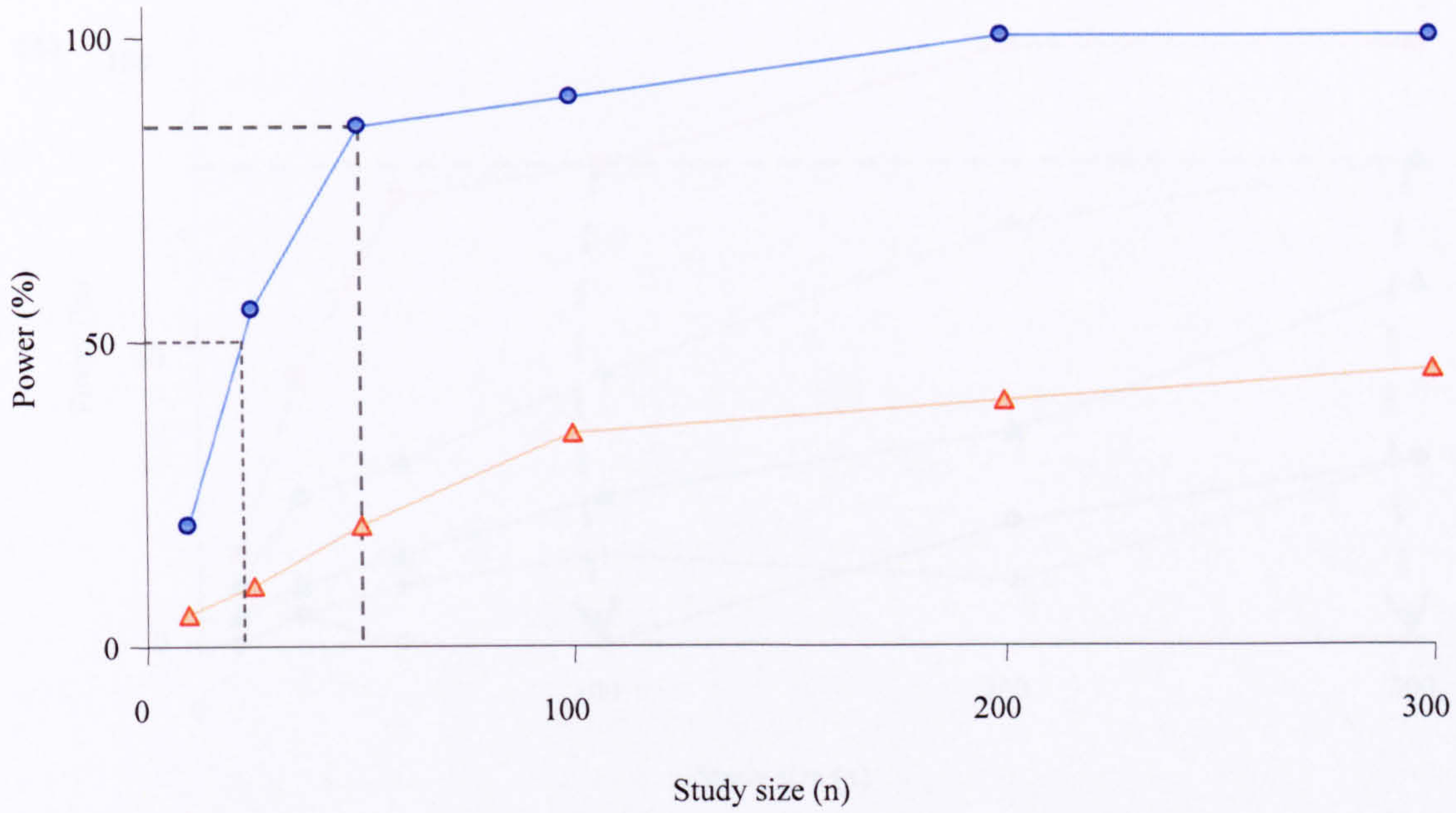


Figure 8.7 The power (%) of simulated studies to show significant differences in AUC (●) and AUEC (△) of TLB between the wild type and a combination of all other *CYP2C9* genotypes *vs* the number of subjects in each study (n). The dashed lines represent 80% power, commonly used in *in vivo* studies, and 50% power, above which, there is more chance of success than failure.

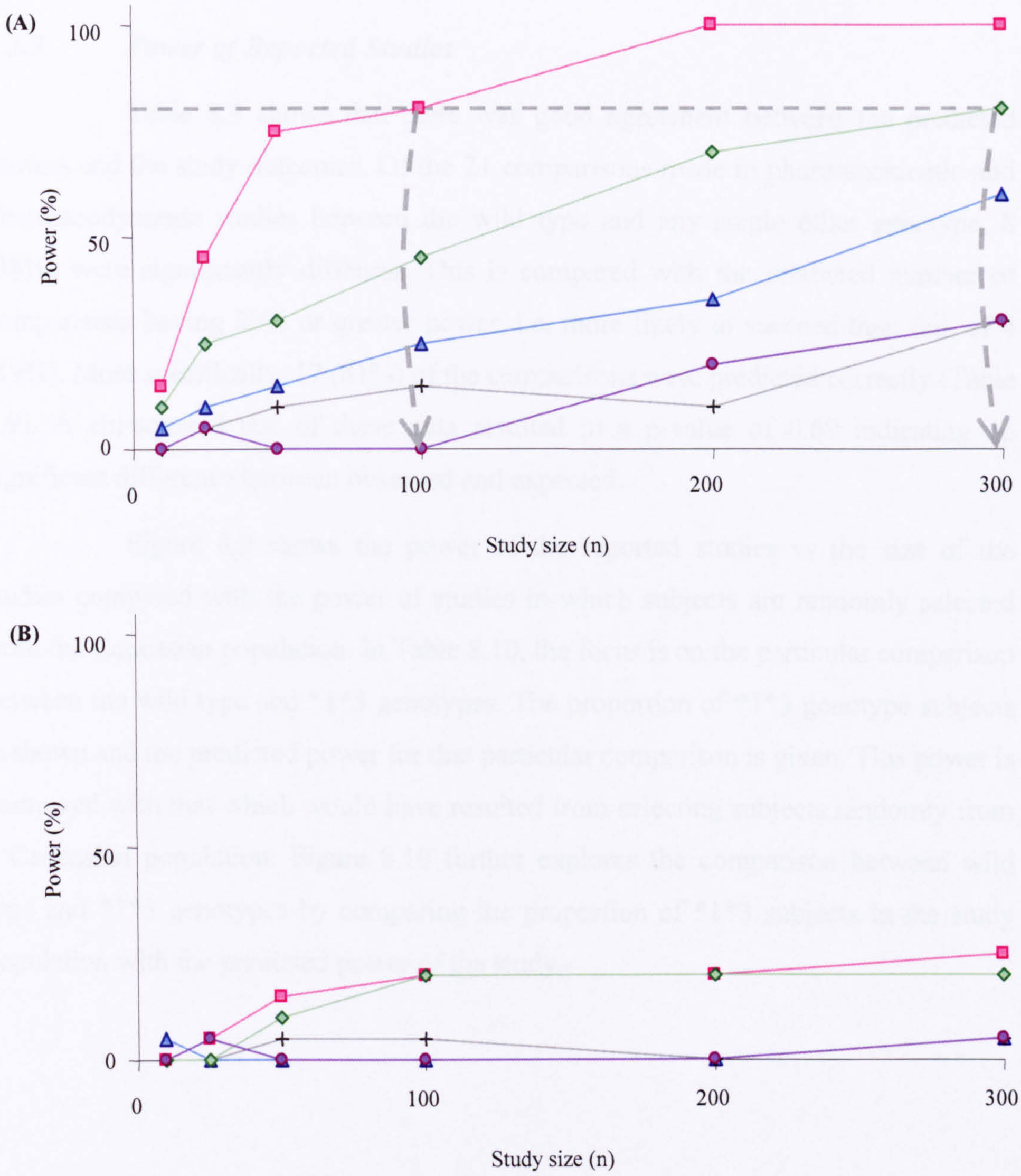


Figure 8.8 The power (%) of simulated studies to show significant differences in (A) AUC, and (B) AUEC between the wild type and any single other *CYP2C9* genotype *vs* the number of subjects in the study population (n). Wild type is compared with the following: *1/*2 (▲), *1/*3 (■), *2/*2 (+), *2/*3 (◆), *3/*3 (●). Dashed line represents 80% power which is typically used in designing clinical studies, the arrows demonstrate the number of subjects required to achieve this power.

8.3.3 Power of Reported Studies

Table 8.9 shows that there was good agreement between the predicted powers and the study outcomes. Of the 21 comparisons made in pharmacokinetic and pharmacodynamic studies between the wild type and any single other genotype, 8 (38%) were significantly different. This is compared with the predicted number of comparisons having 50% or greater power, i.e. more likely to succeed than fail, of 4 (19%). More specifically, 17 (81%) of the comparisons were predicted correctly (Table 8.9). A chi-squared test of these data resulted in a p-value of 0.69 indicating no significant difference between observed and expected.

Figure 8.9 shows the power of the reported studies *vs* the size of the studies compared with the power of studies in which subjects are randomly selected from the Caucasian population. In Table 8.10, the focus is on the particular comparison between the wild type and *1*3 genotypes. The proportion of *1*3 genotype subjects is shown and the predicted power for that particular comparison is given. This power is compared with that which would have resulted from selecting subjects randomly from a Caucasian population. Figure 8.10 further explores the comparison between wild type and *1*3 genotypes by comparing the proportion of *1*3 subjects in the study population with the predicted power of the study.

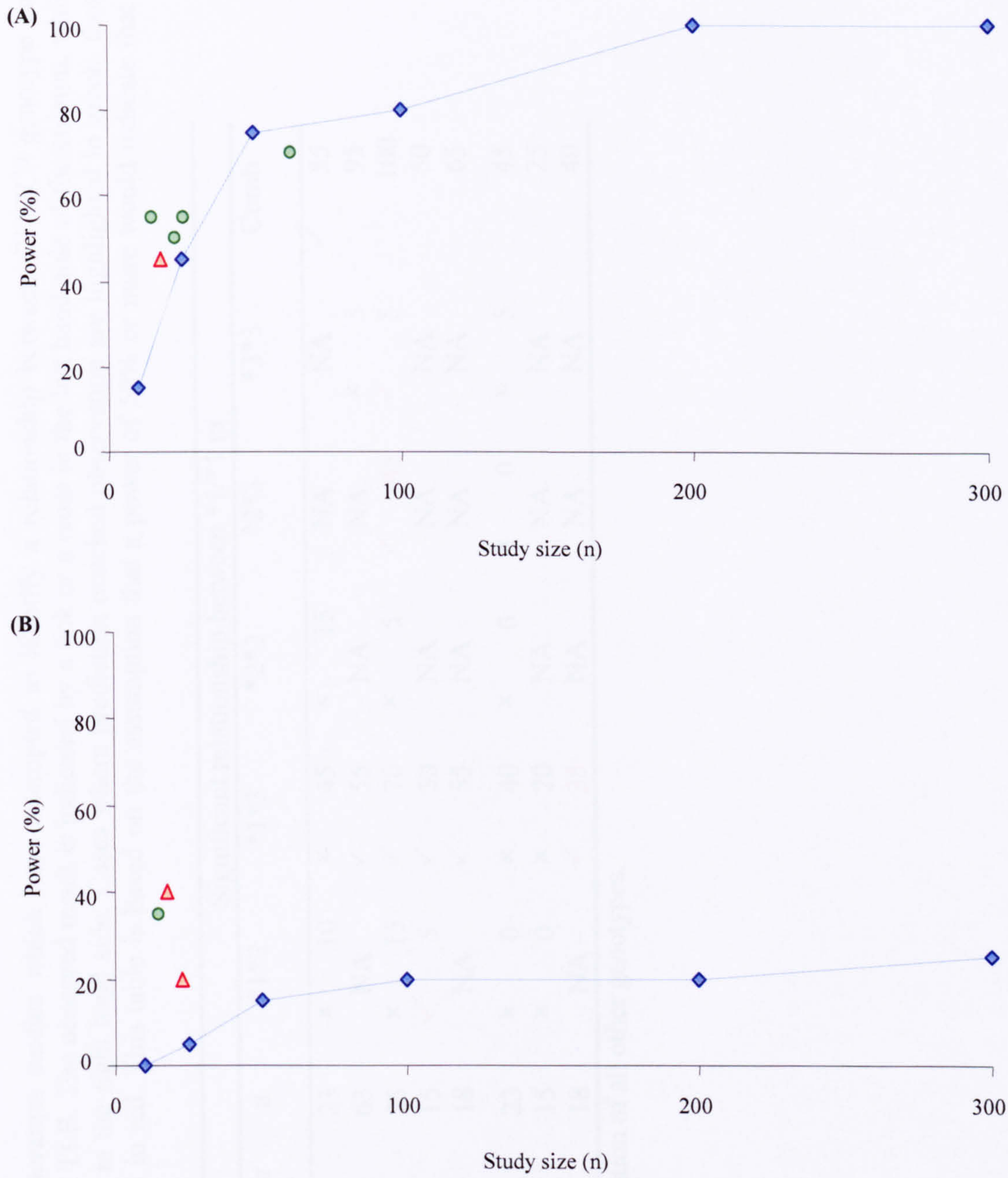


Figure 8.9 The power (%) of simulated studies to show significant differences in (A) AUC and (B) AUEC of TLB between different *CYP2C9* wild type and *1*3 genotypes *vs* the number of subjects (n). The predicted power of both studies involving subjects randomly selected from the Caucasian population (◆) and those using the proportion of *1*3 subjects detailed in the *in vivo* studies are shown (the green and red symbols indicate whether the *in vivo* study was positive (○) or negative (△)).

Table 8.9 A summary of the results of literature studies which have attempted to identify a relationship between *CYP2C9* genotype and pharmacokinetics or response to TLB. The observed result is indicated by a tick or a cross in the left hand side of the column, while the predicted power is recorded in the right hand side. Cases where prediction matched observation are highlighted in green, those where it did not are highlighted in red. This table is based on the assumption that a power of 50% or more would indicate that a positive result is expected.

			Significant relationship between *1/*1 *vs*					
Reference	PK or PD?	n	*1*2	*1*3	*2*2	*2*3	*3*3	Comb
Jetter *et al.*, 2004	PK	23	× 10	× 45	× 15	NA	NA	✓ 55
Wang *et al.*, 2005	PK	63	NA	✓ 55	NA	NA	× 5	95
Kirchheiner *et al.*, 2002	PK	23	× 15	✓ 70	× 5	✓ 45	✓ 5	100
Lee *et al.*, 2002b	PK	15	✓ 5	✓ 50	NA	NA	NA	50
Shon *et al.*, 2002	PK	18	NA	✓ 55	NA	NA	NA	65
Kirchheiner *et al.*, 2002	PD	23	× 0	× 40	× 0	× 0	× 5	45
Lee *et al.*, 2002b	PD	15	× 0	× 20	NA	NA	NA	25
Shon *et al.*, 2002	PD	18	NA	✓ 35	NA	NA	NA	40

'Comb' – wild type *vs* a combination of all other genotypes.

Table 8.10 Results of the published studies which have attempted to identify a significant difference in TLB pharmacokinetics or pharmacodynamics between *CYP2C9* wild type and *1*3 genotype. The powers of the studies estimated by the current study are shown along with the predicted power had subjects been selected randomly from the Caucasian population. Results highlighted in green indicate that the observed result matched the calculated power, red indicates the opposite.

		n				Power for WT *vs* *1*3 comparison	
Reference	PK or PD?	*1*3	Total	% *1*3	WT *vs* *1*3	For this study	For Caucasian pop (*1*3 = 11.6%)
Jetter *et al.*, 2004	PK	3	26	11.5	✓	55	45
Wang *et al.*, 2005	PK	9	63	14.3	✓	70	76
Kirchheiner *et al.*, 2002	PK	4	23	17.4	✓	50	40
Lee *et al.*, 2002b	PK	5	15	33.3	✓	55	25
Shon *et al.*, 2002	PK	6	18	33.3	×	45	30
Kirchheiner *et al.*, 2002	PD	4	23	17.4	×	20	5
Lee *et al.*, 2002b	PD	5	15	33.3	✓	35	3
Shon *et al.*, 2002	PD	6	18	33.3	×	40	4

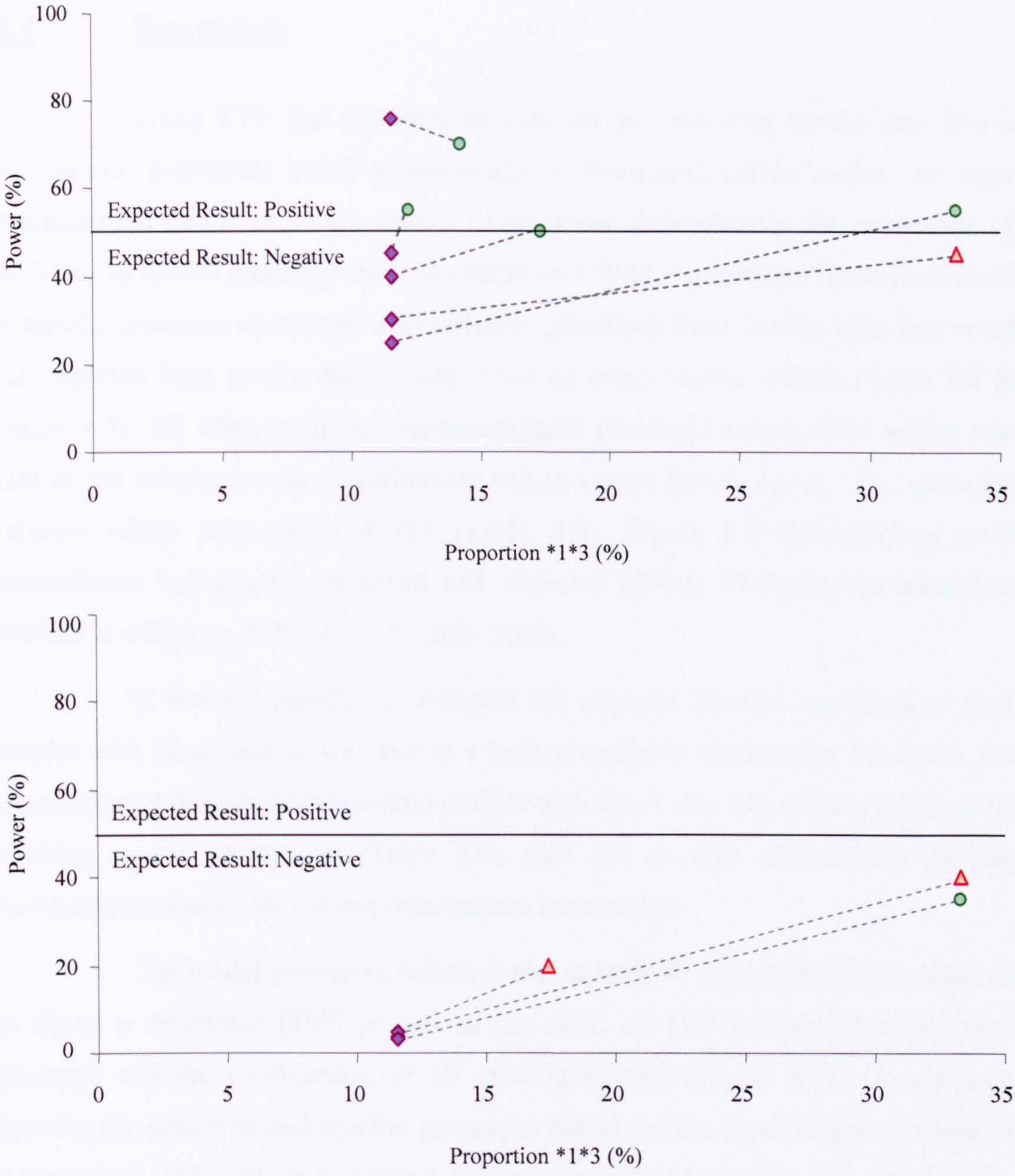


Figure 8.10 The power (%) of (i) simulated studies that use subjects randomly selected from a Caucasian population (◆), and (ii) simulated studies that use proportions of *1*3 subjects comparable to those reported in the *in vivo* studies (symbol indicates whether the outcome of the *in vivo* study was positive (○); or negative (△)) to detect significant differences in (A) the AUC, and (B) the AUEC of TLB *vs* the proportion of *CYP2C9* *1*3 subjects employed in the study (%).

8.4 Discussion

Using CTS that incorporated data on *in vitro* drug metabolism into a mechanistic population based pharmacokinetic-pharmacodynamic model, we have examined the power of *in vivo* studies to determine differences in the disposition of TLB and its insulin secretory effect in relation to *CYP2C9* genotype. The derivation of metabolic clearance associated with different genotypes from *in vitro* data recovered that reported from *in vivo* studies with good accuracy (within 2-fold) (Table 8.8 & Figure 8.3). All other predicted pharmacokinetic parameter values were within two fold of the weighted mean experimental values except for the $t_{1/2}$ in *3*3 genotype subjects which was within 4-fold (Table 8.8). Figure 8.5 demonstrates good concordance between the observed and expected plasma TLB concentration-time profiles in wild type, *1*2 and *1*3 individuals.

It was not possible to compare the response (insulin secretion) *vs* time outputs with those seen *in vivo* due to a lack of available information. However, the consistency of the concentration-time profiles with the *in vivo* data (Figure 8.5) and the resulting power estimations (Table 8.9) with the *in vivo* observations provide convincing evidence that the response outputs are accurate.

The model projection indicated that at least 50 subjects would be required to detect a difference (80% power) in the AUC of TLB between the wild type genotype and the combination of all other genotypes (Figure 8.7). Comparisons between the wild type and specific genotypes would require much higher numbers of subjects (e.g. 100 subjects to achieve 80% power in discriminating pharmacokinetics between wild-type and *1/*3 genotype; Figure 8.8 (B)).

Five studies assessing the impact of genetic variation in CYP2C9 on TLB pharmacokinetics have been reported. One of these, Lee *et al.* (2002) used only 15 subjects but 5 of these were wild type and these were compared with the same number of *1*3 genotype subjects. According to our simulations, this combination resulted in a power of 55% to observe a difference in pharmacokinetics between the two genotypes and accordingly, a difference was observed (Table 8.9 & Figure 8.9). Four other studies were successful in discerning a difference between the wild type and some of the genotypes but not others (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Shon *et al.*, 2002; Wang *et al.*, 2005). For example, according to our simulations, three of the studies had 55% or greater power to differentiate the pharmacokinetics between the

wild type and the *1/*3 genotype (Table 8.9). Accordingly, each of the studies was successful in observing a significant difference between these genotypes (Jetter *et al.*, 2004; Kirchheiner *et al.*, 2002; Wang *et al.*, 2005) (Table 8.9). One study did not observe such a difference with a predicted power of 45% (Shon *et al.*, 2002).

With regard to pharmacodynamic outcome, the model projection indicated that even with 300 subjects, the power to detect a difference in AUEC between wild type and the combination of all other genotypes did not increase above 45% (Figure 8.7).

Three literature studies have described attempts to differentiate the pharmacodynamics of TLB between different *CYP2C9* genotypes. Only one of the three was successful in this aim. Our simulations indicated that none of the reported studies were sufficiently powered to detect the contrast between wild type and the combination of all other genotypes (Table 8.9). Furthermore, as with the pharmacokinetic studies, the power for comparisons between specific genotypes was much lower (Figure 8.8 (B)). The outcome of most of the reported studies were consistent with the calculated powers (Table 8.9) except for one result from the Shon *et al.* study (2002) which demonstrated a significant difference in the pharmacodynamics of TLB between the wild type and the *1/*3 genotype with a predicted power of 35%. This may be a result of an overprediction of pharmacodynamic variability in the model. However, given the success of the model with regard to the majority of the predictions, another possible reason may be the significance level used in the reported studies of 5%. A consequence of this significance level is that 5% of observations will result in false positive results.

Table 8.10 shows that had the aforementioned studies relied on natural occurrences of CYP2C9 genotypes in the Caucasian population, they would have had lower power and been less successful in determining a significant difference in pharmacokinetics between the genotypes. This is also demonstrated by Figure 8.10 which shows the general increase in power that occurs when studies are enriched with regard to the *1*3 genotype.

It should be noted that the endpoint employed in this study, i.e. insulin secretion, is used as a surrogate for the true clinical endpoint resulting from treatment with TLB i.e. decrease in blood glucose concentration. Literature studies of TLB pharmacodynamics generally measure blood glucose concentration. To allow comment on the power of such studies, it has been necessary to assume that the power to

differentiate differences in insulin secretion between genotypes is comparable to that to differentiate blood glucose concentration. However, if the model had been extended to allow prediction of blood glucose levels, the power to differentiate differences in such pharmacodynamic response between individuals would have been lower. Since the power to detect differences in pharmacodynamics is already well below the 80% level (Figure 8.7) it was deemed unlikely that much more information would be gained from this. The use of the pharmacodynamic model demonstrated in this chapter may be simplistic but it has given an indication of its possible uses and applications.

No false positive outcomes have been predicted using this model. However, 5 false negative cases have been predicted. A possible reason for these false negative outcomes could be related to the fact that the effect –time profiles in different *CYP2C9* genotypes are relatively similar during early time points when compared to the later time points (Figure 8.6). Given that the AUECs in the current study were calculated only up to 3h, it follows that this may be a reason for the low calculated powers. However, the *in vivo* studies tend to use similar time scales and so consistency should still be expected.

In conclusion, the results of this study have provided some explanation as to why there is an apparent discrepancy in the results of published *in vivo* studies to discern a difference in the pharmacokinetics and/or pharmacodynamics of TLB between *CYP2C9* genotypes. Where some studies are successful (particularly pharmacokinetics studies), others (pharmacodynamic studies) fail. This may be due to the fact that the sizes of studies, particularly pharmacodynamic studies, are generally low compared to what would be required to achieve 80% power to discern such differences.

CHAPTER 9

General Discussion

9 DISCUSSION

9.1 Background

This chapter briefly summarises the work described in this thesis and makes suggestions for subsequent work. Section 9.2 discusses the main findings of the experimental work using IVIVE within clinical trial simulation to investigate the impact of CYP polymorphisms on the pharmacokinetics and pharmacodynamics of various drugs. Section 9.3 discusses the results of one aspect of the literature review involving the reporting of pharmacogenetic studies in the literature. Section 9.4 is an outline of further work that could be carried out to extend the work in this thesis.

As outlined in the Introduction (Section 1.4.1), there has been much concern expressed within the pharmaceutical industry with regard to the increasing cost and perceived decreasing productivity of the drug development process (FDA, 2004). It has been suggested that many of the studies carried out both during and after the drug development process may be avoided without any compromise in safety or efficacy and in their commentary, Johnsson & Sheiner (2002) advocated 'smarter' clinical trial design.

Recently, there have been calls to improve these aspects of drug development by utilising the processes of modelling and simulation more effectively (FDA, October 1, 2004; Jonsson & Sheiner, 2002; Williams *et al.*, 2006). At present, there is increasing interest in the use of clinical trial simulation in the drug development process as these are powerful tools to integrate all the available information relevant to clinical trial design. Indeed, the FDA's latest draft guidance on drug interaction studies states that "simulations can provide valuable insights into optimizing the study design" (FDA, October 1, 2004).

In the early stages of drug development it may not be possible to build a full picture of events that may happen in real clinical trials since many pieces of information may be missing. However, incorporating all available knowledge can help to ask "what if" questions and to understand the importance of missing information. Once all relevant data are gathered it becomes possible to characterise individuals most

likely to be "at risk" or those expected to gain the "highest benefit". This has got potential cost saving implications.

To our knowledge, there have not been any systematic attempts to use clinical trial simulation for identifying the possible impact of genetic polymorphisms in drug metabolising enzymes. The functional and clinical relevance of genetic polymorphisms in major drug metabolising enzymes such as the CYP enzymes are the bases for a large volume of research. A number of studies have shown the effects of functional allelic variants of CYP enzymes on inter-individual variation in plasma drug concentrations. However, the consequence of these genetic variations for the pharmacodynamics of drugs is unclear and the promise of pharmacogenetics for individualised medicine, at present, remains unfulfilled. While retrospective trials and case studies are abundant in the literature, they are often contradictory and prospective clinical trials are rare. In the absence of such prospective studies, clinical trial simulation provides a feasible alternative. In this thesis we have used dextromethorphan, warfarin, midazolam, omeprazole and tolbutamide as model drugs to examine some applications of clinical trial simulation in determining pharmacokinetic and pharmacodynamic differences between different CYP phenotypes/genotypes.

We have shown that clinical trial simulation is a useful tool for identifying *a priori* underpowered and thus, unsuccessful studies which are likely to be a waste of resources. The results of the current studies show how *in vitro* data can be integrated together with information on variability in genetic, physiological and demographic features into clinical trial simulation. This can help to design and implement more effective and conclusive clinical studies.

9.2 Discussion of Results

9.2.1 CYP2D6 & Dextromethorphan

The simulations in this chapter incorporated a two-compartment pharmacokinetic model for both parent drug and metabolite with first order absorption. This was linked *via* an effect compartment to an inhibitory E_{max} model where the parent drug and the metabolite competitively inhibited cough response.

As discussed in Chapter 2 (Section 2.4.3.1), there is an apparent inconsistency between literature studies of the effect of CYP2D6 phenotype on the pharmacokinetics of dextromethorphan which show significant differences between phenotypes and the equivalent pharmacodynamic studies which appear to show no such difference. The simulations carried out in this section provided some explanation for this observation by demonstrating that the latter studies were severely underpowered to detect differences in the pharmacodynamics of dextromethorphan between CYP2D6 EMs and PMs.

Some possible reasons for the large numbers of subjects that would be required to detect such differences were also put forward. These included (i) the contribution of other, non-polymorphic, enzymes to the overall metabolism of dextromethorphan, (ii) the pharmacological activity of dextrorphan, the main metabolite of dextromethorphan and its contribution to the antitussive activity of dextromethorphan, and (iii) the large interindividual variability in pharmacodynamic parameters describing the antitussive effect of dextromethorphan. The importance of these three factors was demonstrated by further simulations that showed the hypothetical change in power which would occur if any of the conditions were altered.

However, the relative pharmacological activity of dextrorphan compared to dextromethorphan had only a small influence on the power of studies to detect differences in the AUEC of DEX when it was reduced to 0% or increased to 100% and 200%. This result is interesting given the keen attention which is paid to active metabolites during the drug development process.

9.2.2 CYP2C9 & (S)-Warfarin

In addition to differing from the previous section in the particular substrate and CYP enzyme investigated, this section of the work also differed in that the pharmacokinetic-pharmacodynamic model incorporated a one-compartment model for concentrations of *(S)*-warfarin with first order absorption after a lag time. This was linked to an indirect model of anticoagulant response to the drug.

Simulating the pharmacokinetics and pharmacodynamics of warfarin in virtual populations of individuals with different *CYP2C9* genotypes revealed some interesting results. It is clear that the power to detect differences between specific genotypes was as much related to the frequency of the genotypes in the population as it was to the activity of the genotype relative to the wild-type.

Good consistency was evident between the results observed in 19 literature studies and those predicted by the current simulations. The simulations have helped to explain the apparent inconsistency between the success of studies in determining a significant effect of *CYP2C9* genotype on warfarin pharmacodynamics and the perceived failure to define a similar relationship between *CYP2C9* genotype and warfarin pharmacokinetics. The latter inconsistency may be related to the limited numbers of subjects employed in pharmacokinetic (and to some extent pharmacodynamic) studies and the low prevalence of some of the rarer *CYP2C9* genotypes in the Caucasian population.

It is probable that, in the case of warfarin, all but the largest studies will be underpowered at least for determining differences between some of the genotypes. For this reason, the use of populations which are enriched with respect to certain genotypes, allowing a great increase in statistical power using lower smaller study populations is a possible solution to the problem. However, the use of such recruitment methods does have some practical and ethical problems associated with it.

9.2.3 CYP3A5 & Midazolam

In order to simulate concentration- and response- time profiles for MDZ, a two-compartment pharmacokinetic model was used with first order absorption after a lag time for both parent drug and metabolite. This was linked to response using an effect compartment and an excitatory E_{max} model where the parent and the metabolite compete to exert their effect on the central nervous system as measured by the EEG. Although this model is very similar to that employed for modelling the pharmacokinetics and pharmacodynamics of DEX, there is a more significant contribution from gut first pass metabolism in this case, which has necessitated differences in the metabolite pharmacokinetic model.

All except one of the studies investigating the impact of the CYP3A5 polymorphism on the pharmacokinetics of MDZ have failed to detect any influence of genotype or phenotype. The only study which did detect an impact was the largest, employing 63 subjects. However, the current study predicted that the aforementioned studies had powers of 0 – 20%. This is not consistent with the one successful study. Although the success of this study may be the result of a type one error, it is also likely that the model is at fault in that it does not take into account polymorphic expression of CYP3A5 within the gut. Had this been considered, the calculated powers may have

been slightly higher and the predictions more accurate. This is discussed further in Chapter 6; Section 6.4.

9.2.4 CYP2C19 & Omeprazole

In this section a one compartment model with first order absorption was used to model the pharmacokinetics of OMZ. This was linked directly to a mechanism based (indirect) inhibitory model for the effect of OMZ on acid secretory response to the drug. The pharmacokinetic-pharmacodynamic model did not incorporate any lag time or effect compartment, which is unusual for an indirect model since the responses, by definition do not occur instantly as the effect is indirect. In addition to the latter limitation, there were other problems associated with the response model including the lack of incorporation of any feedback mechanism so that the response was able to increase indefinitely towards infinity. Furthermore, the elimination rate of both active and inactive H^+K^+-ATPase was assumed to be the same. All these factors potentially contributed to an inaccuracy in the power predictions. Nevertheless, the overall consistency between the predictions and the outcomes observed *in vivo* was good.

The importance of study enrichment for increasing the statistical power of studies to detect CYP-mediated differences in the pharmacokinetics or pharmacodynamics of drugs can be clearly observed in this chapter. Large increases in power were evident when studies were enriched with CYP2C19 poor metabolisers compared to the powers that were calculated for studies where subjects were selected randomly from a Caucasian population.

9.2.5 CYP2C9 & Tolbutamide

This section of the work was similar to Chapter 5 (*(S)*-warfarin) in that both Chapters investigated the effect of *CYP2C9* genotypes on the pharmacokinetics of a substrate. However, there were two main differences between them. Firstly, the pharmacokinetic-pharmacodynamic model for this chapter incorporated a two-compartment model with first order absorption after a lag time linked *via* an effect compartment to a stimulatory E_{max} model whereby TLB stimulated insulin secretion from both the systemic and the effect compartments. A second difference between this model and the *(S)*-warfarin model was in the decreased activity of *CYP2C9* mutants relative to the wild type genotype. Evidence was available to suggest that the percentage reduction in the intrinsic clearance between different genotypes was

different depending on the substrate being investigated (Lee *et al.*, 2002; Xie *et al.*, 2002). Therefore, a separate analysis was undertaken for TLB and the resulting reductions in intrinsic clearance by genotype for both substrates are different to those observed for *(S)*-warfarin (Table 9.1). It should also be noted that as was the case for the *(S)*-warfarin IVIVE model, possible differences in the expression of *CYP2C9* between genotypes were not taken into account due to lack of available data or evidence of such differences (Chapter 5; Section 5.4 for details).

Good agreement was apparent between the predicted powers of the reported *in vivo* studies and the outcomes observed in those particular studies. The simulations thereby helped to explain some of the inconsistencies whereby some studies have detected differences in the pharmacokinetics or pharmacodynamics of TLB between certain *CYP2C9* genotypes whereas others have not.

A further outcome of this section of the thesis was the observation of differences in power between enriched studies and those that utilise subjects selected randomly from the Caucasian population. The work has shown that increases in power to differentiate differences between *CYP2C9* genotypes can be expected when studies are enriched with respect to those genotypes.

9.2.6 Study Limitations

The present study does not address variability in the fu_B of the model drugs owing to lack of prior information. Such variability will influence variability in some pharmacokinetic parameters, particularly in F_H, and therefore AUC, C_{max}, and t_{max}. However, the outcome of the pharmacodynamic simulations is not expected to be influenced by a lack of individual variability in fu_B in our model. This follows because unbound plasma drug concentrations in different individuals are not affected, and they reflect differences only in intrinsic clearance.

A further limitation involves the intense sampling of data that has been necessary to analyse the large amount of information resulting from the simulations detailed within this thesis. Such techniques are time-consuming and restricting when compared with population modeling methods. However, such methods have not been the subject of this thesis since the majority of literature studies of the impact of CYP polymorphisms on the pharmacokinetics and pharmacodynamics of drugs have been carried out using similar methods to those simulated in this work.

Table 9.1 Relative activity of each of the common *CYP2C9* genotypes in the metabolism of SWF and TLB.

	Activity Relative to *1*1	
Genotype	*(S)*-Warfarin	Tolbutamide
*1*1	100	100
*1*2	85	84
*1*3	55	60
*2*2	70	68
*2*3	40	44
*3*3	10	20

9.3 Bias in Reporting of Negative Outcomes within Pharmaco-genetic Studies

At various stages throughout this thesis, it has been necessary to comment on the relationship between the genetics of certain CYP enzymes and drug pharmacokinetics and pharmacodynamics. As was pointed out in the Literature Review (Section 2.1), current opinion on the usefulness and applicability of pharmacogenetics to drug development is varied and many are inclined towards the view that "the promise of pharmacogenetics remains largely unfulfilled" (Tucker, 2004). Because of such opinion, it is interesting to look at the reporting of pharmacogenetic studies in relation to CYP enzymes in the literature. Tables 2.10 and 2.11 in the Literature Review detail studies which have investigated the relationship between CYP polymorphisms and drug pharmacokinetics (Table 2.10) and pharmacodynamics (Table 2.11). The journals in which the studies were reported are listed. It is interesting to note in which journals the proportions of studies with positive and negative results are reported and this was investigated in an extension to the work detailed below.

When the results reported in specific journals are analysed, the percentage of negative results does not necessarily reflect the average over all journals. For example, while 36% (8/22) of the pharmacokinetic reports presented in the European Journal of Clinical Pharmacology were negative (Figure 9.1) which constitutes higher than average (29%; 38/184) negative reporting, the corresponding number for the Journal of Clinical Pharmacology was only 13% (1/8) (Figure 9.1) which suggests an over-reporting of positive results in studies of the influence of CYP polymorphisms on drug PK.

Analysis of the results of pharmacodynamic studies reported in specific journals revealed that again, the percentage of negative results does not necessarily reflect the average over all journals. For example, whilst only 23% (3/13) of the reports presented in Pharmacogenetics were negative (Figure 9.2), which is much lower than the average value (37%; 41/111), 39% (14/36) of those published in Clinical Pharmacology and Therapeutics were negative (Figure 9.2).

Although no general trend for higher numbers of positive results being reported in high impact journals is seen, it is apparent that there may be some bias and

this may be the result of two factors: (i) certain journals may be biased towards publishing positive results, or (ii) investigators may be reluctant to submit negative results to certain journals because of a perceived lower chance of acceptance.

Such underreporting of negative results by certain journals could potentially lead readers to assume that CYP polymorphisms are of greater importance for drug PK than is really the case. This becomes an even greater problem when the possibility of certain studies never being reported due to their portrayal of negative results is taken into account. It is clear that in many cases, the 'promise' of pharmacogenetics portrayed by some high impact journals may remain 'unfulfilled' by the evidence when all studies are taken into account.

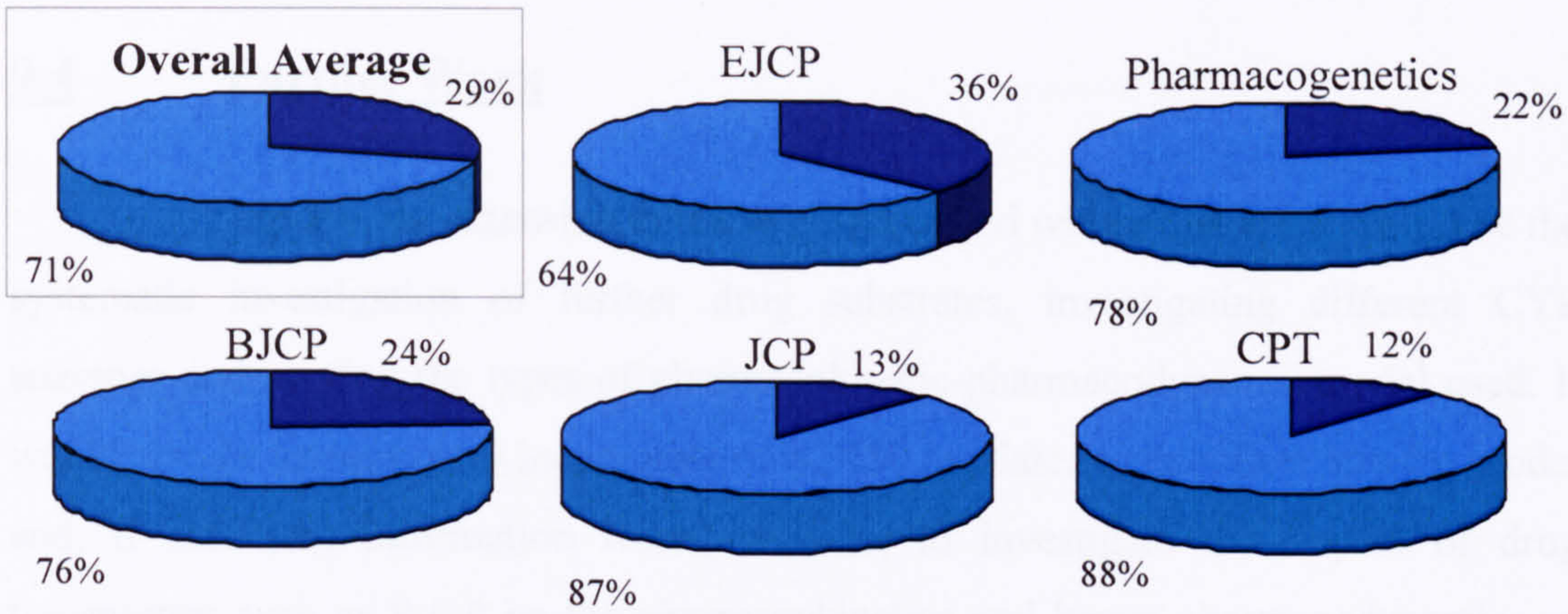


Figure 9.1 The percentage of studies into the effect of CYP polymorphisms on drug pharmacokinetics which report negative (■) and positive (■) results in different journals compared to the percentage across all journals. EJCP = European Journal of Clinical Pharmacology; BJCP = British Journal of Clinical Pharmacology; JCP = Journal of Clinical Pharmacology; CPT = Clinical Pharmacology & Therapeutics.

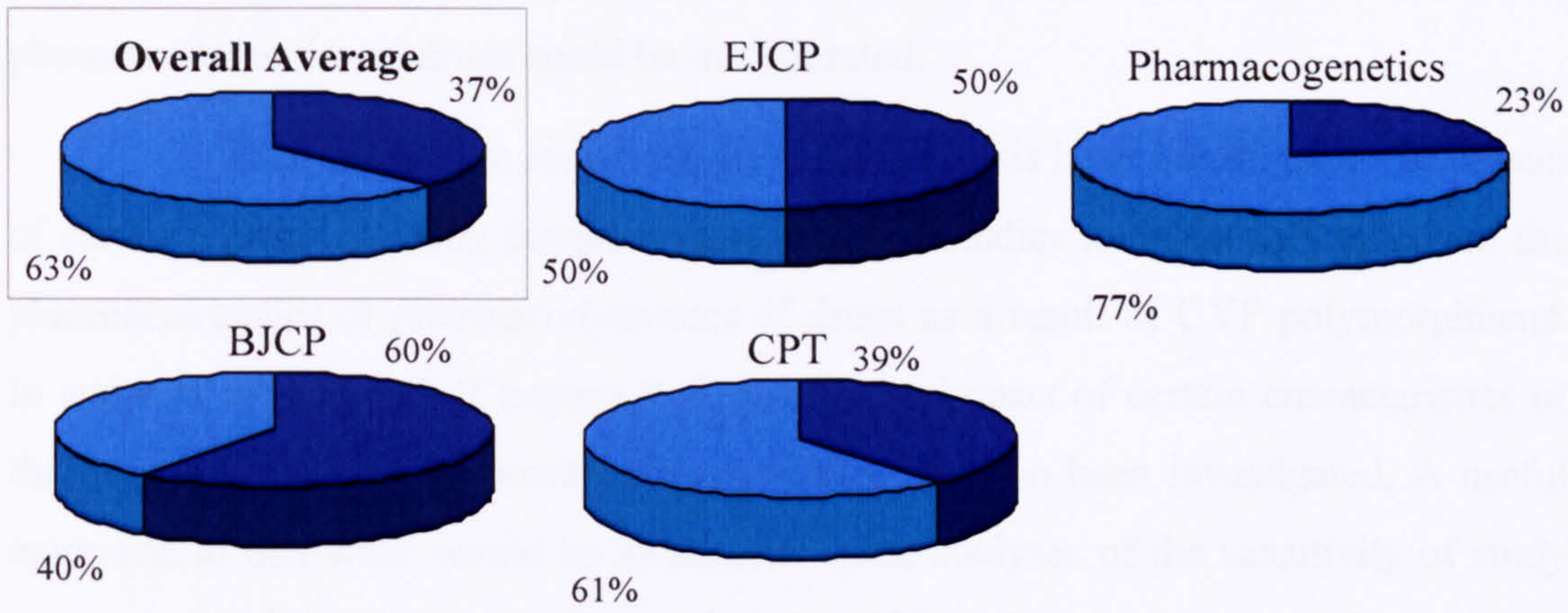


Figure 9.2 The percentage of studies into the effect of CYP polymorphisms on drug pharmacodynamics which report negative (■) and positive (■) results in different journals compared to the percentage across all journals. EJCP = European Journal of Clinical Pharmacology; BJCP = British Journal of Clinical Pharmacology; CPT = Clinical Pharmacology & Therapeutics.

9.4 Further Work

An obvious extension to the work described within this thesis would be the systematic investigation of further drug substrates, investigating different CYP enzymes and varying the types of pharmacokinetic-pharmacodynamic model used. It would also be interesting to incorporate non-P450 mediated metabolism into the model and, if sufficient information were available, to investigate the impact of drug transporters such as P-GP on the pharmacokinetics and hence pharmacodynamics of drugs.

In addition to investigating the impact of study size and enrichment on the power of clinical trials, it would also be of interest to incorporate other aspects of study design into the model such as demographic or physiological characteristics of the study participants or the timing and frequency of the drug dosage. Furthermore, the impact of disease states or ethnicity of individuals on the pharmacokinetics and pharmacodynamics of drugs could be incorporated.

Each of the five results chapters of this thesis have investigated the impact of certain aspects of study design on the power of studies to detect differences in the pharmacokinetics or pharmacodynamics of drugs as a result of CYP polymorphisms. In some of the chapters (Chapters 4, 5 and 6), the impact of certain characteristics of the drug substrate on the power of such studies has also been investigated. A useful extension to this work would be to conduct some analyses of the sensitivity of study power to certain drug characteristics. These could be utilised during drug development to judge the impact of a polymorphic metabolic pathway on drug response and toxicity before making go/no go decisions.

An issue that has not been addressed by the work within this thesis is the impact of genetic variability in drug metabolism on the extent of drug-drug interactions and their consequences for drug toxicity and response. Such models could be used in a similar way to those detailed in this thesis to investigate the impact of certain drug characteristics and aspects of study design on the power of studies to detect differences in the magnitude of drug-drug interactions between individuals of different CYP phenotype/genotype. It would be possible to investigate the impact of CYP polymorphisms on the likelihood of non-response to drugs or drug toxicity as a result of interactions.

Furthermore, recently there has been a move within the literature towards examining the impact of pharmacodynamic polymorphisms on drug response. This has been touched on in Chapter 5 with the VKORC1 polymorphism simulations. However, where data is available it would be possible to incorporate a great many more pharmacodynamic polymorphisms and investigate the impact of these on drug response. A further dimension to such work would be to compare the impact of pharmacokinetic polymorphisms with those in pharmacodynamics.

Finally, in the current work, a number of parameters were taken from *in vivo* studies (e.g. V) however the most important determinant of exposure (CL) was simulated using *in vitro* data. In general, it may be possible to use further *in vitro* information. For example, estimates of volume of distribution in individuals could be made from physicochemical characteristics and variations in organ sizes and composition (Poulin & Theil, 2002) and for some drugs, it is possible to use *in vitro* receptor binding data to estimate pharmacodynamic effects associated with a given concentration at the receptor site (Jonker *et al.*, 2005). Thus, in the future, clinical trial simulation may increasingly use prior *in vitro* data. The approach exemplified by the current study, to calculate statistical power avoids assumptions about the distributions and variation of primary pharmacokinetic parameters required by less mechanistic models used as the basis of clinical trial simulation.