LEADING ARTICLE

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Pharmacogenetic Aspects of Drug-Induced Torsade de Pointes

Potential Tool for Improving Clinical Drug Development and Prescribing

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Abstract

Drug-induced torsade de pointes (TdP) has proved to be a significant iatrogenic cause of morbidity and mortality and a major reason for the withdrawal of a number of drugs from the market in recent times. Enzymes that metabolise many of these drugs and the potassium channels that are responsible for cardiac repolarisation display genetic polymorphisms. Anecdotal reports have suggested that in many cases of drug-induced TdP, there may be a concealed genetic defect of either these enzymes or the potassium channels, giving rise to either high plasma drug concentrations or diminished cardiac repolarisation reserve, respectively. The presence of either of these genetic defects may predispose a patient to TdP, a potentially fatal adverse reaction, even at therapeutic dosages of QT-prolonging drugs and in the absence of other risk factors. Advances in pharmacogenetics of drug metabolising enzymes and pharmacological targets, together with the prospects of rapid and inexpensive genotyping procedures, promise to individualise and improve the benefit/risk ratio of therapy with drugs that have the potential to cause TdP. The qualitative and the quantitative contributions of these genetic defects in clinical cases of TdP are unclear because not all of the patients with TdP are routinely genotyped and some relevant genetic mutations still remain to be discovered.

There are regulatory guidelines that recommend strategies aimed at uncovering the risk of TdP associated with new chemical entities during their development. There are also a number of guidelines that recommend integrating pharmacogenetics in this process. This paper proposes a strategy for integrating pharmacogenetics into drug development programmes to optimise association studies correlating genetic traits and endpoints of clinical interest, namely failure of efficacy or development of repolarisation abnormalities. Until pharmacogenetics is carefully integrated into all phases of development of QT-prolonging drugs and large-scale studies are undertaken during their post-marketing use to determine the genetic components involved in induction of TdP, routine genotyping of patients remains unrealistic.

Even without this pharmacogenetic data, the clinical risk of TdP can already be greatly minimised. Clinically, a substantial proportion of cases of TdP are due to the use of either high or usual dosages of drugs with potential to cause TdP in the presence of factors that inhibit drug metabolism. Therefore, choosing the lowest effective dose and identifying patients with these non-genetic risk factors are important means of minimising the risk of TdP. In view of the common secondary pharmacology shared by these drugs, a standard set of contraindications and

warnings have evolved over the last decade. These include factors responsible for pharmacokinetic or pharmacodynamic drug interactions. Among the latter, the more important ones are bradycardia, electrolyte imbalance, cardiac disease and co-administration of two or more QT-prolonging drugs.

In principle, if large scale prospective studies can demonstrate a substantial genetic component, pharmacogenetically driven prescribing ought to reduce the risk further. However, any potential benefits of pharmacogenetics will be squandered without any reduction in the clinical risk of TdP if physicians do not follow prescribing and monitoring recommendations.

Prescribing drugs during routine clinical practice is a relatively empirical trial and error process consisting of selecting a drug and recommending a relatively rigid 'standard' dose schedule for every patient. These dose schedules, investigated during drug development, are based on population mean data and usually ignore the large interindividual variability that is present within a population.

The International Conference on Harmonisation (ICH) guideline[1] on 'Dose-Response Information to Support Drug Registration' recommends that in using dose-response information, the influences of various factors should be identified where possible. Pharmacokinetics and pharmacodynamics, the two components of a dose-response curve, are both subject to large interindividual variability. This variability arises from their modulation by factors such as age, gender, co-medications or the presence of concurrent diseases, e.g. renal or hepatic dysfunction. This variability also arises from genetic influences that regulate the expression of drug metabolising enzymes (pharmacokinetic variability) or the function of various pharmacological targets (pharmacodynamic variability). The presence of variant alleles often exerts influences that usually far exceed those due to the other covariates stated above.

It is estimated that the human genome has about 50 000–100 000 functional single nucleotide polymorphisms (SNPs) [variations in the DNA in which a single base pair varies]. These SNPs give rise to variant alleles responsible for genetic polymorphisms within a population and may account for genetically mediated interindividual differences in response to clinically prescribed drugs.[2] The need to study genetically determined biochemical variations that characterise humans was first considered almost a century ago.[3,4]

In terms of genetic influences on drug response, two models exist – high genetic and low environment model versus low genetic and high environment model. For many drugs with a shallow concentration-response curve, genetic factors seem to matter only a little, while for others, genetic differences between individuals account for a very significant fraction of the overall variation in drug response. A typical example of an abnormal response that is almost exclusively genetically determined is the prolonged apnoea that follows administration of suxamethonium chloride (a muscle relaxant) to individuals who inherit a variant form of plasma butyrylcholinesterase (designated atypical cholinesterase). Subsequently polymorphism in the metabolism of isoniazid by *N*-acetyltransferase 2 (NAT2) explained the susceptibility of some individuals to drugs metabolised by acetylation, e.g. peripheral neuropathy, hepatitis or poor anti-tuberculous response following administration of isoniazid or haematological reactions or poor therapeutic response to dapsone. Beginning in the late 1970s, major advances in pharmacogenetics followed the discovery of genetic polymorphism in enzymes that catalyse phase I metabolism (cytochrome P450 [CYP]). This discovery not only explained further the individual susceptibility to drug reactions and lack of efficacy, but also provided a mechanistic and rationale basis for metabolic drug interactions.

At a pharmacokinetic level, use of pharmacogenetics has already resulted in great improvement of cancer therapy with mercaptopurine and azathioprine. These drugs are metabolised by thiopurine S-methyltransferase (TPMT) that is expressed polymorphically in a population. At a pharmacodynamic level, great advances have been made in uncovering the genetic and molecular bases of con-

genital long QT syndrome (LQTS). Identification of mutations at different genetic loci of ion channels has shown LQTS to be highly heterogeneous and have begun to explain the significant differences in the clinical features of individuals with it. Not only are there are gene-specific differences in the triggers for cardiac events but for some forms of LQTS, there are also gene-specific differences in response to changes in lifestyle and to therapy.

Anecdotal observations of sporadic cases have raised the expectation that application of pharmacogenetics will result in the choice of the right drug at the right dose at the outset of therapy for each patient, thus maximising the probability of improved efficacy and minimising the probability of an adverse drug reaction. There is, however, an urgent need to explore whether in clinical practice pharmacogenetics will deliver these anticipated benefits and to put these potential benefits in perspective with regard to non-genetic factors that also influence drug response.

Over the last 10 years, the potentially fatal adverse effect of many non-antiarrhythmic drugs on the QT interval of the surface ECG has attracted considerable clinical and regulatory interest.[5-7] This paper focuses on drug-induced QT interval prolongation and torsade de pointes (TdP), to put in perspective the relative contributions of genetic and non-genetic factors in clinical practice.

1. Drug-Induced Torsade de Pointes (TdP)

1.1 Clinical and Regulatory Concern

Drug-induced prolongation of the QT interval is a typical type A pharmacological adverse reaction; usually resulting from concentration-dependent block of potassium channels by most QT-prolonging drugs. Despite its clinical and regulatory significance, this potentially proarrhythmic effect of drugs on QT interval prolongation is not well understood. QT interval prolongation *per se* is not proarrhythmic and does not influence cardiac performance. Class III antiarrhythmic drugs are designed to prolong the rate corrected QT interval (QTc) and increase myocardial refractory period, thereby exerting their therapeutic benefit. Unfortunately, however, QTc interval prolongation is observed with a large number of non-class III antiarrhythmic drugs. When prolonged excessively, it often leads (under the right circumstances) to potentially fatal ventricular tachyarrhythmias, particularly a polymorphic form known as TdP. Thus, the balance between the therapeutic antiarrhythmic and the potentially fatal proarrhythmic prolongations of the QT interval is a delicate one.

Since the measured QT interval varies with heart rate, it requires correction to obtain a rate-corrected QT interval – the QTc interval. Issues surrounding the measurement of QT interval, the rate correction formula that is most appropriate and the magnitude of QTc interval prolongation that is likely to induce TdP are complex and the reader is referred to other detailed reviews on this subject.[5-7] Quantitatively, the proarrhythmic threshold is not a sharp one and TdP can occur after only a minimal prolongation of the QTc interval. The risk of TdP, however, usually begins when the QTc interval is about 500ms and rises exponentially thereafter.

Clinical manifestations of TdP, which is usually a transient tachyarrhythmia, may include palpitation. When TdP is sustained, symptoms arising from impaired cerebral circulation such as dizziness, syncope and/or seizures may manifest. TdP subsequently degenerates into ventricular fibrillation in about 20% of cases[8] and, not uncommonly, sudden death may occur.[9] The overall mortality from TdP is of the order of 10–17%.[8,10]

Not surprisingly, this potentially fatal proarrhythmic effect of many non-antiarrhythmic drugs on the QT interval of the ECG has attracted considerable clinical and regulatory interest over the last decade. Regulatory rejection of new drugs or restrictions in the use of many old and other new drugs over the last decade because of their 'QTc liability' has had a very profound influence on drug development. There are justifiable clinical and regulatory expectations of a better pre-approval characterisation of new chemical entities (NCEs) for this potential risk. This expectation also applies to old drugs already on the market should any of them unexpectedly prove to be proarrhythmic. Among the many examples of such drugs are pimozide, terfenadine, thioridazine and cisapride. One important aspect in

characterising this risk is the investigation of the role of genetic factors.

In December 1997, the Committee for Proprietary Medicinal Products (CPMP) of the European Union (EU) adopted a significant document concerning pre-approval evaluation of an NCE for its potential to prolong the QT interval; 'Points to Consider: The Assessment of the Potential for QT Interval Prolongation by Non-Cardiovascular Medicinal Products'.[11] This document describes a strategy that EU regulators recommend for this purpose. There are initiatives currently in progress at the ICH level aimed at harmonising this strategy internationally.

1.2 Basic Electrophysiology

The QT interval of the ECG reflects the duration of ventricular action potential that is determined by a delicate balance between inward and outward currents, especially during phases 2 and 3 of the action potential. Major ion currents involved during the depolarisation and repolarisation phases of a ventricular action potential are shown in figure 1. Reduction in the major outward current, mediated by the rapid component of the delayed rectifier potassium channels (I_{Kr}), results in prolongation of the QT interval. Although reduction in I_{Kr} may result for many reasons, the most frequent cause at present is the administration of many clinically useful drugs.[7] Drugs reduce this current mainly by their effect on human *KCNH2* gene (human ether-a-go-go-related gene [HERG]) α-subunits of the I_{Kr} channel.

Two main hypotheses have been proposed to explain the underlying electrophysiological mechanism for the induction of TdP. The two hypotheses are not mutually exclusive and may even be complementary. Delayed repolarisation gives rise to the development of early after-depolarisations (EADs) at the Purkinje fibre level. The emergence of these EADs is favoured by calcium loading during the late phase 2 of the prolonged action potential due to the first short cycle of the short-long-short series that generally precedes TdP. The amplitude of EADs is cycle-dependent and there is a strong correlation between the preceding RR interval and the ampli-

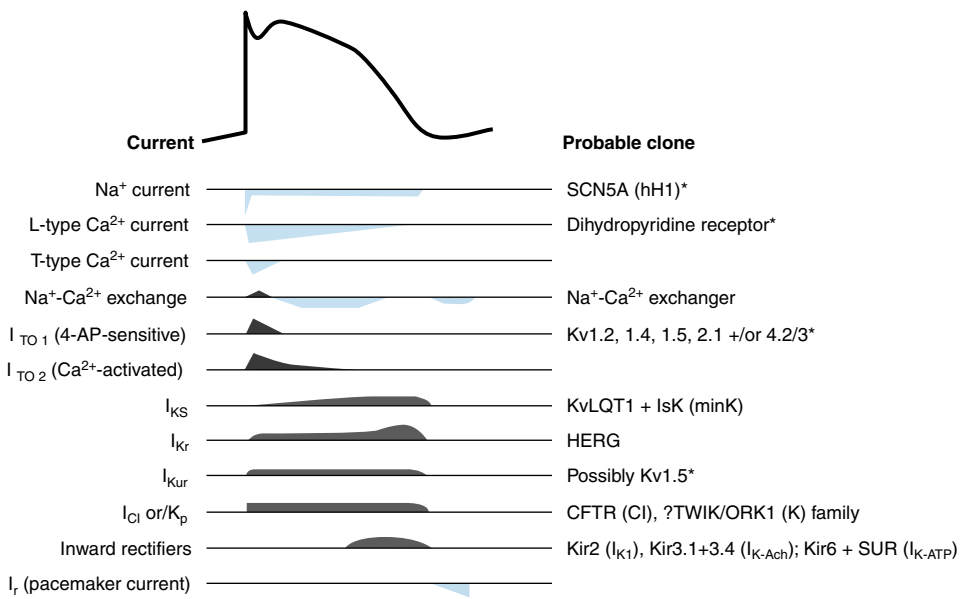

Fig. 1. Cardiac ionic currents and respective ion channel clones responsible for generation of the action potential. Inward currents are drawn in blue outward currents in black. The amplitudes are not to scale (from Priori et al.,[12] with permission from Elsevier). * Subunits also identified.

tude of EADs that follow. When the amplitude of the EADs reaches a critical threshold, a repetitive burst of electrical activity is triggered, which forms the basis of TdP. The other hypothesis proposes an increase in transmural dispersion of action potential duration throughout the ventricle and this may facilitate transient functional block. Drugs that block I_{Kr} cause significantly greater prolongation of the action potential duration in Purkinje fibres and the M-cells (special ventricular myocytes found in the mid-myocardial region) than in other myocytes layers. This is the consequence of relative scarcity of other major cardiac repolarising channels in Purkinje fibres and the M-cells. Thus, a uniform decrease in I_{Kr} function results in the transmural dispersion of action potential duration within the ventricular wall.

1.3 QT Interval as a Surrogate of TdP

Numerous clinical and experimental data have established that QTc interval prolongation is a major precursor of drug-induced TdP. It is, however, an imperfect (but at present the best available) surrogate marker of the risk of TdP. The potential for emergence of EADs and induction of TdP following prolongation of the QTc interval varies greatly. Not all drugs that prolong the QT interval to the same extent carry the same risk of causing TdP. The incidence of TdP is estimated to be 0.5–8.8% with quinidine[13] and 2.6–4.1% with sotalol.[14] The incidence is higher in combination preparations of sotalol that include a thiazide diuretic, which induces hypokalaemia,[15] and lower with racemic sotalol in contrast to *S*-sotalol because of the β-adrenoceptor blocking activity of *R*-sotalol present in the former. Other ancillary properties of the drug (e.g. α- or β-adrenoceptor or calcium channel blocking activities) greatly modify the risk of TdP at a given duration of QT interval.[16-19] Selective, but not non-selective, I_{Kr} blockers are known to induce TdP in anaesthetised rabbits during α_1-adrenoceptor stimulation.[17] The use of β-adrenoceptor blocking drugs, with the addition of α-blocking drugs when necessary, has been effective in the treatment of patients with congenital LQTS[18] and the response to adrenergic modulation in these patients appears to be genotype specific.[19]

Not every patient who has a prolongation of the QT interval to the same extent will develop TdP. The clinical outcome is modulated by not only the drug concerned and its plasma concentration but also a number of other host factors. Furthermore, the risk of developing TdP is not fixed. An individual can tolerate a QT-prolonging drug well for many months only to be at risk due to an inter-current event such as the development of hypokalaemia due to diarrhoea and vomiting, or introduction of an interacting co-medication.

1.4 Drug Withdrawals Due to TdP

Prenylamine, an effective antianginal drug, was the first drug to be withdrawn from the market (in 1988) because of its high potential to cause TdP.[20] Table I lists drugs that have been withdrawn from various markets during the period 1990–2001. These include eight drugs withdrawn because of their propensity to prolong QT interval with or without TdP. Eleven (33%) of the 33 major drugs were withdrawn during this period because of their potential for drug interactions or prolongation of the QT interval and/or TdP. Two additional drugs (encainide and flosequinan) were also removed from the market for their proarrhythmic potential.

Drug-induced QT interval prolongation seems to be a modern 'epidemic'. This potentially fatal adverse reaction is not only associated with cardiovascular drugs but also with over 90 non-cardiovascular drugs.[7] In one survey of 2194 cases of TdP in the US FDA database, the major drug classes involved were cardiac (26.2%), CNS (21.9%), antiinfectives (19.0%) and antihistamines (11.6%). Of these, 92.8% were reported between 1989–98 in contrast to only 7.2% between 1969–88.[10]

Since QT interval prolongation is not an ideal surrogate of the risk of TdP, withdrawals of drugs that simply prolong the QT interval illustrate the need to balance carefully the risks versus benefits and the availability of alternative agents. When found to induce TdP frequently, drugs such as prenylamine, terodiline terfenadine, astemizole, cisapride and levacetylmethadol were all withdrawn from the market when their benefit/risk ratio was determined to be adverse and alternatives were available. On the other hand, arsenic trioxide was approved and is still on market despite a known high

Table I. Reasons for the withdrawal of drugs from the market since 1990

Drug	Year of withdrawal	Reason(s) for withdrawal
Dilevalol	1990	Hepatotoxicity
Triazolam	1991	Neuropsychiatric reactions
Terodiline[a]	1991	QTI prolongation and TdP
Encainide	1991	Proarrhythmic effects
Fipexide	1991	Hepatotoxicity
Temafloxacin	1992	Hypoglycaemia, haemolytic anaemia and renal failure
Benzarone	1992	Hepatotoxicity
Remoxipride	1993	Aplastic anaemia
Alpidem	1993	Hepatotoxicity
Flosequinan	1993	Excess mortality, possibly due to arrhythmias
Bendazac	1993	Hepatotoxicity
Soruvidine	1993	Myelotoxicity following DI
Chlormezanone	1996	Hepatotoxicity and severe skin reactions
Tolrestat	1996	Hepatotoxicity
Minaprine	1996	Convulsions
Pemoline	1997	Hepatotoxicity
Dexfenfluramine	1998	Cardiac valvulopathy and pulmonary hypertension
Fenfluramine	1998	Cardiac valvulopathy and pulmonary hypertension
Terfenadine[a]	1998	DI, QTI prolongation and TdP
Bromfenac	1998	Hepatotoxicity following prolonged administration
Ebrotidine	1998	Hepatotoxicity
Sertindole[a]	1998	QTI prolongation and potential for TdP
Mibefradil	1998	Rhabdomyolysis following DI Concerns on potential DI including risk of TdP
Tolcapone	1998	Hepatotoxicity
Astemizole[a]	1999	DI, QTI prolongation and TdP
Trovafloxacin	1999	Hepatotoxicity
Grepafloxacin[a]	1999	QTI prolongation and TdP
Troglitazone	2000	Hepatotoxicity
Alosetron	2000	Ischaemic colitis
Cisapride[a]	2000	DI, QTI prolongation and TdP
Droperidol[a]	2001	QTI prolongation and TdP
Levacetylmethadol[a]	2001	DI, QTI prolongation and TdP
Cerivastatin	2001	Rhabdomyolysis following DI

a Drugs withdrawn specifically due to the risk of TdP.

DI = drug interactions; **QTI** = QT interval; **TdP** = torsade de pointes.

frequency of TdP associated with its use. Likewise, pimozide and thioridazine continue to be available. More importantly, because of its low potential for extrapyramidal adverse effects and lack of reports of TdP despite marked prolongation in QT interval, it is planned to allow the re-introduction of sertindole.

2. Clinical Implications of Pharmacogenetics

To date, most pharmacogenetic studies have focussed on drug metabolising enzymes. The enzymes most frequently involved in the primary metabolism of drugs are CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The influence of pharmacogenetics in determining drug response is best illustrated by drug metabolising enzyme such as CYP2D6 and by pharmacological target such as the potassium channels. Both exhibit genetic polymorphisms.

2.1 Genetic Factors in Pharmacokinetics with Reference to CYP2D6

Depending upon the ability of individuals to mediate CYP2D6-dependent hydroxylation of the (now obsolete) antihypertensive drug debrisoquine, two population phenotypes have been identified – extensive metabolisers (EMs) or poor metabolisers (PMs).[21] This polymorphism results from autosomal recessive inheritance, in a simple Mendelian fashion, of alleles at a single locus mapped to chromosome 22q13.1. Individuals heterozygous for the defective allele are EMs with some impairment in effecting this reaction, indicating a gene dose effect. Since the wild-type allele (*CYP2D6**1) responsible for normal functional capacity is dominant, only those individuals carrying two CYP2D6 inactivating alleles (e.g. *CYP2D6**3, *CYP2D6**4, *CYP2D6**5 or *CYP2D6**6) are phenotypic PMs.[22] Some phenotypically EM individuals inherit alleles (e.g. *CYP2D6**10 and *CYP2D6**17) that express the enzyme with reduced or altered affinity for certain CYP2D6 substrates.[23-25] Within the EMs, there is another subgroup, termed the ultra-rapid metabolisers, resulting from multiple copies of the alleles *CYP2D6**1 or *CYP2D6**2 for normal metabolic capacity.[26] Alleles *CYP2D6**35 and *CYP2D6**41 are also associated with ultra-rapid metabolism.

Table II. Pharmacokinetic consequences of CYP2D6 polymorphism

Pharmacokinetic parameter	Consequences for PMs vs EMs
Bioavailability	2- to 5-fold
C_{max}	2- to 6-fold
AUC	2- to 5-fold
Half-life	2- to 6-fold
Metabolic clearance	0.1- to 0.5-fold

AUC = area under the plasma concentration-time curve; **C_{max}** = peak plasma concentration; **CYP** = cytochrome P450; **EMs** = extensive metabolisers; **PMs** = poor metabolisers.

Although CYP2D6 accounts for only 2% of the total liver CYP content, it is responsible for the metabolism of well over 20% of the drugs eliminated by metabolic clearance.[27] CYP2D6 polymorphism is the most widely studied genetic polymorphism and CYP2D6 isozyme has been shown to control the oxidative biotransformation of well over 60 drugs to date. These include antiarrhythmics, β-blockers, antihypertensives, antianginals, antipsychotics, antidepressants, analgesics as well as a number of other miscellaneous drugs.[27,28]

The pharmacokinetic consequences of polymorphism in CYP2D6, summarised in table II, are that relative to EMs, the PMs experience far greater exposure to the parent drug[29] while the reverse is true for the metabolites generated by this enzyme. PMs may of course activate alternative, otherwise dormant and possibly less effective, pathways and yield otherwise atypical metabolites.

The importance of this polymorphism arises from the fact that the substrates of CYP2D6 are typically cardiovascular and psychoactive drugs, most with narrow therapeutic index with a narrow margin between the toxic and the effective therapeutic concentrations. These drugs are also generally intended for long-term administration, thus adding a further clinical concern. The clinical consequences of CYP2D6 polymorphism for some drugs are shown in table III. It has now been shown that PMs are at risk of a number of adverse effects of drugs that are primarily metabolised by CYP2D6. In contrast, many EMs (but especially the ultra-rapid metabolisers) are at risk of exaggerated pharmacological effects of the metabolite but much attenuated effects of the parent drug. CYP2D6 polymorphism has efficacy implications as well. PMs are at a risk of lack of efficacy when the therapeutic effect of a drug is mediated principally by its CYP2D6-generated metabolite.

2.2 Genetic Factors in Pharmacodynamics with Reference to Long QT Syndrome

Among the pharmacological targets widely studied in terms of genetic influences are the polymorphisms related to asthma,[54,55] cardiac failure,[56,57] depression[58-60] and cardiac arrhythmias.

Voltage-gated potassium channels, more specifically those related to LQTS, are among the arrhythmia-related pharmacological targets that have been studied most extensively.[61] There is a great diversity of genes that control the expression of these potassium channels.[62] In view of the complexity of structure and nomenclature, it is appropriate to des-

Table III. Clinical consequences for PM and ultra-rapid EM phenotypes of CYP2D6

Clinical consequences for PMs	
Increased risk of toxicity	
Debrisoquine	Postural hypotension and physical collapse[30]
Sparteine	Oxytocic effects[31]
Perphenazine	Extrapyramidal symptoms[32]
Flecainide	?Ventricular tachyarrhythmias[33]
Perhexiline	Neuropathy and hepatotoxicity[34,35]
Phenformin	Lactic acidosis[36]
Propafenone	CNS toxicity and bronchoconstriction[37,38]
Metoprolol	Loss of cardioselectivity[39]
Nortriptyline	Hypotension and confusion[40]
Terikalant	Excessive prolongation in QT interval[41]
Dexfenfluramine	Nausea, vomiting and headache[42]
L-tryptophan	Eosinophilia-myalgia syndrome[43]
Indoramin	Sedation[44]
Thioridazine	Excessive prolongation in QT interval[45]
Failure to respond	
Codeine	Poor analgesic efficacy[46]
Tramadol	Poor analgesic efficacy[47]
Opioids	Protection from oral opioid dependence[48]
Clinical consequences for ultra-rapid EMs	
Increased risk of toxicity	
Encainide	?Proarrhythmic effects[49]
Codeine	Morphine toxicity[50]
Failure to respond	
Nortriptyline	Poor efficacy at normal dosages[51,52]
Propafenone	Poor efficacy at normal dosages[53]

CYP = cytochrome P450; **EMs** = extensive metabolisers; **PMs** = poor metabolisers.

Table IV. Long QT syndrome (LQTS) genes, locations, gene products and currents

Gene	Chromosomal location	Protein (and amino acids)	Chain subunit	Associates with	Current	Clinical syndrome
KCNQ1	11p15.5	KvLQT1 (676)	α	minK	I_{Ks}	LQT1
KCNE1	21q22.1	minK (129)	β	KvLQT1	I_{Ks}	LQT5
KCNE2	21q22.1	miRP1 (123)	β	HERG	I_{Kr}	LQT6
KCNH2	7q35–36	HERG (1159)	α	miRP1	I_{Kr}	LQT2
SCN5A	3p21–24	(2016)	α		I_{Na}	LQT3
KCNJ2	17q23	(427)			$I_{Kir2.1}$	LQT7
ANK2	4q25–27	Cardiac repolarising current due to sodium pump, sodium-calcium exchanger and inositol-1,4,5-triphosphate receptors				LQT4

HERG = human ether-a-go-go-related gene; $I_{Kir2.1}$ = inward rectifying potassium current; I_{Kr} = rapidly activating outward-rectifying potassium current; I_{Ks} = slowly activating outward-rectifying potassium current; I_{Na} = depolarising sodium current.

cribe the cardiac potassium channel genes, their products, chromosomal location, current mediated and the corresponding clinical LQTS (table IV).

Delayed rectifier current is the most important repolarising current and it has at least three distinct components (I_{Kur}, I_{Kr} and I_{Ks}). The ultra-rapidly activating I_{Kur} current is mediated by a channel formed by co-assembly of Kv1.5 α-subunits (613 amino acids) encoded by the gene *KCNA*5.[63] Four HERG (*KCNH2)* α-subunits assemble with MiRP1 (*KCNE2)* β-subunits to form I_{Kr} (the rapidly activating delayed rectifier current).[64] The response of HERG (*KCNH2)* to drugs is modulated by MiRP1 *(KCNE2*) β-subunits of I_{Kr}. Four KvLQT1 (*KCNQ1)* α-subunits assemble with minK (*KCNE1)* β-subunits to form I_{Ks} (the slowly activating delayed rectifier current).[65] Mutations of these subunits lead to dysfunctional channels, reduced I_{Kr} or I_{Ks} current and a clinical syndrome of congenital prolongation of the QT interval, with all its consequences. Romano-Ward syndrome[66] is a more familiar example of LQTS.

LQTS is a heterogeneous group of genetic disorders caused by mutations at five different loci at the least. Three of the congenital LQTS, LQT1, LQT2 and LQT5, result from mutations of potassium channel subunits while the fourth one, LQT3 results from mutations of a cardiac-specific sodium channel, *SCN5A*. LQT7 results from mutations of the gene coding for cardiac (and skeletal) inward rectifier potassium channel (Kir2.1) – the loss of function that arises prolongs the terminal phase of the cardiac action potential. LQT6 results from mutations in *KCNE2* that encodes for MiRP1 β subunit. LQT4 results from mutation of gene (*ANK2*) coding for ankyrin-B, a member of a family of membrane adapters.[67] This mutation produces disruption in the cellular organisation of the sodium pump, sodium-calcium exchanger and inositol-1,4,5-triphosphate receptors, all ankyrin-B binding proteins. Whereas LQT1, LQT2, LQT5, LQT6 and LQT7 arise from loss of function of potassium channel, LQT3 results from gain in function of sodium channel – all nonetheless resulting in prolonged action potential duration and diminished repolarisation reserve. One large study in 580 patients with LQTS has identified a QTc interval greater than 500ms as a major factor for high risk of cardiac events, although the risk was also modulated by gender and LQTS genotype.[68] Gender has different genotype-specific modulating effects in LQT1 and LQT2 but has no effect at all in LQT3.[68,69]

A large number of families with mutations of KCNQ1 (leading to LQT1) have been described. Most of these have their own unique family-specific mutations. However, there is at least one frequently mutated region (called a 'hot-spot') of this gene. This gene is now believed to be the most commonly mutated gene in LQTS. The second most frequently involved gene in LQTS is KCNH2 which encodes hERG channel. There are no specific 'hot spots' in this gene and mutations scattered widely along the gene have been described. Of the 177 mutations known to lead to LQTS (as at April 2000), 45% affected HERG (*KCNH2*), 42% affected KvLQT1 (*KCNQ1*), 8% *SCN5A*, 3% minK (*KCNE1*) and 2% MiRP1 (*KCNE2*).[70] About one-third of the HERG mutations are in the pore region and these are associated with markedly increased risk for arrhythmias compared with HERG non-pore mutations.[71] One

specific mutation of hERG (2690A→C; K897T) results in hastening of cardiac repolarisation and therefore, a slightly shorter QTc interval. A large population study showed a significant association between this polymorphism and QTc interval with CC homozygotes having a significantly shorter QTc (388.5ms) compared to AA homozygotes (398.5ms) and heterozygotes (AC, 397.2ms).[72] In many patients with congenital LQTS, it has not been possible to detect any mutations of the genes that are known to code for ion channels. It is thought that at present, at least 50% of the patients with LQTS have no, as yet, identifiable genetic defect. It was only recently that the genetic and molecular basis of LQT4 was uncovered[67] and the number of mutations discovered continues to rise rapidly. Indeed, the application of pharmacogenetics in minimising the risk of drug-induced QT interval prolongation may be limited by the fact that many families appear to have 'private' family mutations. Despite sharing the same clinical LQTS phenotype, these 'private' mutations do not occur widely among other patients or carriers.

An interesting electrophysiological counterpart of LQT3 is the Brugada syndrome that results from loss of function or rapid recovery of sodium channel.[73] This is also associated with higher risk of proarrhythmic events and sudden death following administration of class I (sodium channel-blocking) antiarrhythmic drugs. Whereas these drugs almost normalise QT interval in LQT3, their effects can be catastrophic in Brugada syndrome. Indeed, a controlled administration of the short acting drug ajmaline is used to unmask latent Brugada syndrome. Bezzina et al.[74] screened SCN5A in a large 8-generation family characterised by a high incidence of nocturnal sudden death, and QT interval prolongation and the 'Brugada ECG' occurring in the same participant. An insertion of three nucleotides, predicted to cause insertion of aspartic acid, was linked to the phenotype, and identified in all electrocardiographically affected family members. ECGs were obtained from 79 adults with a defined genetic status (43 carriers and 36 non-carriers). In affected individuals, PR and QRS durations, and QT intervals were prolonged with ST segment elevation in the right precordial leads. Twenty-five family members died suddenly, 16 of them during the night. Channel expression studies confirmed that LQT3 and Brugada syndromes might also share a common genotype although they are usually different allelic disorders. Recently, a number of other SCN5A mutations have been described and these are thought to be associated with loss of function and non-progressive cardiac conduction defects,[75,76] bradycardia and sinus node dysfunction[77] and with severe cardiac conduction disturbances and degenerative changes in the conduction system.[78]

Congenital LQTS is estimated to have a frequency of 1 in 5000 individuals in the US.[79] However, in view of the low penetration of many of these mutations, the population with potassium channels with dysfunctional or altered properties may be substantially larger than diagnosed by ECG recordings alone. Relatively large numbers of individuals who carry these 'clinically silent' variants of LQTS genes have been identified.[80-82] Among the family members of sporadic cases of LQTS, the frequency of these 'silent carriers' could be as high as 33%.[82] In one study, there was an overlap of QTc intervals in 126 (63%) of the 199 participants (83 carriers and 116 non-carriers).[80] Not all gene carriers have symptoms and only the DNA markers make it possible to make a genetic diagnosis in these individuals. Despite a normal ECG phenotype, the affected individuals have a 'diminished repolarisation reserve'. They are highly susceptible to drug-induced QT interval prolongation and/or TdP and a substantial proportion of these might represent cases of *forme frust* of the congenital LQTS.

The frequency with which mutations of ion channels contribute to drug-induced TdP remains speculative. Despite several years of investigation, there has not been adequate persuasive evidence so far to link the vast majority of patients with drug-induced QT prolongation and proarrhythmic effects to an underlying genetic defect. As will be discussed later, by far the greater number of cases of TdP in routine clinical practice is related to drug interactions. The link between genetic mutations and drug-induced TdP is of course plausible and will no doubt become clearer as the majority of the genes expressing ion channels are identified and patients with drug-induced prolongation of QT interval and/or TdP are routinely genotyped.

Table V. Main cytochrome (CYP) enzymes involved in the metabolism of drugs that prolong QT interval

CYP enzyme	Distribution	Chromosomal location	Alleles known (10 April 2003)	Main QT-prolonging drugs metabolised
CYP2B6	Polymorphic	19q13.2	16	Efavirenz, methadone
CYP2C19	Polymorphic	10q24.1-q24.3	19	Nelfinavir, citalopram
CYP2D6	Polymorphic	22q13.1	73	Most antipsychotics, antidepressants and antianginals, encainide, flecainide, lorcainide, ajmaline, indoramin
CYP3A4	Unimodal	7q21.1	25	Most class III antiarrhythmics, antihistamines, cisapride, pimozide, ziprasidone, levacetylmethadol, methadone, tacrolimus, tamoxifen, clarithromycin, erythromycin, halofantrine, quinidine, bupivacaine

2.3 Genotyping Versus Phenotyping

In the context of pharmacogenetics, although the emphasis is on genotyping of patients, phenotyping too is a potentially valuable and at times more effective tool. Patients may be phenotyped for their drug metabolising capacity using appropriate substrate drugs as metabolic probes (e.g. dextromethorphan for CYP2D6). Classification of an individual as either an EM or a PM is based on estimation of drug in the serum at a predetermined time point or of the parent drug and its metabolite in urine sample collected over a defined period.[83] The major advantage of genotyping is the lack of interference from interacting drugs that need not be discontinued. For example, in the presence of a metabolic inhibitor of CYP2D6, genotyping a patient will correctly identify an EM whereas phenotyping may result in misclassification of an EM as a (phenocopy) PM if there is co-medication with a CYP2D6 inhibitor. For most pharmacological targets, genotyping is at present the only available option to explore the role of genetic factors. Recently, an epinephrine (adrenaline) challenge test has been described as a means of establishing an electrocardiographic diagnosis in silent LQT1 mutation carriers.[84]

3. Genetic Influences in Drug-Induced TdP

Genetic influences underlying drug-induced QT interval prolongation can best be illustrated by polymorphisms in CYP2D6 and ion channels, especially the potassium channels.

Table V shows the main CYP enzymes involved in the oxidative primary metabolism of the commonly used QT-prolonging drugs or drug classes. Often other CYP enzymes may also contribute modestly to metabolic elimination of many of these drugs. Although CYP3A4 metabolises a large number of these commonly used drugs and its activity varies widely between individuals, it does not display a clinically relevant genetic polymorphism. A closely related gene CYP3A5, with almost identical substrate specificity as CYP3A4, is polymorphically expressed in intestine and fetal livers but is detectable in only 10–20% of adult livers. CYP3A4 is, however, an important enzyme with regard to drug-drug interactions (see section 4). CYP2C9, CYP2C19 and CYP2D6 display well-characterised genetic polymorphisms and in the context of genetic influences, CYP2D6 is by far the most important since it metabolises a substantial number of QT-prolonging drugs that are administered long-term.

3.1 Pharmacokinetic Genetic Influences on Drug-Induced TdP

The metabolism of a number of QT-prolonging drugs, especially the antipsychotics, antidepressants and cardiovascular agents, is predominantly under the control of CYP2D6. These include terikalant, thioridazine, sertindole, risperidone, indoramin and nortriptyline. The risk of TdP following the use of these drugs may therefore be genetically determined in some patients.

QT interval produced by terikalant, a class III antiarrhythmic drug, has been shown to correlate well with CYP2D6 metabolic ratio.[41] This genetically determined risk has resulted in termination of further development of this compound. There is no information on how many other compounds have been dropped from development due to their CYP2D6-mediated metabolism.

Until recently, thioridazine was a widely used antipsychotic drug. It has been associated with a high risk of TdP. Although other antipsychotics

such as pimozide, sertindole, droperidol and haloperidol have also been documented to cause TdP and sudden death, the most marked risk is believed to be associated with thioridazine.[85] There are several reports of syncope, cardiovascular collapse and sudden death in patients receiving thioridazine.[86]

Thioridazine produces a dose-related effect on ventricular repolarisation, primarily due to the parent drug but with a contribution from the metabolites.[87] Compared with EMs of CYP2D6, PMs attain higher serum levels of thioridazine with a 2.4-fold higher peak plasma concentration (C_{max}) and a 4.5-fold larger area under the plasma concentration-time curve (AUC) associated with a 2-fold longer half-life.[88] Two side-chain metabolites of thioridazine, mesoridazine and sulforidazine, appear more slowly in serum in PMs, implicating CYP2D6 in their formation.[89] Compared with EMs, PMs attain a higher C_{max} and 3.3-fold higher AUC of a third metabolite (a ring-sulphoxide of thioridazine). The formation of this metabolite in CYP2D6 PMs is catalysed by CYP2C19[90,91] – an enzyme that also displays genetic polymorphism. These three metabolites may also prolong the QT interval. Despite this complex metabolic profile and presence of circulating cardiotoxic metabolites, CYP2D6 PM individuals may be at risk of adverse reactions to thioridazine,[92] including the prolongation of QT interval. CYP2D6 status has been suggested to be an important determinant of the risk for thioridazine-induced QTc interval prolongation.[45] The difficulty of interpreting such complex pharmacological data is compounded by a recent contradictory report that CYP2D6 genotype does not substantially affect the risk of thioridazine-induced QTc interval prolongation.[93] Individuals with reduced CYP2C19 activity (either due to genetic factors or following co-administration of a metabolic inhibitor such as fluvoxamine) develop higher plasma levels of thioridazine and its CYP2D6-generated metabolites and may also be at risk of toxicity.[91] However, at present there are no data on the effect of thioridazine on QT interval in individuals with reduced CYP2C19 activity.

Terodiline was first marketed in 1965 as an antianginal agent in Scandinavia.[94] It was later redeveloped and approved in 1986 for clinical use in urinary incontinence. Beginning in 1989, reports of TdP began to appear[95-99] and following a warning in July 1991, many additional, mostly retrospective, reports followed swiftly. By September 1991, there were 69 reports of serious terodiline-induced cardiac arrhythmias and the drug was immediately withdrawn from the market in September 1991.[100] An analysis of predisposing factors in these 69 reports revealed 12 cases (18%) in whom there were no clinically identifiable risk factors at all.

In vivo, the proarrhythmic effect of terodiline is mediated by (+)-(R)-terodiline.[101] Although much of the clinical data are incomplete or often difficult to reconcile, available evidence suggests that the major enzyme involved in the metabolism of (+)-(R)-terodiline is CYP2D6.[102,103] A role of other hitherto uncharacterised factors, probably also genetic, cannot be excluded. Interestingly, CYP2D6 has been shown to metabolise R-enantiomer of tolterodine, a structural analogue of terodiline.[104,105] CYP3A4-mediated dealkylation provides the main route of elimination of tolterodine in PMs of CYP2D6.[106]

One study has suggested that the presence of *CYP2C19**2 allele may contribute to adverse cardiac reactions to terodiline.[107] The susceptibility role of *CYP2C19**2 does not explain either the absence of terodiline-induced cardiotoxicity among the Japanese or the high frequency of anticholinergic effects of (+)-(R)-terodiline in Scandinavia. In these countries, the frequencies of *CYP2C19**2 allele are 0.29 to 0.35 (high) and no more than 0.08 (low), respectively.[108,109] Information on the genotypes of patients who developed TdP without any risk factors but in whom plasma terodiline concentrations were markedly elevated[98,99] would have been helpful in elucidating the role of genetic susceptibility to terodiline-induced arrhythmias. As stated earlier, about 18% of patients with terodiline-induced arrhythmias have no identifiable risk factors.

While it is true that the dosages used in Sweden and Japan were generally lower than those used in the UK, this CYP2D6-mediated metabolism of (+)-(R)-terodiline might also explain the striking inter-ethnic differences in the incidence of ventricular arrhythmias associated with its use.[110] Whereas 9% of the UK population are PMs,[21] the corresponding figures for Sweden and Japan are 6.8% and less than 1%, respectively.[111,112] In addition, inter-ethnic dif-

ferences in the frequency of alleles with altered functional activity and of gene duplication, multiplication or amplification may also contribute to this inter-ethnic difference in terodiline-induced arrhythmias.[113,114] CYP2D6-mediated metabolism would also indicate a higher potential for drug-drug interaction in the UK between terodiline and other QT interval prolonging substrates of CYP2D6, such as antipsychotics, antidepressants and other antiarrhythmic drugs.[115]

Prenylamine was also withdrawn from the market because of its high potential to induce TdP, often with a fatal outcome.[20] First approved in 1960s for angina pectoris, prenylamine first began to be associated with prolongation of the QT interval and TdP as late as 1971.[116] By 1988, there were 158 cases of polymorphous ventricular tachycardia and prenylamine was withdrawn world-wide soon after its removal from the UK market. Thirty of 109 patients had received prenylamine as the only medication. The vast majority of the patients were taking a 'standard' daily dose of 180mg.[110]

The proarrhythmic effect of prenylamine in humans is probably mediated by its (+)-(S)-enantiomer.[117,118] (−)-(R)-prenylamine shortens the action potential duration to a minor extent. Metabolism of (+)-(S)-prenylamine also appears to be genetically controlled. The high average value of plasma half-life of the (+)-(S)-enantiomer in one study[119] was mainly a consequence of the extremely long plasma half-life of 82 and 83 hours in two of the eight volunteers. The drug had an average half-life of only 11 hours in the remaining six participants. This study had preceded the first description of CYP2D6 polymorphism and, therefore, none of these participants had been phenotyped for their metabolic capacity. However, it is worth speculating whether the two individuals were PMs of CYP2D6. Prenylamine, a structural analogue of terodiline and tolterodine, fulfils all the structural requirements of a CYP2D6 substrate. It seems probable that the PM phenotype is associated clinically with impaired metabolism of (+)-(S)-prenylamine with resultant prolongation of the QT interval and/or induction of arrhythmias.

As noted earlier, the Brugada syndrome, resulting from loss of function or rapid recovery of sodium channel, is associated with a higher risk of arrhythmias and sudden death following administration of class I antiarrhythmic drugs. In this context, the finding of increased mortality following the administration of flecainide or encainide, relative to placebo, observed in the Cardiac Arrhythmia Suppression Trial may be highly relevant.[120] Genetic factors operating at two levels may have contributed to this – both flecainide and encainide block the sodium channels, are metabolised by CYP2D6 and prolong QT interval – the metabolite being responsible for encainide.

Sertindole, an atypical antipsychotic, is also associated clinically with marked prolongation of QTc interval and is metabolised by CYP2D6.[121] It is an excellent example of how CYP2D6-mediated metabolism may not always be relevant in drug-induced QT interval prolongation. Electrophysiological studies have shown that CYP2D6-generated metabolites of sertindole are also active in blocking HERG potassium current at nanomolar concentrations. However, they do not induce EADs that are the precursors of TdP.[122] Its powerful α-blocking activity seems to afford a relative protection against degeneration of prolonged QT interval into TdP.[123-125] First approved and marketed in the EU in 1996, sertindole was removed from the market in 1998 because of concerns over its cardiac safety (high frequency of recipients experiencing QTc interval prolongation). Following an in-depth review of its electrophysiological and autonomic pharmacology, efficacy and safety in 2001, it is to be reintroduced albeit under carefully monitored postmarketing surveillance. As with sertindole, the QT interval prolongation by haloperidol does not seem to be related to CYP2D6 genotype despite the fact that haloperidol is substantially cleared by CYP2D6 and there are significant pharmacokinetic differences between EMs and PMs.[126]

Procainamide is metabolised by NAT2 and the slow acetylators are at a greater risk of procainamide-induced systemic lupus erythematosus-like syndrome. Although procainamide is a class I antiarrhythmic drug, its acetylated metabolite, *N*-acetylprocainamide is powerful class III drug known to induce TdP. The role of acetylator genotype in the aetiology of TdP following clinical use of procainamide has not been investigated.

Both sertindole and procainamide illustrate an important point when applying pharmacogenetics to drug development. Pharmacogenetic influences on drug metabolism, and therefore the response to a drug, need to be considered in context of the activity of metabolites relative to that of the parent drug.

3.2 Pharmacodynamic Genetic Influences on Drug-Induced TdP

The most striking example of genetically conferred pharmacodynamic susceptibility to drug-induced TdP is the gender distribution – two-thirds of the cases occur in females.[127] There is no evidence to suggest that this gender-associated difference is related to differences in drug exposure, in the number of drugs prescribed (polypharmacy), in drug pharmacology or other possible differences in the way the adverse reactions are reported. Female gender is associated with a longer QTc interval at baseline and an exaggerated response to drugs that block I_{Kr}. This results most likely from a specific regulation of ion channel expression by sex steroids. Estrogens facilitate bradycardia-induced prolongation of the QT interval and the emergence of arrhythmia whereas androgens shorten the QT interval and blunt the QT response to drugs. Experimental evidence suggests that gender differences in specific cardiac ion current densities are responsible, at least in part, for the greater susceptibility of females for developing TdP arrhythmias.[128-132]

However, genetically conferred pharmacodynamic susceptibility to TdP due to abnormal channels has also been frequently reported. Even in the absence of any risk factor, there are reports of TdP following administration of QT-prolonging drugs at their recommended doses that are otherwise generally well tolerated by a wider patient population. Many CYP3A4-metabolised QT-prolonging drugs have been reported to induce TdP in some individuals at the usual therapeutic doses even in the absence of a metabolic inhibitor. Just as drugs can inhibit one or more drug metabolising enzymes to produce a phenocopy of a PM, it follows that drugs can also inhibit one or more ion channels to produce a phenocopy of a congenital channelopathy.

It is difficult to speculate on the extent to which genetic mutations of potassium channels may have conspired with impaired CYP2D6-mediated metabolism in the induction of QT interval prolongation and TdP by terodiline, prenylamine, thioridazine and other CYP2D6 substrates. A role of other hitherto uncharacterised factors, probably also genetic, cannot be excluded. The presence of modifier gene(s) is suggested by the observation that even the carriers from families with the same HERG (*KCNH2*) mutations have a highly variable clinical expression.[133] The point to be emphasised is that pharmacogenetic studies rarely, if ever, investigate these kinds of interactions.

One of the earliest publications of mutations of potassium channels predisposing to drug-induced TdP, reported this arrhythmia in a patient with congenital long QT interval following a relatively low dose of vincamine.[134] Stratmann and Kennedy also noted that 49% of the 63 cases of TdP in which the information was available had pre-existing prolongation of QTc interval before the administration of the drug that precipitated TdP.[135]

Donger et al.[136] analysed the relationship between phenotypes and underlying defects in KvLQT1 (*KCNQ1*) in 20 families with Romano-Ward syndrome. They identified three families with a missense mutation, Arg555Cys. Drugs known to affect ventricular repolarisation had triggered most of the cardiac events occurring in these families.

In early clinical trials, halofantrine, an effective antimalarial agent, was shown to induce dose-related lengthening of the PR and QT intervals in all 61 patients treated, while none of the 53 patients receiving mefloquine developed similar changes.[137]

By June 1995, the FDA had received 17 reports of halofantrine-induced QT interval prolongation (with or without TdP).[138] Interestingly, seven (41%) of these patients had congenital LQTS and/or a family history of it. Although none of these patients were genotyped for mutations of potassium channel, Monlun et al.[139] had previously reported a 29-year-old woman who developed TdP following treatment with halofantrine for malaria. She was found to have congenital QT prolongation (Romano-Ward syndrome), which was also present in two of her relatives. Castot et al.[140] have also reported a similar case.

Of particular interest, however, is the description by Piippo et al.[141] of two patients with halofantrine-induced QT interval prolongation, TdP and recur-

rent syncope. Both had mutations of *SCN5A* of LQT3 syndrome – indicating a role of mutated sodium channels in induction of drug-induced TdP. Recently, Makita et al.[142] have also reported a similar patient who had a silent mutation of *SCN5A* and developed QT prolongation and TdP following administration of a daily dose of cisapride 5mg, a gastric prokinetic drug that is known to block I_{Kr}. These sodium channel mutations that predispose to drug-induced TdP may be more common than has hitherto been appreciated. Splawski et al.[143] have reported a mutation in codon 1102 (Ser1102Tyr) of *SCN5A* in 13 (56.5%) of the 23 patients with arrhythmias or presenting with symptoms suggestive of arrhythmias or considered at risk of arrhythmias. A family study of one of the cases (an African-American) revealed that 11 of the 23 family members had prolonged QT interval (≥460ms) or a history of syncope and they all carried this allele (five in heterozygous and six in homozygous state). In a control population, this allele was observed in 19.2% (90/468) of West African and Caribbean individuals (85 in heterozygous and five in homozygous state) and in 13.2% (27/205) of African Americans (26 in heterozygous and one in homozygous state). Only one of the 123 Hispanic participants carried this allele (heterozygous state). Ser1102Tyr was not observed in any of the 511 Caucasian and 578 Asian control individuals.

Napolitano et al.[144] reported an elderly female patient with documented cardiac arrest following treatment with cisapride and a transiently prolonged QT interval. On mutational analysis of the known LQTS-related genes, she was found to have a heterozygous mutation in the pore region of KvLQT1 (*KCNQ1*). This mutation was also present in her two adult asymptomatic sons who had a normal QT interval. Similarly, Koh et al.[145] reported TdP with a therapeutic dose of terfenadine in a patient with LQTS. In one series of 17 patients with drug-induced long QT syndrome, mutational analysis of the five genes known to cause congenital LQTS revealed four patients (23%) who were carriers of a mutation.[146]

These isolated reports indicate that some cases of drug-induced QT prolongation and/or TdP may indeed depend on a genetic substrate. In one analysis of 341 reports of cisapride-induced ventricular arrhythmias, there were 38 (11%) cases in whom there were no identifiable risk factors or contraindications.[147] Among the 159 cases of QTc prolongation or TdP associated with cisapride reported to Uppsala Monitoring Centre as of 1999, about half reported no interacting medication, and by May 2000, six of the 20 deaths associated with cisapride occurred at dosages of 40mg or less in absence of known interactions.[148]

Abbott et al.[64] reported three missense mutations associated with LQTS and ventricular fibrillation in the *KCNE2* gene for MiRP1. One variant, associated with clarithromycin-induced arrhythmia, increased channel blockade by the drug. Sesti et al.[149] also examined *KCNE2* (gene for MiRP1) in 98 patients with drug-induced LQTS, and identified three individuals with sporadic mutations and a patient with sulfamethoxazole-associated LQTS who carried a SNP found in approximately 1.6% of the general population. It was concluded that allelic variants of *KCNE2* (MiRP1) contribute to a significant fraction of cases of drug-induced LQTS and that the common sequence variations that increase the risk of life-threatening drug reactions can be clinically silent before drug exposure.

Yang et al.[150] have identified functionally important DNA variants in genes encoding K^+ channel ancillary subunits in 11% of an acquired LQTS cohort. Further screening studies aimed at the coding regions of *KCNQ1* (KvLQT1), *KCNH2* (HERG) and SCN5A in the same cohort of 92 patients revealed missense mutations (absent in controls) in five of the 92 patients. *KCNQ1* and *KCNH2* mutations (one each) reduced K+ currents *in vitro*, suggesting that these mutations enhanced the risk for acquired LQTS. Overall, the data provided evidence that DNA variants in the coding regions of LQTS genes, predisposing to acquired LQTS, can be identified in approximately 10–15% of affected subjects, predominantly in genes encoding ancillary subunits.

The Survival With Oral d-Sotalol (SWORD) study compared (+)-(S)-sotalol with placebo in preventing sudden arrhythmic deaths in patients with a recent or remote myocardial infarction and a left ventricular ejection fraction <40%. A number of patients in this study were excluded during the run-in dose-titration period because of excessive QTc interval prolongation. Despite this, the study was

stopped prematurely because of 65% greater mortality following the active treatment relative to placebo.[151] Of course, great care needs to be exercised not to assume automatically that all adverse outcomes from drugs that potentially prolong the QT interval are directly related to TdP or other arrhythmias. On re-analysis of the data, there was little objective evidence to support the notion that the increased mortality was due to TdP or any specific proarrhythmic mechanism. However, the possibility cannot be excluded that some patients who developed excessive prolongation of QT interval during the run-in period and were consequently excluded may have had genetic predisposition to QT interval prolongation and were therefore, candidates for TdP.

Mutations of genes coding for β-adrenoceptor may also confer a proarrhythmic risk. One study compared the frequencies of five recognised non-synonymous coding region polymorphisms in genes encoding β_1-adrenoceptors (Ser49Gly and Gly389Arg) and β_2-adrenoceptors (Thr164Ile, Arg16Gly and Gln27Glu)[152] in 93 patients with drug-induced QT interval prolongation and three control groups. One of the control groups was composed of 66 patients who had tolerated QT-prolonging drugs without drug-induced QT interval prolongation. None of the five common polymorphisms in the β-adrenoceptor genes was found to predict drug-associated TdP, although the Gly16/Gln27 haplotype emerged as a potential risk factor. This finding is important since β-blockers are used widely in the treatment of LQTS with a significant reduction in cardiac events in these patients and patients with mutations may be considered as 'endogenously β-blocked'. It is known that patients carrying mutations in the *KCNQ1* gene (LQT1) respond better to β-blockers than those with *KCNH2* mutations (LQT2) [92% vs 20%].[153]

4. Non-Genetic Influences in Drug-Induced TdP

Since genotyping is a relatively new tool, it is difficult to ascertain what proportion of patients with drug-induced TdP in clinical practice has a genetic substrate. Drug metabolism and pharmacological targets may also be modulated by a number of non-genetic factors such as co-morbidity (e.g. hepatic, renal or cardiac disease) or by co-medications. The interaction between the genotype and these extrinsic factors is an important factor that is likely to limit the potentially beneficial applications of pharmacogenetics.

4.1 Pharmacokinetic Non-Genetic Influences on Drug-Induced TdP

Drug-drug and drug-disease interactions are proving to be a major problem not only in clinical medicine generally but also with regard to drug-induced QT interval prolongation. In an analysis of 2194 cases of TdP, 11.7% were associated with drug interactions and a further 9.2% with overdoses.[10]

Inhibition of drug metabolising enzymes by concurrent administration of other drugs is an important point of intersection between pharmacogenetics and drug response. In principle, the phenotype of a patient is not an immutable parameter as is the genotype. An individual of EM genotype can be readily converted into an individual of PM phenocopy by concurrent administration of an inhibitor. A number of substrates and non-substrate drugs inhibit a given CYP enzyme. For example, quinidine, fluoxetine and a range of other drugs convert a genotypic CYP2D6 EM into a phenocopy PM while fluvoxamine or omeprazole convert a CYP2C19 EM into a PM. Similarly, ketoconazole or clarithromycin inhibit CYP3A4, fluconazole inhibits CYP2C9 and furafylline or fluvoxamine inhibit CYP1A2. A natural consequence of this iatrogenic 'phenocopying' is that many individuals may be prescribed 'normal' dosages on the basis of their genotype. However, when temporarily co-prescribed these metabolic inhibitors, they develop high plasma concentrations of the parent drugs, exposing them to high-dose pharmacology of the drugs concerned.

As stated earlier, CYP3A4 metabolises a large number of commonly used drugs that prolong QT interval. This enzyme is subject to inhibition by a number of drugs, most especially azole antifungals and macrolide antibiotics. Even grapefruit juice is known to inhibit this enzyme and may interact with drugs such as halofantrine to induce QT interval prolongation.[154-156] For example, grapefruit juice increased halofantrine-induced maximum QTc interval prolongation from a mean of 17 ± 6ms to 31 ± 12ms.[154] However, interaction studies between

grapefruit juice and either terfenadine or cisapride have failed to show a consistent effect on QTc interval.[155,156] With terfenadine, there is a modest increase in QTc interval when the two are taken together but not if the grapefruit juice is taken 2 hours after terfenadine.[157]

CYP3A4 activity is also highly susceptible to liver disease.[158,159] In contrast, CYP2D6 activity is relatively refractory to the presence of liver disease.[160,161] The effect on CYP2C19 activity is intermediate between that on CYP3A4 and CYP2D6.[161] For those QT-prolonging drugs that are primarily eliminated unchanged through the renal route (e.g. dofetilide, gatifloxacin, levofloxacin), renal dysfunction is another important risk factor.

Not surprisingly, the majority of cases of TdP are reported in association with CYP3A4 substrates (such as terfenadine and cisapride) in the presence of these interacting variables, predominantly azole antifungals and macrolide antibiotics. Eleven (44%) of the 25 patients with terfenadine-induced TdP were taking these metabolic inhibitors compared with only two who had pre-existing prolongation of the QT interval and none without any obvious risk factor.[162] Similarly, 32 (56%) of the 57 cases of QT interval prolongation and/or TdP in association with cisapride were also taking inhibitors of its metabolism.[163] In the more recent analysis,[147] there were 126 (37%) patients taking cisapride concurrently with CYP3A4 inhibitors. These high frequencies of drug interactions with terfenadine and cisapride contrast with the finding with halofantrine. While seven (41%) of the 17 patients who experienced QT interval prolongation (with or without TdP) following halofantrine administration had congenital LQTS and/or a family history of it, only four had hypokalaemia and none were on an obviously interacting drug.[138] However, a number of these patients were also taking mefloquine (another antimalarial) that is known to potentiate halofantrine-induced QT interval prolongation.

4.2 Pharmacodynamic Non-Genetic Influences on Drug-Induced TdP

A number of non-genetic factors also alter the responsiveness of ion channels to QT-prolonging drugs. The susceptibility of the female gender to drug-induced QT interval prolongation and TdP is further increased during the menstrual cycle.[164] Potassium channels are often down-regulated in diseased myocardium, explaining why individuals with cardiac failure are at a greater risk of TdP from drugs that prolong the action potential duration.[165] Changes in internal environment such as hypokalaemia may trigger or further lower the threshold to the arrhythmia.[166] Among other factors that increase the risk of TdP at a pharmacodynamic level are bradycardias with or without heart blocks and pre-existing prolongation of QTc interval.[9] There is also a wider appreciation of clinical conditions with increased pharmacodynamic susceptibility to this effect. QTc interval prolongation is associated with a variety of diseases. These include sudden deaths (usually labelled as sudden unexplained cardiac deaths) and a number of cardiovascular as well as non-cardiovascular 'natural' diseases – for example, cardiomyopathy,[167-169] cardiac failure,[14,170] myocardial infarction,[171] sudden infant death syndrome,[172] diabetic autonomic neuropathy,[173-175] hypoglycaemia,[176] cirrhosis[177] and a number of other conditions associated with autonomic failure.[178,179] In all of these conditions, QTc interval prolongation has been identified as a risk factor for malignant ventricular tachyarrhythmias. An earlier study had also reported a higher prevalence of QTc interval prolongation in patients with HIV infection compared with other hospitalised patients (28.6% vs 7%).[180] In this context, any reports of TdP associated with protease inhibitors may raise some concern. However, any such reports must be viewed with a careful risk/benefit perspective.

One well-known class of drugs associated with QT interval prolongation is the quinolone antibiotics. Broadly speaking, those quinolones that are thought to have a higher potential for inducing repolarisation abnormalities (e.g. sparfloxacin, moxifloxacin, gatifloxacin, levofloxacin) are metabolised either hardly at all or by conjugation (glucuronidation, sulfation or acylation). CYP-mediated metabolism is not a feature of these compounds. It appears that some of them are actively secreted via hepato-biliary transport into the canaliculi[181] – a process that may be disrupted in liver disease. Although conjugation reactions display genetic polymorphisms, their role in determining drug response, especially the fluoroquinolones with poten-

tial to cause TdP, has not been investigated. Reports of TdP due to these antibiotics, usually administered short-term, are often associated with increased pharmacodynamic susceptibility due to non-genetic factors such as heart disease (especially heart failure), electrolyte imbalance, coadministration of other QT-prolonging drugs and/or bradycardia.[182-184]

Often, the trigger to TdP is the concurrent administration of two drugs, each of which by itself has a very low, if any, potential to induce TdP. This pharmacodynamic drug-drug interaction between two QT-prolonging drugs is proving to be of great concern.[115,185] Electrolyte imbalance (5–19%) and concurrent administration of another QT-prolonging drug (5–12%) were also identified as major risk factors in cisapride-induced TdP and other ventricular arrhythmias.[147,163] The majority of the 69 patients with cardiotoxicity due to terodiline had non-genetic risk factors such as hypokalaemia or concurrent use of cardioactive medications, antidepressants, antipsychotics or diuretics.

4.3 Regulatory Concerns on Drug Interactions

Such is the concern regarding drug interactions that the CPMP has adopted a guideline on 'Drug Interactions'.[186] This outlines recommendations for interaction studies on NCEs on the basis of their physico-chemical, pharmacokinetic and pharmacodynamic properties as well as their co-medication potential. It discusses the potential of an NCE for interactions at the levels of drug absorption, metabolism and renal excretion. According to this guideline, an interaction is considered 'clinically relevant' when: (i) the therapeutic activity and/or toxicity of a drug is changed to such an extent that a dosage adjustment of the medication or medical intervention may be required; and (ii) concomitant use of the two potentially interacting drugs could occur when both are used as therapeutically recommended.

This guideline also recommends that when performing mechanism-based *in vivo* studies, consideration should be given to genotyping the subjects at the beginning of the study if any of the enzymes mediating the metabolism of the interacting drugs are polymorphically distributed in the population. As an extension to this, the genotype should also be ascertained of the donor liver used for *in vitro* microsomal studies.

In recent years, our understanding of absorption and excretion processes has increased greatly and interactions with transporters mediating these processes will also become as important as those at drug metabolising enzymes. For example, even at low dosages cimetidine is a selective inhibitor of renal cation transporter. Dofetilide, a potent QT-prolonging class III antiarrhythmic agent indicated for atrial fibrillation, is largely eliminated unchanged through active tubular secretion. When dofetilide was administered with cimetidine, there was a clinically significant increase in exposure to dofetilide. Dofetilide-induced prolongation of the QTc interval was enhanced by cimetidine; the mean maximum change in QTc interval from baseline was increased by 22% and 33% with 100mg and 400mg of cimetidine, respectively.[187]

There is a complex relationship (beyond the scope of this review) between CYP3A4, renal and hepatic anion and cation transporters and *MDR1* (multi-drug resistance gene encoding for P-glycoprotein transporter expression).[188] These transporters too display genetic polymorphisms with large inter-ethnic differences.[189-192]

5. Genotyping and Reduction of Risk of TdP

Very broadly, about 40–50% of the cases of drug-induced QT interval prolongation and/or TdP result from drug-drug interactions with metabolic inhibitors, 10% are associated with electrolyte imbalance, and 10% with concurrent use of other QT-prolonging drugs. Approximately 10–20% of cases have no obvious risk factors. There is a high probability of a genetic substrate in these patients who have no obvious risk factors.[110,147,150] However, the frequency of this genetic substrate remains speculative and requires more systematic and prospective studies during pre-approval clinical trials and the post-marketing period. If the prevalence of persons affected by LQTS is estimated to be 1 in 5000 persons, the prevalence of carriers of silent mutations may be even higher. Until large studies are available, pre-prescription genotyping of patients is not a practical proposition.

However, even without pharmacogenetic data, it is already possible to significantly reduce the risk of TdP in clinical practice by simple adherence to the prescribing information.

First of all, drugs that prolong the QT interval should be used carefully only for the indications approved. Secondly, the choice of the starting dose must be the lowest potentially effective dosage. In respect of selecting the most appropriate dosage, pimozide and astemizole provide valuable lessons. Pimozide has a half-life of approximately 55 hours in most individuals. This is highly variable, reaching as long as 150 hours in some patients even in the absence of any inhibitors of its metabolism. It was introduced originally at a starting dosage of 2–4 mg/day with a slow upward titration to a maximum daily dose of 10mg. Subsequently, the starting dosage was increased to 20 mg/day, the slow titration schedule was removed and the maximum daily dose was increased to 60mg. Following reports of QTc interval prolongation and TdP, the dosage schedule was re-amended to recommend an initial starting dosage of 2 mg/day with a very shallow dose titration to a maximum daily dose of 16–20mg. Trials investigating the use of pimozide in schizophrenia in the US had to be suspended in 1981 following sudden deaths of two patients during acute titration of pimozide to 70–80 mg/day.[193] Overdose with astemizole is often associated with cardiac arrhythmias.[194] Astemizole was originally approved at a dosage of 10 mg/day, but it has a long half-life, requiring many days before steady state is achieved. Given that desmethylastemizole with its cardiotoxic potential has a much longer half-life than astemizole,[195] the perils of recommending a loading dose of astemizole soon became evident. A recommendation to administer astemizole at a loading dose of 30mg daily for 1 week followed by 10 mg/day had to be revised to remove the loading dose recommendation following reports of cardiac arrhythmias.[196]

Unless the dose is carefully selected, the advantages from pharmacogenetic targeting of drugs may be lost. In principle, recommending higher dosages than therapeutically warranted is equivalent to administering an otherwise 'normal' dosage in the presence of a metabolic inhibitor or genetic mutation. Traditionally, sponsors of new drugs have always promoted higher than optimal dosages – the so-called maximum tolerated dosages – to maximise efficacy. In one study, all 499 labels of drugs approved by the FDA between 1 January 1980 and 31 December 1999 were examined for significant dosage changes. Of the 354 evaluable labels, 73 (21%) registered dosage changes.[197] Of these, 58 were safety-motivated dosage reductions. The relative risk of dosage change was the highest for drugs approved originally during the period 1995–99 relative to those approved during 1980–84. In another study, it was reported that of the 48 drugs examined, about 40 were found to be just as effective at doses of ≤60% than those recommended.[198]

Probably the most effective strategy in reducing the clinical risk of TdP is the observance of contraindications – pharmacokinetic and pharmacodynamic factors that constitute important risk factors. At a pharmacokinetic level, these include the concurrent use of metabolic inhibitors and the presence of liver and/or renal diseases. Pharmacodynamic risk factors include diseases (listed above) that are associated with QT interval prolongation, bradycardia, use of diuretics that may lead to electrolyte imbalance and concurrent use of QT-prolonging drugs. Finally, those patients who are at risk should be regularly monitored for QTc interval prolongation, bearing in mind that the risk in any individual patient may vary temporarily due to an inter-current event. Ideally, if a patient is a candidate for a QT-prolonging drug, QTc interval should be monitored: (i) at baseline; (ii) at steady state post-dose and at each incremental dose; (iii) when there is an inter-current change in risk; and (iv) if the patient develops symptoms of tachycardia or impaired cerebral circulation. If baseline QTc interval is prolonged, treatment should be approached with caution. If the patient develops a QTc interval ≥500ms, therapy should be reviewed and possibly discontinued.

It is evident that if therapy is to be individualised and improved on the basis of the patient's genotype/phenotype, drug development programmes need to investigate the influence of genetic factors when evaluating an NCE for its: (i) pharmacokinetics; (ii) dose-response relationships; (iii) drug interaction potential; and (iv) inter-ethnic differences in drug response.

6. Regulatory Framework for Pharmacogenetics in Clinical Trials

Since an individual's response to drugs may be profoundly influenced by genetic factors, regulatory authorities now increasingly require information on genetically mediated variability in the patient population in clinical trials. Arising from these inter-individual differences in pharmacology, areas of new drug applications (and even for older drugs should a significant safety issue emerge) that are likely to attract close regulatory scrutiny include the investigation of genetic influences on dose-response relationships and the recommended dose schedules. Genotypes of the patients who fail to respond, or who withdraw from clinical trials due to adverse events, are also likely to be of great regulatory interest.

A number of guidelines from CPMP and ICH make direct or indirect references to the need for exploring genetic factors when developing NCEs (table VI). In order to characterise the true consequences of genetic variability, it is important to investigate not only the inter-individual variability in pharmacokinetics of a drug but also the extent of its intra-individual variability. This is best done by studies of replicate design in a panel of genotyped healthy volunteers. Indeed, the CPMP guideline on pharmacokinetic studies in man[199] has recommended that metabolic studies should indicate whether the metabolism of a drug may be substantially modified in a case of genetic enzyme deficiency and whether within the dose levels normally used, saturation of metabolism may occur, thereby resulting in non-linear kinetics.

Table VI. Pharmacogenetics and Committee for Proprietary Medicinal Products (CPMP) and International Conference on Harmonisation (ICH) Guidelines

Genetic factors in pharmacokinetics
Pharmacokinetic studies in man[199]
Drug interactions[186]
ICH – ethnic factors in the acceptability of foreign clinical data[200]
Bioavailability and bioequivalence[201]
ICH – dose-response information[1]
"….metabolic polymorphism…."
Genetic factors in pharmacodynamics
ICH – dose-response information[1]
"Variability in pharmacodynamic response…"

In April 1997, the FDA issued their guidance note[202] 'Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies *In Vitro*'. This guideline states; "Identifying metabolic differences in patient groups based on genetic polymorphisms, or on other readily identifiable factors such as age, race, and gender, could help guide the design of dosimetry studies for such populations groups. This kind of information will also provide improved dosing recommendations in product labelling, facilitating the safe and effective use of a drug by allowing prescribers to anticipate necessary dose adjustments. Indeed, in some cases, understanding how to adjust dose to avoid toxicity may allow the marketing of a drug that would have an unacceptable level of toxicity were its toxicity unpredictable and unpreventable."

The Japanese Ministry of Health, Labour and Welfare have also issued guidelines[203,204] that recommend genotyping in all drug development programmes for drugs metabolised by CYPs.

The relative frequency of the DNA variations varies in different populations. For example, the frequency of CYP2D6 PM phenotype is much higher in populations of western Caucasian origin (5–10%) than in Far East and Asian ethnic groups (0–2%). It is found to be the highest (19%) in San Bushmen of South Africa among ≈60 population groups so far studied. Whereas the frequency of PMs of CYP2C19 is low in Western Caucasians and Africans (2–4%), it is high among Orientals (about 15–25%) and reaches as high as 70% on the two islands of Vanuatu in Eastern Melanesia.[114,205]

Inter-ethnic variations are also present in the mutations of a number of pharmacological targets. Studies are under way to determine whether or not there are any inter-ethnic differences in mutations of various genes encoding for potassium channels. At least for five of the 20 different SNPs detected in one study, there were no apparent differences in their frequencies between the Caucasian and the Japanese populations.[206-208] However, a recent population-based analysis has reported that the incidence of hereditary LQTS in Japan seems comparable to that in western countries.[209] Although the genotypes were mainly LQT1 and LQT2, LQT3 and other types were rare. Mutations found in Japanese LQTS families were found to be mostly novel

and different compared with those reported in other ethnic groups or countries. Another study suggested that the prevalence of LQTS may be as high as 1 in 1164 Japanese school children.[210] Genetic screening of the symptomatic Brugada syndrome and suspected cases revealed SCN5A mutations in approximately 12%. A case of idiopathic ventricular fibrillation was found to have a novel mutation in SCN5A, in which the expressed current showed marked suppression of channel function.

The significance of this global heterogeneity in the frequency of alleles that may influence drug response lies in the facts that: (i) there is an increasing trend towards globalisation of drug development with clinical trials often conducted in geographical populations which may be distant and not the ultimate target of the drug; (ii) more and more of the new drugs are found to be substrates of polymorphic drug metabolising enzymes and/or targeted towards receptors that are polymorphically expressed; and (iii) that modern drugs are more potent with narrow therapeutic indices, with relatively small differences between therapeutic and toxic concentrations in an individual. Inter-ethnic differences in responses to a number of drugs are well known.

The ICH guideline[200] on 'Ethnic Factors in the Acceptability of Foreign Clinical Data', recommends the sponsors and the regional regulatory authority in a new region to assess an application for the ability to extrapolate to the new region, those parts of the application based on studies from the foreign region. To this end, it is recommended that the submission should include: (i) adequate characterisation of pharmacokinetics, pharmacodynamics, dose-response, efficacy and safety in the population of the foreign region; and (ii) characterisation of pharmacokinetics, pharmacodynamics and dose-response in the new region. The guideline recognises the role of genetic factors, and the slope of the dose-response curve, in determining whether the drug is likely to show significant ethnic differences during clinical use. When inter-ethnic differences are anticipated, additional studies may be required.

7. Pharmacogenetic Strategies in Drug Development

7.1 Current Pharmacogenetic Testing in Clinical Trials

To comply with regulatory guidelines, sponsors of NCEs already conduct formal phase I studies to characterise genetic influences on the pharmacokinetics of the NCEs. Unfortunately however, the findings are rarely carried forward to improving the designs and inclusion criteria of phase II dose-finding or phase III pivotal studies.

Genotyping is most unusual in phase II dose-finding studies and this deficiency adversely influences the selection of the most appropriate dose for phase III pivotal studies. Whereas most ultra-rapid metabolisers of CYP2D6 require doses of 300–500 mg/day (even higher in rare individuals) of nortriptyline, PMs need only 20–30 mg/day for therapeutic effect with a whole range of dosages between these two extremes.[211] A recent study of 23 patients characterised for their CYP2D6 status has shown that to maintain the plasma concentrations of perhexiline within the therapeutic, non-toxic range (0.15–0.60 mg/L), PMs require a daily dose of 10–25mg while the corresponding requirements for the EMs and ultra-rapid EMs are 100–250mg and 300–500mg, respectively.[212] Given the common practice of recommending the highest tolerated dosages to maximise efficacy, it is not surprising that during the post-marketing period, downward dosage requirements are a norm for many drugs.[197]

Patients in phase III studies are seldom, if ever, genotyped. Even those that withdraw from the studies because of failure of efficacy or development of a serious adverse drug reaction do not attract any further attention.

7.2 Future Pharmacogenetic Trends in Clinical Trials

The industry is now investing an enormous effort and resources aimed at integrating pharmacogenetics across the whole of the drug development programme. In order to explore the role of pharmacogenetics in drug response, sponsors are now including a genetic extension to the usual protocols of clinical trials, which would allow them to collect

and store blood samples for future genotyping. Patients would be required to give informed consent for the main study protocol and would be encouraged to give a separate consent to being included in the genetic extension of the study. They will have the option to refuse consent to this if they so desire without prejudicing their enrolment in the main study.

The blood samples of those consenting would be stored and analysed at a later date for a genotype profile of the patients. At the completion of studies, an analysis of phenotype/genotype relationship may provide a genetic marker associated with toxicity or failure to respond.

7.3 Selective Genotyping/Phenotyping in Clinical Trials

While a genome-wide scan of all patients enrolled in clinical trials is not currently practical, an ethically acceptable and potentially productive approach is to phenotype or genotype patients only for specific traits (those relevant to the pharmacology of the drug). This would allow intensive study of specially targeted subjects/patients enrolled in phase I/III studies. This approach has the benefits of providing data on random trial population that is not selected by pre-screening for genotype and also a more direct evidence linking genotype with pharmacology and clinical outcomes of primary interest.

This approach is also highly cost effective. Short of phenotyping or genotyping the entire population randomised into clinical trials, it is possible to obtain valuable data from intensive genetic and pharmacological studies of specially targeted populations as shown in table VII. Even on a more limited scale, valuable information for hypothesis generation can be obtained from appropriate *post hoc* genetic and pharmacokinetic studies in the individuals who withdraw from clinical trials due to type A adverse events or lack of efficacy. However, it is an unfortunate fact that these individuals, once withdrawn, attract little attention of the sponsors or the investigators of the clinical trials. In the context of drug-induced QT interval prolongation, individuals who are outliers in terms of categorical responses (QTc interval prolongation of ≥60ms above the baseline or those who develop a QTc interval of ≥500ms) to normal dosages can be genotyped and compared with those who do not.

Table VII. Subjects/patients specially targeted for genotype/phenotype and intensive pharmacological studies

Genotype/phenotype all individuals in:
Pharmacokinetic and pharmacodynamic studies
Drug interaction studies
Studies in special populations
Genotype/phenotype all individuals in dose-response studies
These studies should be large enough to include the whole range of variability in sufficient numbers
Genotype/phenotype those who:
Are outliers in phase I/II studies
Withdraw from phase III studies due to failure of efficacy
Withdraw from any study due to type A adverse events
Intensive pharmacological studies in those who:
Are outliers in phase I/II studies
Withdraw from phase III studies due to failure of efficacy
Withdraw from any study due to type A adverse events

A simplified and rapid detection assay for genetic polymorphisms influencing drug metabolising enzymes has been reported recently.[213] This PCR-based high-throughput assay is capable of simultaneously detecting SNPs in various drug metabolising enzymes such as CYP2A6, CYP2B6, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A5, NAT2, TPMT, UGT1A1 (UDP-glucuronosyltransferase) and MDR1 as well as a number of pharmacological targets. A new fluorescence PCR strategy has also been reported recently for CYP2C9 and MDR1 alleles.[214] For potassium channels, techniques are already being developed for rapid analysis of the entire *KCNQ1* and *KCNH2* genes and the protein encoding part of the *KCNE1* and *KCNE2* genes, thus allowing for rapid identification of LQT gene defects.[215]

7.4 Limitations of Pharmacogenetics During Drug Development

With respect to the application of pharmacogenetics in clinical trials and routine clinical practice, there are a number of unresolved regulatory and clinical issues. One approach that has been advocated, based on candidate gene information, is to conduct phase III studies of enrichment design. This design seeks to randomise only patients of selected genotype[216] and exclude those who are unlikely to

benefit or are likely to develop adverse reactions. This design is claimed to: (i) provide a more robust evidence of efficacy with smaller number of patients in studies of shorter duration; (ii) increase subject safety; and (iii) eliminate the need to monitor drug plasma concentrations.[217]

From a regulatory perspective, enrichment design studies, however, will not provide adequate safety data even in patients randomised by genotype, if the studies are small and of short duration. Excluding individuals who may not respond therapeutically or who carry some mutations potentially responsible for toxicity will almost certainly lead to: (i) an overestimation of efficacy by excluding potential therapeutic failures; (ii) an erroneous assessment of population variability; and (iii) underestimation of potential risks by excluding those most at risk (e.g. possibly in the SWORD study referred to earlier). It is even uncertain which individuals would be candidates for exclusion because very few drugs are metabolised by a single enzyme and there are many alleles of varying functional activity at a given locus. Furthermore, exclusion by pre-screening genotyping will be ineffective if: (i) the pharmacology of the parent drug and its metabolite contribute to therapeutic effect; and (ii) the drug response has a shallow concentration-response curve. More importantly, they limit the scope for investigating the safety and efficacy of alternative dose schedules in genotypes excluded from the studies. Prospective genotyping should be employed to ensure the inclusion of important phenotype subgroups.[216]

Probably the most important concern when integrating pharmacogenetics in drug development is detection of mutations associated with rare or delayed adverse effects (which are usually the serious ones). To truly harness the potential benefits, pharmacogenetic studies will have to continue for well beyond the approval of a drug into the post-marketing surveillance period.

8. Conclusions

The consequences resulting from drug-drug or drug-disease interactions and promotion of an inappropriately high dosage emphasise the complex interaction between genetics and many non-genetic factors in improving and individualising therapy.

There is little doubt that carefully planned integration of pharmacogenetics in clinical trials is well overdue. If the data provides robust evidence for the utility of routine genotyping for the drug concerned, the appropriate recommendation can be included in the prescribing information. This has already been done for a number of drugs such as thioridazine, perphenazine, celecoxib, escitalopram and, most recently, atomoxetine.

Unfortunately, such data exist for very few drugs and it may be too early to routinely genotype patients before prescribing even those drugs that display polymorphic metabolism. Integration of pharmacogenetics in clinical trials and post-marketing surveillance of drugs should generate data of clinical value. When the data are robust and genotyping tests readily available, there is a great opportunity for not only improving the risk/benefit of many drugs, but also greatly reducing the economic and healthcare burdens arising from adverse drug reactions.

Whether or not the anticipated advances resulting from genome-wide pharmacogenetic studies translate into safe and effective individualised therapy remains to be seen. In principle, genotype-based prescribing ought to be more effective in improving response rates, and decreasing adverse effects. However, there would have to be a considerable change in drug promotion and prescribing cultures.

The benefits of pharmacogenetics will be squandered if dosages promoted are inappropriately high and drug interactions are not fully characterised by sponsors and appreciated by prescribing physicians. Equally, one needs to be confident that pharmaceutical companies will be ready to depart from the traditional paradigm of ever enlarging the target population of their drugs; pharmacogenetically driven prescribing will have the reverse effect without necessarily assuring a longer market life for a drug.

Physicians responsible for prescribing medications and monitoring patients will have their responsibilities too. Pharmacogenetic targeting of drugs will be without any benefits if prescribers do not comply with contraindications regarding co-prescription of interacting drugs and recommendations on appropriate monitoring of patients. Recent studies on continued co-prescription of cisapride with contraindicated drugs (ranging from 5.2% in one

study to 33.6% in another) despite strong regulatory warnings is a typical example.[218,219] Ultimately, in July 2000, cisapride was withdrawn from many markets while its use very restricted in others. Similarly, there is a report of patients on high-dose neuroleptics not being monitored with regular ECGs despite national guidelines recommending a regular ECG monitoring of these patients.[220]

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