LEADING ARTICLE

Drug Safety 2004; 27 (15): 1171-1184
0114-5916/04/0015-1171/$31.00/0

Linking Pharmacovigilance with Pharmacogenetics

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Abstract

The ability to identify individuals who are susceptible to adverse drug reactions (ADRs) has the potential to reduce the personal and population costs of drug-related morbidity. Some individuals may show an increased susceptibility to certain ADRs through genetic polymorphisms that alter their responses to various drugs.

We wished to establish a methodology that would be acceptable to members of the general population and that would enable estimation of the risks that specific genetic factors confer on susceptibility to specific ADRs. Buccal swabs were selected as a minimally invasive method to obtain cells for DNA extraction. We wished to determine whether DNA of sufficient quantity and quality could be obtained to enable genotyping for two different polymorphic genes that code for enzymes that are widely involved in drug disposition.

This article describes a small pilot study of methodology developed in the New Zealand Intensive Medicines Monitoring Programme (IMMP) to link prescription event monitoring (PEM) studies with pharmacogenetics. The methodology involves a nested case-control study design to investigate whether patients with genetic variants in P-glycoprotein (P-gp) and cytochrome P450 (CYP) 2C9 are more susceptible to psychiatric or visual disturbances following cyclo-oxygenase-2 inhibitor use (ADR signals identified in the IMMP database) than matched control patients taking the medication without experiencing any ADRs.

It was concluded that the use of buccal swabs is acceptable to patients and provides DNA of sufficient quantity and quality for genotyping. Although no differences in the distribution of genotypes in the case and control populations were found in this small study, case-control studies investigating genetic risks for

ADRs using drug cohorts from PEM studies are possible, and there are several areas where population-based studies of genetic risk factors for ADRs are needed.

Examples are discussed where research in large populations is required urgently. These are: (i) genetic variations affecting P-gp function; (ii) variations affecting drugs metabolised by CYP2C9 and other polymorphic CYP enzymes; (iii) genetic variation in β-adrenergic receptors and adverse outcomes from β-adrenoceptor agonist therapy; and (iv) genetic variation in cardiac cell membrane potassium channels and their association with long QT syndromes and serious cardiac dysrhythmias.

Such studies will help to identify factors that increase the risk of unwanted outcomes from drug therapy. They will also help to establish in what circumstances genotyping should be performed prior to commencing drug treatment and in tailoring drug treatment for individual patients.

The ability to identify individuals who are susceptible to adverse drug reactions (ADRs) has the potential to reduce the personal and population costs of drug-related morbidity. Genetic polymorphisms may alter the pharmacokinetics or pharmacodynamics of a given drug in ways that affect the response to the drug concerned and create an increased susceptibility to certain ADRs.

This article is based on a presentation made as part of a symposium on 'Risk Factors in Pharmacogenetics' during the Annual Scientific Meeting of the International Society of Pharmacovigilance (ISoP) held in Marrakesh, Morocco in October 2003. The paper describes the methodology developed in the New Zealand Intensive Medicines Monitoring Programme (IMMP) aimed at linking pharmacoepidemiology with pharmacogenetics in order to estimate the degree to which specific genetic factors alter susceptibility to some ADRs, and the results from a small pilot study are given. Future directions are discussed and examples considered of important areas where genetic studies in prescription event monitoring (PEM) cohorts are opportune and are underway using the IMMP cohorts and the cohorts from the Drug Safety Research Unit (DSRU) in England.

1. The New Zealand Intensive Medicines Monitoring Programme

The New Zealand IMMP began in 1977[1] and was designed to play a significant part in monitoring for previously unrecognised ADRs.[2] Generally, medicines are selected for inclusion in the IMMP if they are of a new class and are likely to be used widely. A near complete record is obtained of all patients who have been prescribed these medicines by recording all prescriptions dispensed throughout the whole monitoring period. Monitoring is usually continued for 4–5 years, which is sufficient time to accumulate a cohort of adequate size for epidemiological investigations. The methodology utilises the technique now known as PEM. A similar methodology is used by the DSRU in England.[3] In the IMMP, prescription records for the monitored medicines are received from dispensing pharmacists in community and hospital pharmacies throughout the country. The details recorded in each drug cohort includes prescribers' names and addresses; the names, addresses, dates of birth and sex of patients who have been prescribed the medicines; details of each prescription (medicine, dose, date and quantity dispensed) and indication for treatment.

Adverse events are defined as any untoward experience whether or not the event is thought to be drug related.[4] These events include changes in a pre-existing condition or abnormally changed laboratory values and all deaths. The method is based on sending questionnaires to the prescribers at regular intervals following receipt and data entry of new prescription details. Doctors are requested to record all events noted in the patient records from the time of commencement of the drug or the latest prescription, whichever is appropriate. These forms are computer generated and include all prescribing and patient details and the first and most recent dispensing dates for reference. Doctors may also record events on IMMP duplicate prescriptions or send spontaneous reports on the standard 'yellow' card.

Because the IMMP databases include records of monitored medicines over a known time period, including those from patients who have *not* experienced an adverse event, the incidence of events can be calculated. The details of age, sex, dose, treatment duration and indication for treatment allow the identification of risk factors using cases and controls from the individual cohorts. The study of risk factors can include genetic variation that may increase susceptibility to the adverse event.

2. Investigations into Genetic Factors Affecting Drug Response

The field of research that uses large-scale, systematic genomic approaches to identify multiple drug response marker genes is known as pharmacogenomics. It is likely that this approach will be increasingly used to identify genetic factors that may influence an individual's response to a particular drug and thus assist in determining the optimum drug regimen for that individual. Pharmacogenomic approaches are also increasingly being used by the drug industry to predict potential problem areas during drug development.[5] However, to date, most investigations have focused on variability in single genes predicted to contribute to inter-individual differences in drug responses (i.e. pharmacogenetics).

Several pharmacogenetic studies have provided evidence that in specific populations some subjects have particular single nucleotide polymorphisms (SNPs) or a set of closely linked SNPs on a chromosome (haplotypes) that influence their response to specific drugs.[6-12] It is thus important to identify whether specific SNPs or haplotypes may lead to clinically important differences in drug response and ADRs. We have previously demonstrated that pharmacogenetic studies are feasible in patients reported with adverse reactions.[13] However, because these earlier studies required physicians to collect blood samples for DNA extraction, the number of patients willing to participate, and so subject themselves to an invasive sampling procedure, was small. In the pilot investigation described in this article, we wished to assess whether buccal cell samples could yield enough DNA of sufficient quality for genotyping in larger population studies.

As part of the normal monitoring of rofecoxib and celecoxib (cyclo-oxygenase [COX]-2 inhibitors), the New Zealand IMMP has received a number of reports of visual[14] and psychiatric[15] disturbances that appear after COX-2 inhibitor use and resolve following withdrawal.[14] The strong evidence of causality from these reports and details of the types of visual and psychiatric disturbances associated with COX-2 inhibitor use have been published.[14,15] We hypothesised that there may be identifiable genetic factors that increased the risk of such events in these patients. The pilot study was therefore designed to investigate whether selected genetic polymorphisms contribute to the risk of developing visual or psychiatric disturbances following the use of rofecoxib or celecoxib.

3. The Pilot Study

This study investigated the potential role of genetic polymorphisms in P-glycoprotein (P-gp) and cytochrome P450 (CYP) 2C9 in the visual and psychiatric disturbances experienced by some patients prescribed COX-2 inhibitors.

National ethical approval was given for the use of the IMMP data in this study and for the sampling and genotyping of patients.

3.1 Rationale

For most drug-related visual distubances and for psychiatric disturbances to occur, the drug concerned must cross the blood-brain barrier. Several variants of the transporter protein P-gp show reduced ability to extrude drugs and other substrates from cells.[12,16] It is not yet known whether COX-2 inhibitors are substrates for P-gp, but if this is the case, the presence of variant P-gp forms may lead to an increase in drug absorption or movement across the blood-brain barrier with resulting high plasma or brain concentrations; should the distribution of poor P-gp transport efflux genotypes be greater in these cases, this would highlight the need for *in vitro* studies to confirm whether the COX-2 inhibitors are substrates for P-gp. As prostacyclin plays an important role in the control of retinal blood flow[17] and COX-2 inhibitors reduce prostacyclin synthesis in the vascular endothelium,[18] an altered transport of celecoxib or rofecoxib may contribute to the visual or psychiatric disturbances.

Patients experiencing the events may have inherited a gene that codes for variant CYP2C9 enzymes that have been demonstrated to have a reduced ability to metabolise certain drugs, including celecoxib[19] and some other NSAIDs.[20,21] Therefore, it is possible that genetic variability in proteins involved in the uptake or metabolism of COX-2 inhibitors may contribute to an increased risk of developing visual and/or psychiatric disturbances.

The pilot case-control study was designed, involving patients from the IMMP who were currently taking rofecoxib or celecoxib, to test the hypothesis that variant forms of P-gp and/or CYP2C9 are associated with the rare visual or psychiatric disturbances reported. Patients were genotyped for selected SNPs in the genes encoding P-gp (*ABCB1*) and CYP2C9 (*CYP2C9*). Previous experience using DNA extracted from blood samples yielded a very low number of patients willing to participate.[13] Therefore, it was considered essential for a study of this nature that another method of DNA collection was adopted, and a simple buccal swab sampling method was selected.

The primary aims of the pilot study were: (i) to determine whether pharmacogenetic studies using a minimally invasive method of obtaining DNA are feasible, using a PEM database as a method of recruiting cohorts of patients exposed to the same drug, both with and without specific reactions; and (ii) to determine whether a buccal swab sampling method would enable the collection of sufficient cells from inside the cheek for the extraction of DNA and subsequent *ABCB1* and *CYP2C9* genotyping.

3.2 Methodology

3.2.1 Recruitment of Patients

Subjects experiencing psychiatric or visual disturbances with celecoxib or rofecoxib that were of similar type to those previously reported by the IMMP[14,15] were selected from the IMMP event databases. Patients who were not experiencing any adverse events were selected from the IMMP cohorts as controls. Prescribers were asked to indicate whether patients (cases and controls) were taking specific listed medications (amiodarone, fluconazole, paroxetine, probenecid, sertraline, trimethoprim and cotrimoxazole [trimethoprim/sulfamethoxazole]) that might possibly interact with P-gp or CYP2C9 substrates. None of the patients were

taking these medications during the times that celecoxib or rofecoxib was prescribed.

The control patients were matched by date of birth (within 5 years), sex and geographical location. Where possible, two control patients who were prescribed the same COX-2 inhibitor by the same doctor were matched for each case. For each case, we provided the patient's doctor with the identification of three possible controls from the IMMP cohorts and requested that he/she select the two most appropriate. If the doctor could not identify any suitable controls, we sought controls from other doctors located in the same practice or general area. As some patients had died and others were considered too frail or ill to participate, a total of 37 cases and 91 controls were selected and invited to enrol.

3.2.2 Obtaining the Buccal Cell Samples

General practitioners (GPs) were identified from the IMMP database and contacted by mail with an explanation of the purpose of the investigation and were asked for approval to approach their patient(s). Once the GP's approval had been obtained, patients (37 cases and 91 controls) were contacted by mail with an explanation of the study, invited to participate and asked to provide consent for their participation. The patients were informed that participation would be at no cost to them and would involve making an appointment with their doctor so that a swab could be taken from inside their cheek to obtain a cell sample for DNA extraction.

Once informed consent had been received from the patients (consent was received from 17 cases and 31 controls), the GPs were asked to return additional details of the reported event, concurrent medications and conditions. They were then sent kits for the collection of buccal cell samples (figure 1). These were coded prior to being sent to the GPs so that the subsequent genotyping was blind. The GPs were requested to post the samples, using the reply paid courier packs provided in the kit, to the genetics laboratory for DNA extraction and genotyping. The results were sent to Dunedin, New Zealand, for interpretation and analysis in collaboration with the laboratory in Christchurch, New Zealand.

3.2.3 Genotyping of Samples

Genotyping for selected *CYP2C9* (*1,*2 and *3) and *ABCB1* (3435C>T) variants was performed using methods already established within the Christchurch laboratory.[22,23] The method of buccal cell sampling allows the recovery of sufficient cells from which to isolate DNA. The DNA was extracted using the method described by Richards et al.,[24] coded by number and date and stored at −20°C until analysed.

3.2.4 Analysis of Results

Differences in the frequency of variants between cases and controls and between cases taking celecoxib and rofecoxib were tested using relative risks with confidence intervals. Chi-squared tests were also applied.

Fig. 1. Population genotyping kit sent to general practitioners (GPs) for obtaining buccal cell samples for extracting DNA. The kit contains a cytology brush, a 2mL screw top tube containing sodium hydroxide solution, an instruction sheet for the GP and a prepaid courier pack for sending the sample to the Gene Structure and Function Laboratory, Christchurch School of Medicine and Health Sciences.

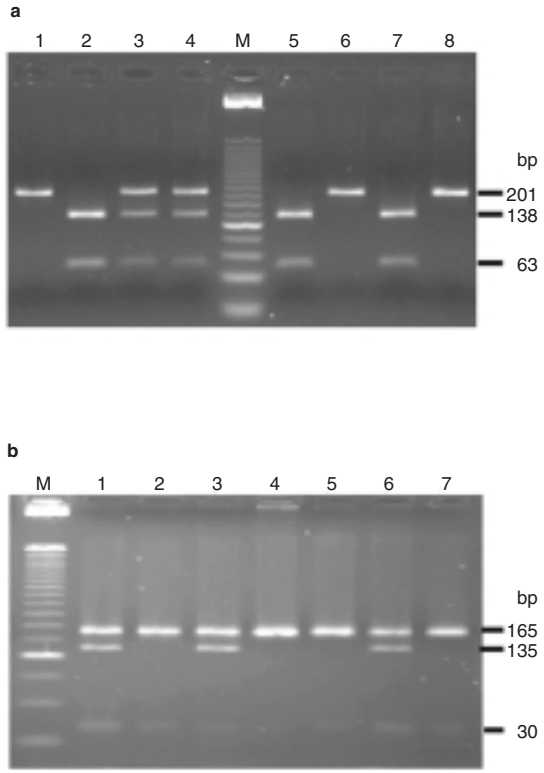

Fig. 2. Genotyping assays for the detection of the *ABCB1* polymorphism 3435C>T and *CYP2C9**3. **(a)** The region of the *ABCB1* gene containing the single nucleotide polymorphism 3435C>T was amplified from patient buccal DNA using polymerase chain reaction (PCR). The resulting 201 base pair (bp) PCR products were digested with the restriction endonuclease *Dpn*II, which only cuts the C allele to yield 138bp and 63bp fragments. Lanes 1, 6 and 8 are homozygous for the T allele; lanes 3 and 4 are heterozygotes, and lanes 2, 5 and 7 are homozygous for the C allele. **(b)** Similarly, DNA samples were screened for the *CYP2C9**2 (representative gel not shown) and *CYP2C9**3 alleles using PCR followed by digestion of the PCR products with different restriction endonucleases. Lanes 1, 3 and 6 are heterozygous for *CYP2C9**3; and lanes 2, 4, 5 and 7 are homozygotes for the wildtype (*CYP2C9**1) allele. No *CYP2C9**3 homozygotes were found in the pilot study. The PCR fragments for both assays were separated on 3% agarose and sized using a 25bp DNA ladder (M).

3.2.5 Feedback to Patients and General Practitioners

Letters were sent to the GPs and patients informing them of the individual results. Patient letters included a recommendation that if the patients wished to discuss the wider implication of their results with respect to other medicines that they might be prescribed in the future, they should contact their GP. In addition to the letters containing their individual patients' results, the GPs were also sent a document with an explanation and list of other medicines that might be affected by the genetic variants of CYP2C9 and P-gp that were identified for their patients.

3.3 Results

Of the 37 case and 91 control patients who were approached to participate in the study, genotyping was carried out on 15 case and 24 control patients. Consent forms from the remaining nine patients were received too late for the genotypes to be included in this study. The DNA obtained from buccal cells was of sufficient quality in all samples tested to enable *ABCB1* and *CYP2C9* genotyping (figure 2). In the small sample size genotyped, the distributions of variant genotypes were similar between cases and controls and across drugs (table I).

3.4 Discussion

Genetic polymorphisms may contribute to inter-individual differences in drug response by altering pharmacokinetics (e.g. where drug blood concentrations may be affected by variation in drug-metabolising enzymes or in transport proteins involved in drug uptake) or by altering pharmacodynamics (e.g. where variation in receptors, ion channels or intracellular enzymes or in other cell/tissue components may lead to altered drug response).

There are several studies that indicate that polymorphisms in genes coding for proteins involved in pharmacokinetic and pharmacodynamic processes influence the effects of administered drugs, thereby altering susceptibility to ADRs. However, most of these studies have compared small, selected samples of the population, such as those with or without particular variant genes (e.g. studies comparing 'extensive metaboliser' with 'poor metaboliser' populations) and using short-

Table I. Results from genotyping cases taking celecoxib or rofecoxib and experiencing psychiatric or visual adverse effects and controls taking celecoxib or rofecoxib without experiencing psychiatric or visual adverse effects[a]

Event class	Adverse effect	Gene	Case genotype	Control genotype 1 (genotype 2)
Celecoxib				
Psychiatric	Depression	*ABCB1*	C/T	C/T
		CYP2C9	*1*1	*1*1
	Depression	*ABCB1*	C/T	C/T
		CYP2C	*1*2	*1*1
	Depression	*ABCB1*	**T/T**	C/T
		CYP2C	*1*1	*1*1
	Hallucinations	*ABCB1*	C/C	**T/T**
		CYP2C9	*1*2	***1*3**
	Suicidal ideation	*ABCB1*	**T/T**	C/C
		CYP2C9	*1*1	*1*1
Visual	Abnormal vision	*ABCB1*	**T/T**	**T/T (**C/T)
		CYP2C9	*1*2	*1*1 (*1*2)
	Visual field defect	*ABCB1*	C/T	**T/T**
		CYP2C9	*1*1	*1*1
Rofecoxib				
Psychiatric	Confusion	*ABCB1*	C/T	C/T
		CYP2C9	1/1	*1*2
	Depression	*ABCB1*	**T/T**	C/T (C/C)
		CYP2C9	***1*3**	***1*3 (***1*1)
	Restlessness	*ABCB1*	C/T	C/T (**T/T)**
		CYP2C9	*1*1	*1*1 (*1*1)
	Low concentration	*ABCB1*	C/T	C/T
		CYP2C9	*1*1	*1*2
	Delirium	*ABCB1*	C/C	**T/T**
		CYP2C9	***1*3**	***1*3**
	Anxiety	*ABCB1*	**T/T**	C/T
		CYP2C9	*1*1	***1*3**
Visual	Blurred vision	*ABCB1*	C/T	C/T
		CYP2C9	*1*1	*1*1
	Teichopsia	*ABCB1*	C/C	C/C (C/T)
		CYP2C9	*1*1	*1*1 (*1*2)

a Control genotypes 1 and 2 indicate matched controls. A further five controls were genotyped that had been matched to cases that did not consent to genotyping. The genotypes of these control patients were: C/C *1*1; C/T *1*1; CT/*1*2; TT/*1*1; and TT/*1*3. In only four cases were two matched controls per case able to be obtained for genotyping. Allelic variants that may lead to altered plasma or CNS levels of substrates are shown in bold.

term dosage regimens.[25] Therefore, it should be helpful to use epidemiological methods to estimate the risk of increased susceptibility to ADRs due to pharmacogenetic factors, where comparisons are made between matched populations of patients with the selected adverse effect (cases) and those without any adverse effect (controls).

In order to test the feasibility of such a population study, where the minimally invasive method of using buccal swabs was used for obtaining DNA for genotyping, we conducted a small pilot study, using case-control methodology. The pilot study investigated whether selected SNPs within *ABCB1* and *CYP2C9*, which might influence the pharmacokinet-

ics of the COX-2 inhibitors by (a) altering the passage of drugs through biological membranes and/or (b) altering the metabolism of the drugs, were associated with an increase in the susceptibility of patients to psychiatric and visual disturbance. In the case and control samples genotyped (table I), several variants were found that could potentially lead to altered substrate transport by P-gp or metabolism by CYP2C9.

The results from this study suggest that genetic variability in P-gp or CYP2C9 may not contribute to the rare psychiatric or visual disturbances associated with celecoxib and rofecoxib. However, as the sample size in this pilot study was small, there is a possibility of type II error that would be minimised in a large population study. Furthermore, we only examined one of the many SNPs that have been described in *ABCB1* and we cannot rule out the possibility that genetic variability in P-gp does impact on these COX-2 inhibitor-related ADRs. Two recent studies[26,27] investigating sequence diversity in *ABCB1* have identified over 30 different SNP haplotypes of this gene, of which 14 were found to include 3435C>T.[27] Results from these haplotyping studies strongly suggest that although screening for 3435C>T is a good starting point, to fully assess the potential impact of P-gp genetic variability on drug response it is necessary to screen patients for haplotypes rather than individual SNPs.

Despite an apparent lack of association between selected P-gp and CYP2C9 variants and COX-2 inhibitor-induced psychiatric or visual disturbances in this study, we have demonstrated that a less invasive method for obtaining DNA than using peripheral blood samples is acceptable and that genetic studies can be linked with pharmacovigilance data to provide information on genetic variation that may alter susceptibility to ADRs.

Because of health and other reasons, including some recent deaths, we were requested by the GPs not to approach several of the patients selected as cases or controls. However, the number of case patients (15 of the 37 approached [41%]) and control patients (24 of the 91 approached [26%]) who consented to participate and were genotyped was greatly in excess of those who agreed to participate in our earlier genotyping study where DNA was extracted from peripheral blood.[13] A lower percentage of controls than cases agreed to participate. This may have been because of the lack of direct interest due to not having experienced an adverse reaction, in spite of being supplied with the information that knowledge of their genotypes might assist in the choice of other types of medications that could be needed in the future.

Because of time constraints, follow-up of patients who had not returned consent forms was not carried out. However, as several patient consent forms were received following the closing date for the study, it is likely that telephone follow-up would have improved the return rate. It is also possible that methods other than telephone follow-up could increase the return rate. In future investigations we intend to approach patients by asking the prescribers to co-sign and to forward the letters, describing the intention of the study and inviting participation, to their patients. This was an Ethics Committee requirement for a future study and it may also improve patient acceptance of the study objectives. Although we informed patients that doctors' fees would be paid by the IMMP, it may be possible that the offer of a financial reward to patients for participation would increase enrolment. However, in some countries, including New Zealand, the ethics of offering a financial incentive to participate would be subject to intense scrutiny.

Linking pharmacogenetics and pharmacovigilance in epidemiological studies that use non-invasive sampling methods has the potential to clarify the population risk of particular variant genes in increasing susceptibility to particular ADRs. PEM databases, as in the DSRU in England and the

IMMP in New Zealand, have cohorts drawn from the general population who have been prescribed the monitored drug(s), with information on outcomes of therapy and other patient details. Patients with and without particular adverse events can be identified and their genotypes compared. We concur with the opinion expressed by Tucker[28] that "the challenge now is to assemble large, prospective, multidisciplinary, multicentre projects". Case-control studies designed to assess the risk of selected variant SNPs and haplotypes in predisposing towards adverse outcomes from drug therapy in the 'real world' of clinical practice will contribute to the evidence obtained from future large-scale prospective studies.

4. Future Directions

Examples of genes that are known to influence drug responses are provided in several reports.[28] We have selected examples below where research on the population risk of genetic variability in influencing therapeutic effect and the susceptibility to ADRs is underway or is needed urgently. Further detail is provided on genetic variability in P-gp and CYP2C9: examples that may impact on pharmacokinetics. Two further examples where genetic variation may affect pharmacodynamics are also discussed.

4.1 Pharmacokinetic Variation

4.1.1 Variations Affecting P-Glycoprotein Function

P-gp is a transmembrane protein efflux pump encoded by the *ABCB1* gene (formerly known as *MDR1*). As discussed earlier, P-gp functions to extrude many drugs and other 'foreign' lipophilic compounds from the cells back into the extracellular space, thereby preventing substrates from accumulating in critical organs such as the brain.[29,30] P-gp has been isolated in endothelial cells of the gut, kidney, liver and other organs.[12] Because of this, it has an important role in determining the extent of absorption, distribution and elimination of many drugs,[31,32] and this may contribute to serious ADRs or interactions.[33,34] In addition, P-gp expression in endothelial cells of the CNS prevents the penetration of several drugs across the blood-brain barrier.[12,35,36] The *ABCB1* gene is highly polymorphic and many variant forms of P-gp show significantly reduced transporter function. One of the most common of these variant forms has a mutation (3435C>T) in exon 26 of the *ABCB1* gene. Subjects with the 3435T allele produce P-gp with altered substrate transport function compared with subjects with the 3435C allele. In some studies the 3435T allele has been associated with increased P-gp function, whereas in other studies this variant appears to encode a protein with impaired activity.[37-39] It is possible that these discrepancies in relation to 3435T reflect differences in haplotypes. Our pilot study sought to determine whether this known *ABCB1* mutation is associated with increased susceptibility to developing visual or psychiatric effects in patients receiving celecoxib or rofecoxib.

P-gp is known to transport many anticancer drugs[12,16] and several other clinically important drugs including some statins (atorvastatin, lovastatin),[40] digoxin,[41,42] some calcium channel antagonists (diltiazem, verapamil),[43] the angiotensin II receptor antagonist losartan,[44,45] erythromycin,[40] fexofenadine,[46] nortriptyline[47] and phenytoin.[33] These examples have been selected because, in some cases, studies (referenced after drug names) have associated P-gp genetic polymorphisms with adverse effects or potentially important effects on pharmacokinetics. Therefore, they are potentially important candidate drugs for population studies aimed at assessing the association of variant P-gp and ADRs. More complete lists of drugs that are known substrates for P-gp may be obtained from Schwab et al.[16] and Marzolini et al.[12] It is important that the risks of genetic variation in the function of P-gp on adverse outcomes of drugs, including

adverse interactions between drugs, are determined in large populations.

4.1.2 Variations Affecting Drugs Metabolised by Cytochrome P450 (CYP) 2C9 and Other Polymorphic CYP Enzymes

The CYP enzymes are a group of haemoproteins responsible for catalysing the phase I metabolism of almost all drugs.[48] Over recent years emphasis has been placed on the clinical implications of genetic variability in CYP2D6 because this enzyme is involved in the metabolism of a wide range of currently prescribed drugs.[49]

CYP2C9 is one of the four known members of the human 2C family and, although it has received less attention than *CYP2D6*, it is one of the most abundant CYPs found in the human liver.[50-55] *CYP2C9* accounts for about 20% of the total liver CYP content and is responsible for the metabolic clearance of about 10% of all drugs currently in use.[20,54]

Several polymorphisms have been observed in the CYP2C9 enzyme.[54,56] Of these polymorphisms, two alleles referred to as *CYP2C9**2 and *CYP2C9**3 lead to the reduced function of the enzyme. Mutations that alter amino acids in the substrate recognition sites of CYP2C9 proteins (such as the *3 allele) can markedly reduce the metabolic ability of the enzyme[54,56] and have clinically significant effects on the pharmacokinetics of some drugs.[57] Although our small pilot study did not confirm that visual and psychiatric adverse reactions are associated with reduced CYP2C9 metabolism, it remains possible that this is an important risk for susceptibility to adverse gastrointestinal events, including gastrointestinal bleeding and ulceration that may follow celecoxib and other NSAID use. The risk that genetic variants of CYP enzymes pose for NSAID and other drug use needs to be assessed using case-control studies in large populations. In the UK, the DSRU is commencing a study to investigate whether patients identified as being hetero- or homozygous for *CYP2C9**3 have a higher risk of alimentary system ADRs associated with celecoxib, compared with patients identified as carriers for *CYP2C9* alleles other than *CYP2C9**3 (Layton D, DSRU, personal communication).

4.2 Pharmacodynamic Variation

4.2.1 Genetic Variation in β-Adrenergic Receptors: Association with Adverse Outcomes from β-Adrenoceptor Agonist Therapy

β-Adrenoceptor agonists are the most commonly used medication for rapid bronchodilation during acute asthma episodes. However, serious adverse outcomes may occur with the use of β-agonists, including long-acting β-agonists.[58-60] These may involve the respiratory, cardiovascular or neuromuscular systems.

Of particular note are adverse respiratory outcomes that occur following the use of long-acting β-agonists, such as salmeterol and formoterol.[61] When these drugs are used regularly, some patients find that they do not get their usual relief from short-acting β-agonist therapy. In this respect, there is conclusive evidence that subsensitivity of β_2-adrenergic receptors develops when long-acting β-agonists are used regularly.[62] A number of SNPs in the β_2-adrenoceptor (*ADRB2*) gene have been described.[63] To date, the most important of these appear to confer variation in both the acute responses to β_2-receptor stimulation and in their propensity for desensitisation and downregulation.[63-65]

Lima et al.[66] reported that bronchodilator responses, resulting from acute administration of salbutamol, are enhanced in patients with an arginine substitution in position 16 of the *ADRB2* gene (Gly>Arg polymorphism). This raised the possibility that the continuous use of short-acting β_2-agonists or of long-acting β-agonists such as salmeterol or formoterol may result in downregulation of the β_2-receptors. Israel et al.[8] and Taylor et al.[58] have shown a consistent relationship between the presence of the Arg-16 allele and deterioration in lung

function during regular treatment with the short-acting β-agonist salbutamol. In the study by Taylor et al.,[58] the frequency of exacerbations of asthma was increased in patients who were homozygous for the Arg-16 allele. In addition, the benefits of regular salmeterol treatment were much less in homozygous Arg-16 patients than in the other genotypes. Therefore, there is strong evidence to support the hypothesis that adverse outcomes may be determined by *ADRB2* genotype. Research is planned in the IMMP to determine the risk for the development of adverse outcomes from specific genetic variations following treatment with long-acting β-agonists.

As in the case of *ABCB1*, although individual SNPs in *ADRB2* may influence drug response, haplotypes may be more clinically relevant. Haplotype analysis of the *ADRB2* gene and its promoter region takes into account the possibility that combinations of SNPs may confer particular phenotypic effects.[67] In New Zealand, the most common functionally relevant haplotypes are 4/4, 2/2, 4/6, 2/4 and 2/6[68] and the frequency of specific haplotypes is sufficiently common that they can be identified in modestly sized groups of subjects. Therefore, genetic analyses in populations using short- and long-acting β-agonists should provide evidence relating adverse outcomes in patients taking inhaled long-acting β-agonists to combination haplotypes, as well as to the important SNPs. In spite of advances in asthma management, asthma morbidity and mortality remain high. In view of this and because of concerns regarding serious adverse outcomes following long-acting β-agonist use,[60] determination in populations of specific genetic variation in β-receptors and the risks following β-agonist use is needed urgently.

4.2.2 Genetic Variation in Cardiac Cell Membrane Potassium Channels: Association with Long QT Syndromes and Serious Cardiac Dysrhythmias

Several drugs are associated with the occurrence of rare but potentially serious cardiac dysrhythmias. Alteration in the rate of cardiac cell membrane repolarisation is reflected by a prolongation of the QT interval, a common cause of serious dysrhythmias including torsade de pointes.[69] The two most common types of inherited long QT syndromes (LQT1, LQT2) are due to variant genes that affect the function of cardiac cell membrane potassium channels: *KCNQ1* (formerly known as *KVLQT1*) and *KCNH2* (formerly known as *HERG*). A third, less common, type of long QT syndrome (LQT3) is due to variants of a gene (*SCN5A*) that codes for sodium channels (I_{Na}).[70]

K^+ currents that pass through specialised K^+ channels are the predominant outward currents in heart muscle involved in membrane repolarisation. The most predominant K^+ current responsible for ventricular repolarisation is known as I_K, of which there are two distinct components: I_{Kr} and I_{Ks} (rapid and slow). Several mutations exist in the genes that code for the potassium channel proteins, KCNQ1 and KCNH2. Individuals carrying these mutant genes have reduced function in transporting K^+ from the cells and are therefore susceptible to long QT interval and, when taking drugs that also potentially inhibit I_K currents, are at a high risk of QT prolongation and serious arrhythmias.[71] Lists of such drugs may be found in the literature[70,72] and Internet sites (e.g. http://www.torsades.org), which are updated as new examples come to light. They include well known examples, such as terfenadine, cisapride, antipsychotic medications and also drugs such as sibutramine and dexfenfluramine,[73] where investigations in animals have provided an indication of the potential to prolong QT interval.

More research is needed to identify further drugs associated with this serious problem and to measure the risk of a long corrected QT interval (QTc) with these drugs in large populations. Currently, the DSRU, in collaboration with St. George's Hospital Medical School (London, UK), is conducting such research (i.e. the DARE [Drug-Induced Arrhythmia

Risk Evaluation] study). This research will systematically document and follow up incident cases of drug-induced ventricular arrhythmia in England and compare them with matched controls. In addition, blood samples from case and control patients will be analysed for mutations and polymorphisms of the potassium and sodium channel genes implicated in the long QT and similar syndromes.[74] Results from this and similar research should greatly increase the knowledge of this rare, but potentially serious, cardiac risk of some medicines.

5. Conclusion

In spite of a number of association studies where a link has been demonstrated between particular polymorphisms in genes and drug response, there has been a lack of large population studies where other factors have been controlled for that may modify drug responses. The pilot study outlined in this article demonstrates that case-control studies investigating the risk of genetic variation in the wider population, using databases held by centres undertaking PEM studies, are possible. PEM databases, such as those held by the IMMP for New Zealand and the DSRU for England, are able to identify cases and matched controls. Application of buccal cell sampling, as described in our pilot study, should enhance the recruitment of patients to such studies and enable the collection of sufficient DNA for successful genotyping.

There are many areas of concern in pharmacogenetics that are urgently in need of population studies, including those outlined in this article. Such studies will contribute to the evidence on whether or not selected genetic factors represent a risk in the population of unwanted outcomes from drug therapy. Furthermore, the studies will help to establish in which circumstances genotyping should be performed prior to commencing drug treatment and may also help in tailoring drug treatment for individual patients.

Acknowledgements

A generous grant from the New Zealand Pharmacy Education and Research Foundation has enabled us to undertake the experimental work described in this article. We are also grateful to Janelle Ashton for invaluable assistance with this study and to Deborah Layton and Andrew Boshier for further information on pharmacogenetic studies initiated in the Drug Safety Research Unit. Dr Kennedy is a Senior Research Fellow of the Health Research Council of New Zealand. Dr Roberts is the recipient of a Health Sciences Career Development Award of the University of Otago. The New Zealand Intensive Medicines Monitoring Programme (IMMP) has been supported financially by Medsafe of the NZ Ministry of Health and some pharmaceutical companies, in particular, Merck Research Laboratories, USA.

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