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The interface between pharmacoepidemiology and pharmacogenetics

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Abstract

One of the most challenging areas of research in pharmacoepidemiology is to understand why individuals respond differently to drug therapy, both in terms of beneficial and adverse effects. Pharmacogenetics focuses on the question to what extent variability in genetic make-up is responsible for these observed differences. Pharmacoepidemiologic research can contribute to pharmacogenetics by explaining the observed variability in drug response in 'real life' patient populations with known polymorphisms in their genetic profile. Genetic pharmacoepidemiologists also are interested in the distribution of polymorphisms and correlated frequencies of responders and non-responders in the general population, and in searching for unknown genetic links to variability in drug response. In the future, we will probably have fewer drugs that suit all individuals. Genetic pharmacoepidemiology is going to play a major role in the development and evaluation of the concept of 'tailor-made' pharmacotherapy.

Keywords: Pharmacogenetics; Polymorphism; Pharmacotherapy; Pharmacoepidemiology

1. Introduction

Pharmacoepidemiology uses observational methods to quantify drug exposure and to evaluate the effectiveness and safety of drug treatment in the general population (Storm, 2000; Leufkens and Urquhart, 1994). While randomised clinical trials mostly include more-or-less homogeneous groups of patients in terms of disease severity, prognosis, risk factors, etc., and is exposure protocol-driven per definition, the 'real world' of pharmacotherapy represents an erratic natural experiment with a large variability in both drug exposure and effects (Leufkens and Urquhart, 1994). Peter Goodfellow, a senior researcher in the field of pharmacogenomics, recently stated that, on average for each drug, 30% of treated patients show beneficial effects, 30% do not show beneficial effects, 10% only experience side effects, and 30% are non-compliant (which is often related to perceived insufficient effect of the drug or side-effects of drugs) (Feenstra, 1999). From both a medical, societal and economic point of view, this is a waste of resources, and possibly 70% of all patients are unnecessarily exposed to the risk of adverse effects (Evans and Relling, 1999; Vesell, 1997; Mancinelli et al., 2000).

One of the most challenging areas of research in pharmacoepidemiology is to understand why individuals respond differently to drug therapy, both in terms of beneficial and adverse effects (Storm, 2000). Important factors in interpreting the variability in outcome of drug therapy include the patient's health profile, prognosis, disease severity, quality of drug prescribing and dispensing, compliance with prescribed pharmacotherapy and last, but not least, the genetic profile of the patient (Sander, 2000; Vesell, 1997).

Pharmacogenetics focuses on the question to what extent variability in genetic make-up is responsible for the observed differences in therapeutic efficacy, effectiveness and adverse reactions among patients (Nebert 1999; Kleyn and Vesell, 1998). From this perspective, one may expect a natural link between pharmacoepidemiology and pharmacogenetics in elucidating unexplained variance in both drug exposure and therapy outcome, and in developing individualised pharmacotherapy models (Roses, 2000). Pharmacogenomics embraces both these fields encompassing a genome-wide search for genes relevant for the application of drugs in humans, including genes determin-

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ing disease susceptibility and genes causing individual variations in drug response (Evans and Relling, 1999; Nebert, 1999).

Pharmacogenomics guides the selection of gene targets for drug discovery and serves to select the most suitable drugs for individual patient groups (Kleyn and Vesell, 1998; Roses, 2000). Pharmacogenetic research holds promise for the individualisation of drug therapy, so that only individuals who are expected to have an optimal benefit/risk ratio will receive a certain drug. This will lead to a situation in which we can optimise the proportion of patients who will benefit from a certain drug, lower the burden of side effects and eventually improve the cost-effectiveness of individual drugs (Evans and Relling, 1999).

This article provides an overview of current thinking and experience on the application of pharmacogenetic concepts to pharmacoepidemiology. We will start by discussing a number of features of molecular epidemiology, including genetic epidemiology. The relevance of currently available knowledge of gene–drug interactions for pharmacoepidemiology will be discussed in the next section, and we will complete this article with a discussion of future challenges and strategies.

2. Genetic pharmacoepidemiology

Epidemiology includes the study of disease frequency in populations and the investigation of determinants of disease (Rothman and Greenland, 1998). Molecular approaches are being increasingly used in epidemiological studies (Hunter, 1999; Ambrosone and Kadlubar, 1997). This goes beyond the 'use of biomarkers in epidemiologic studies' and requires the integrated effort of scientists across disciplines (epidemiologists, biologists, geneticists, laboratory scientists, clinicians, pharmacologists). Traditionally, epidemiologists looked at exposure-disease occurrences, e.g. in pharmacoepidemiology of drug exposure-disease occurrences (Storm, 2000). Nowadays, the molecular mechanisms that are involved in these associations are perceived as being more important for a proper and unbiased understanding of why certain exposures lead to certain effects (Ambrosone and Kadlubar, 1997).

Genetic epidemiology is a relatively new branch of molecular epidemiology (Khoury and Wagener, 1995; Khoury, 1998). Basically, genetic epidemiology deals with the role of genetic make-up in investigating and understanding direct associations between genetics and diseases and the associations between environmental factors (e.g. drugs), polymorphisms and patient outcome. In pharmacoepidemiology, exposure means drug treatment and patient outcome may include therapeutic response, adverse effects or economic effects. Genetic pharmacoepidemiology will add a new set of explanatory and predictive variables to optimise individualised pharmacotherapy, just as was achieved in the past with established relationships between certain contra-indications, pharmacokinetic profile, interactions, etc., and patient drug response (Evans and Relling, 1999).

2.1. Methodological considerations

Genetic epidemiology applies several study designs that are not equally useful for investigating genetically linked drug responses (Khoury, 1998). There are two main approaches used in genetic epidemiology: family studies (e.g. linkage studies and sibling pair studies) and population studies. Linkage studies are applied in high-risk families where there is a clustering of disease or a pattern of specific drug responses. The increasing availability of genetic markers on different chromosomes can be used to evaluate whether a particular disease cosegregates with a specific marker. Such studies can indicate whether a disease susceptibility gene is located on a particular chromosome (Khoury, 1998). So far, pharmacoepidemiology has shown limited interest in targeting familial patterns of drug response. In the advent of an increased interest in genetic pharmacoepidemiology, this will probably change, although from a practical point of view family-based study designs are not easy to establish (Kleyn and Vesell, 1998). If the aim of the research is to identify the site of a relevant polymorphism on a chromosome, studies of families or isolated populations remain the mainstay of research. This is because a high degree of similarity between DNA is necessary in order to find the piece that makes a difference. In the future, this may no longer be necessary, because more will be known about those parts of DNA that we all share and about the functions of different parts of DNA. An example of a pharmacogenetic family study is the ESPRIT study. Hypertensive sibling pairs were enrolled in a therapeutic trial, designed to assess responses to angiotensin converting enzyme-inhibitor treatment with lisinopril (Hollenberg, 1999).

All traditional population-based epidemiological studies (cross-sectional, cohort, case-control studies) can be used in genetic pharmacoepidemiology studies (Storm, 2000; Rothman and Greenland, 1998). In a cross-sectional study, exposure information and disease information are ascertained at the same moment in time. Usually, this design is highly vulnerable to bias because the time sequence of exposure and outcome is not ascertained. However, this study design is appropriate if there is a known exposure-outcome association, such as parkinsonism as a side-effect of antipsychotics with a certain cytochrome *P*450 2D6 genotype (which is assumed to be stable over time) as important determinant (Andreassen et al., 1997). In a cohort study, a population of exposed and unexposed subjects (in pharmacoepidemiological studies the exposure is usually a drug) is followed over time. These subjects have to be free of the outcome at baseline. The investigator measures and compares the incidence of disease in both groups, resulting in the calculation of the relative risk. In a

case-control study, a source population is defined, cases (subjects suffering from the outcome of interest) in this population are identified and controls may be sampled from the same source population. The investigator measures and compares the exposure status in the history of both cases and controls. From this comparison an exposure odds ratio is calculated. Under normal conditions, when a rare event is the outcome of interest, the odds ratio is a valid approximation of the relative risk (Khoury and Wagener, 1995).

The case-control design is probably the most suitable design for genetic pharmacoepidemiological studies. There are several reasons for this: (1) unlike biological markers of exposure (for example nutrition), genetic markers are stable indicators of host susceptibility over time; (2) the case-control design can help in determining the effects of several genes and gene–drug exposure interactions; (3) case-control studies are suitable for uncommon disease endpoints (for example specific cancers or adverse drug reactions) (Khoury, 1998); and (4) the case-control design is very efficient in terms of number of patients; this is particularly important because of the cost and time involved in determining genetic polymorphisms. The study of Vandenbroucke et al. (1994) on the association between Factor V Leiden, use of oral contraception and venous thrombosis (which is a very rare event in young women) is a good example of such a case-control approach. Whether a genetic polymorphism of interest really influences a drug response can be evaluated by comparing the odds ratios for groups of subjects with the different genotypes. Under the null-hypothesis, the relative risk of such comparisons (as presented as the odds ratio) equals 1.0.

We can anticipate a surge in post-hoc genetic case-control studies when biological material is still available, allowing genotype mapping directed at a specific exposure-outcome association (Kleyn and Vesell, 1998; Evans and Relling, 1999). An example of such a post-hoc approach is the evaluation of the impact of β_2-adrenoreceptor polymorphism and the development of bronchodilator desensitisation in asthma patients (Hancox et al., 1998). The results were not consistent across different study designs. Aziz et al. (1998) were able to explain part of the differences in drug response by the genotype of the subjects in a randomized clinical trial, but Hancox et al. were not able to do so in their posthoc case-control study. This also shows that the puzzle is not that simple. In most chronic diseases, multivariate systems, including large numbers of genetic polymorphisms, influence the clinical effect of drug treatment.

A special epidemiological study approach used in genetic epidemiology is the case-only study (Khoury, 1998). This design can be used when the genetic factor is rare. Random sampling of cases and controls may require prohibitively large sample sizes to reach adequate power to detect a gene–environment interaction (Schmidt and Schaid, 1999). In the case-only method, investigators use case subjects only to assess the magnitude of the association between the exposure of interest (for example a drug) and the susceptibility of the genotype. With this analysis, main effects of the susceptible genotype and environmental exposure cannot be estimated, but a measure of the association is easily computed as a cross-product ratio. If genetic and environmental risk factors (e.g. drug exposure) are independent in the population, the cross-product ratio is equal to the risk ratio. If, additionally, the risk of disease is small at all levels of the study variables, it is approximately equal to the odds ratio for the gene–environment interaction computed from case-control data (Schmidt and Schaid, 1999). An example of the case-only approach is shown in Tables 1 and 2. Table 1 is the case-control study performed by Hwang et al. From Table 2, the case-only odds ratio can be calculated: $(13 \times 36)/(13 \times 7) = 5.1$ (95% confidence interval 1.5–18.5) (Khoury and Flanders, 1996). The odds ratio for the smoking–gene interaction in the case-control analysis was 5.5 (95% confidence interval 2.1–14.6) (Hwang et al., 1995). The results for both analyses were comparable, but the case-only design was much more efficient.

2.2. Confounding in genetic epidemiology

Prevention of or adjustment for confounding is a key concept in epidemiology (Hunter, 1999; Storm, 2000; Rothman and Greenland, 1998). Several sources of confounding in genetic pharmacoepidemiology need to be considered. When genetic studies are performed in open, dynamic populations, there is always the risk of confounding by population admixture. Admixture of a population occurs when subjects of different subpopulations have offspring together. Subpopulations (for example different races) can have a different risk of disease or drug response, leading to confounded comparisons when admixture is not taken into account. An example of confounding by admixture is seen in a study from Knowler et al. They studied the association between $GM^{3;5;13;14}$ and type 2 diabetes mellitus in Native Americans (Knowler et al., 1988). In a sample of 4920 Native Americans of the Pima and Papago tribes, a negative association (relative risk = 0.27, 95% confidence interval 0.18–0.40) between this genotype and diabetes mellitus type 2 was found. One might conclude

Table 1
Case-control analysis of the interaction between maternal cigarette smoking, transforming growth factor alpha (TaqI) polymorphism, and the risk of cleft palate. Adapted from Hwang et al. (1995)

Smoking	Taq1b polymorphism	Cases (*n*)	Controls (*n*)	Odds ratio	95% Confidence interval
no	no	36	167	1.0	Referent
no	yes	7	34	1.0	0.3–2.4
yes	no	13	69	0.9	0.4–1.8
yes	yes	13	11	5.5	2.1–14.6

Table 2
Case-only table of the interaction between maternal cigarette smoking, transforming growth factor alpha (TaqI) polymorphism, and the risk of cleft palate. Adapted from Hwang et al. (1995)

		Genotype (TaqI polymorphism)	
		No	Yes
Exposure (smoking)	No	36	7
	Yes	13	13

that the absence of this gene is a risk factor for diabetes. This conclusion is probably incorrect. $GM^{3;5;13;14}$ is a very rare allele in the Pima and Papago Indians. It is a very sensitive marker for Caucasian admixture in native Americans (Williams et al., 1986). The risk of diabetes varies inversely with the amount of Caucasian admixture (Caucasians have a greater chance on getting diabetes mellitus type 2) and the genotype is a marker for that admixture (Knowler et al., 1988). Such admixture effects may also be expected in genetic pharmacoepidemiological studies.

Another source of possible confounding is the practice of drug prescribing (Leufkens and Urquhart, 1994). Nonrandom variation among physicians in how they take genotype into account when they prescribe a drug might affect treatment outcome in a very complicated fashion. At this time, drug prescription on the basis of genotype is limited to a number of special settings and environments. But this will probably change in the near future when pharmacogenetics will become a more integral part of the practice of medicine. Genetic pharmacoepidemiology will then have to face a peculiar type of 'confounding-by-indication', when some of the research population get drug treatment based ('as indicated') on genotype and others do not (Storm, 2000). For example, imagine that a physician wants to evaluate the risk of adverse effects of antipsychotics. If the physician knows that a patient is a poor metaboliser based on the patients cytochrome *P*450 2D6 profile, then he or she may be aware of the higher risk of an adverse event in such patients and prescribe an atypical antipsychotic agent. Moreover, the chances that the adverse effect will be recognised in an early stage (detection bias!) or that the patient will receive preventive anticholinergic drug treatment in order to cope with these adverse effects (protopathic bias!) are higher than would be the case if the physician did not know the patient's genotype (Storm, 2000). So far, it is too early to estimate the impact of these types of confounding on the validity of genetic pharmacoepidemiologic studies.

2.3. Principles of epidemiologic gene–exposure interactions

There are several types of gene–drug interactions: either a polymorphism is not related to the outcome, but comes only to expression when there is exposure, or a polymorphism itself already is associated with the outcome (Khoury, 1998). It is also possible that the exposure has an influence on the outcome even if the polymorphism is not there. Table 3 lists a series of examples of known polymorphisms. These examples are discussed elsewhere in this paper.

One of the extreme examples is the classic case of glucose-6-phosphate deficiency and the risk of serious anaemia. Patients with an inherited glucose-6-phosphate deficiency are at risk of severe anaemia if they use certain drugs in high dosages. These drugs include antimalaria drugs (Brewer and Zarafonetis, 1967), sulphonamides and salicylates (such as aspirin). As long as these patients are not exposed to these drugs, they have no increased risk of severe anaemia. This means that there is no direct gene-outcome effect. In subjects without the deficiency, there is no increased risk of anaemia if they use these drugs: the direct exposure-outcome effect is 1.0. The multiplicative gene–exposure effect is much higher than 1.0. An example at the other extreme of the spectrum is the Factor V Leiden mutation and risk of venous thromboembolism. The incidence of venous thrombosis among non-users of oral

Table 3
Examples of different gene–drug interactions

Genotype			G6PD-deficiency	CYP2D6 polymorphism (Mancinelli et al., 2000)	APOE * 4 (Slooter et al., 1999)	CETP B2B2 (Kuivenhoven et al., 1998)	Factor V Leiden (Vandenbroucke et al., 1996)
Exposure Outcome			Sulphonamides Anemia	Antipsychotics Parkinsonism	Estrogens Alzheimer	Statins Artherosclerosis	Oral contraceptives VTE
	Exposure	Susceptible genotype	Relative risk	Relative risk	Relative risk	Relative risk	Relative risk
Reference	0	0	1	1	1	1	1
Gene alone effect	0	1	1	1	>1	<1	>1
Exposure alone effect	1	0	1	>1	<1	<1	>1
Multiplicative gene-exposure effect	1	1	$\gg 1$	$\gg 1$	$\ll 1$	1	$\gg 1$

contraceptives is about 0.8 per 10,000 person years. This risk increases to 5.7 per 10,000 person years for carriers of the Factor V mutation. Therefore, the direct gene effect is greater than 1.0. The risk increases to 3 per 10,000 person years for women who use oral contraceptives (direct exposure-outcome effect > 1.0). Among women who have both risk factors (carriers of Factor V Leiden who use oral contraception) the incidence becomes 28.5 per 10,000 person years, so the gene–exposure interaction is much greater than 1.0 (Vandenbroucke et al., 1994). Thus, carriers of the Factor V Leiden should use methods of birth control other than oral contraceptives.

3. Pharmacogenetic pathways

It is estimated that 47–61% of all protein loci are polymorphic (Nebert, 1999). Thus, the mutation of genes that potentially may affect drug response is a common biological phenomenon. The consequences for the drug response will depend on the extent to which the function of the gene product is affected by the mutation. In addition to the magnitude of loss of function, the frequency with which the mutation occurs determines the clinical relevance of genetic variability. Principally, there are three routes by which genes can affect a drug response (Table 4).

3.1. Pharmacokinetic gene–drug interactions

Already in the 1960s and 1970s it was known that there were individual and ethnic variations in drug metabolism (Grahame-Smith, 1999). Now, it is known that gene products relevant for the pharmacokinetics (biotransformation and excretion) of drugs comprise various enzyme systems (e.g. cytochrome *P*450 enzymes), ATP binding cassette (ABC) transporter proteins (proteins involved in the absorption, excretion and transport of drugs across bodily barriers, e.g. the blood–brain barrier), etc. There is ample evidence for the important role of different genotypes that code for these enzymes (Van der Weide and Steijns, 1999). Changes in enzyme activity can cause a substantial variation in the amount of drug present in the body. For example, the cytochrome *P*450 2C9 enzyme is associated with the metabolism of phenytoin. It has been observed that the plasma level of phenytoin varies 16-fold among patients given the same dose of the drug (Bullock, 1999).

Subjects who are homozygous for the cytochrome *P*450 enzyme 2D6 (CYP2D6) null alleles exhibit a 'poor metaboliser'-phenotype, which occurs in 6% to 10% of Caucasians. Other genotypes for this enzyme (on chromosome 22) lead to phenotypes that can be classified as extensive or ultra-rapid metabolisers. Cytochrome *P*450 2D6 is involved in the metabolism of many cardiovascular drugs and antipsychotics. Andreassen et al. performed a case-control study to evaluate the association between cytochrome *P*450 2D6, the use of classic antipsychotic drugs and the risk for extrapyramidal side effects (akathisia, parkinsonism and tardive dyskinesia). There was a non-significant tendency for poor metabolisers to have more severe tardive dyskinesia and parkinsonism in the cross-sectional study. In their case-control data, they saw a non-significant 3-fold higher frequency of poor metabolisers among patients with longitudinal tardive dyskinesia. However, a significant association between poor metaboliser phenotype and the risk of drug-induced parkinsonism was not confirmed (Andreassen et al., 1997).

Another example is the cytochrome *P*450 2C19 enzyme. Homozygosity for the null allele on this gene leads to the 'poor metaboliser' phenotype in 2% to 5% of

Table 4
Examples of pharmacogenetic markers by mechanism

Mechanism and involved polymorphisms	Pharmacogenetic effect
Pharmacokinetic gene–drug interactions	
Cytochrome *P*450 (CYP) 2D6	The CYP 2D6 defect leads to a "poor metaboliser" phenotype in 6–10% of Caucasians. Metabolises 25% of all drugs including many cardiovascular drugs and antipsychotics.
Cytochrome *P*450 2C19	The CYP 2C19 defect leads to a "poor metaboliser" phenotype in 2–5% of Caucasians. These subjects are highly sensitive to omeprazole, diazepam, propranolol and other drugs.
Pharmacodynamic gene–drug interactions	
5-HT receptor, D 4 receptor	Polymorphisms in both serotonin and dopamin receptors have been associated with efficacy of atypical antipsychotics.
Vitamin D receptor	Vitamin D receptor polymorphism is associated with a different response to vitamin D used for increasing bone mineral density.
Gene–drug interactions in the causal pathway of disease	
Adducin polymorphism	Adducin polymorphism is associated with a salt-sensitive form of hypertension. These patients will benefit from diuretic treatment.
Apolipoprotein E	The Apo E ε 4 polymorphism is associated with risk for Alzheimer disease and with a poor response to anticholinesterase treatment.
CETP polymorphism	Association with atherosclerosis progression and with response to HMG-Coa reductase inhibitor therapy.

Format according to Kleyn and Vesell (1998).

Caucasians and in 3% to 23% of Asians. These subjects are highly sensitive to omeprazole, diazepam, propranolol, mephentoin, amitrypiline, hexobarbital and other drugs (Mancinelli et al., 2000). Furuta et al. (1998) conducted a prospective cohort study to investigate whether the cytochrome *P*450 2C19 genotype is associated with the rate of cure of Helicobacter pylori infection achieved with dual therapy with omeprazole and amoxicillin. They found that the cure rate for Helicobacter pylori infection was 28.6% (95% confidence interval 13.1–48.7%) for rapid metabolisers, 60% (95% confidence interval 38.6–83.0%) for intermediate metabolisers and 100% (95% confidence interval 66.4–100%) for poor metabolisers. The genotype of cytochroom *P*450 2C19 was predictive for curing of Helicobacter pylori infection and peptic ulcer in patients who received this dual therapy.

A case-control study conducted by Aithal et al. (1999) showed a significant association between a polymorphism of the cytochrome *P*450 2C9 allele and low warfarin dose requirement (< 1.5 mg). The odds ratio that individuals with a low warfarin dose requirement had one or more cytochrome *P*450 2C9 variant alleles compared to the normal population was 6.2 (95% confidence interval 2.5–15.6). Patients in this low dosage group had an increased risk of major bleeding complications (odds ratio 3.7, 95% confidence interval 1.4–9.5).

Variation in the activity of these drug-metabolising enzymes results in variable pharmacokinetics: rapid metabolisers will be underdosed and poor metabolisers will be overdosed. When multiple drugs are administered to a patient, this variation may result in unpredictable drug–drug interactions (Bailey et al., 1998).

3.2. Pharmacodynamic gene–drug interactions

Gene products expressed as receptors are relevant for the pharmacodynamics of drugs. After entering the body each drug interacts with numerous proteins, such as carrier proteins, transporters and multiple types of receptors. These proteins determine the site of action and the pharmacological response. Thus, polymorphisms in genes encoding for drug targets may affect the response to a drug (Mancinelli et al., 2000).

For example polymorphisms in the coding region and promotor of the serotonin receptor are associated with efficacy of atypical antipsychotic drugs (e.g. clozapine). The evidence base for the use of these drugs is compelling, but various reasons seem to prevent their use in greater numbers of eligible patients. The response to the drugs is variable (between 30% and 60% respond to clozapine) and treatment costs are higher, because it is necessary to screen for effects on blood (counting white blood cells) before and during the use clozapine, than with classic antipsychotics. In a study by Arranz et al. (2000), a combination of six polymorphisms in neurotransmitter–receptor related genes resulted in 76.6% success in the prediction of clozapine response. These results can be implemented in a treatment protocol with a simple test to enhance the usefulness of clozapine in psychiatric treatment. There is also evidence that polymorphisms in the dopamine D4 receptor may explain some of the interindividual variation seen in patient response to clozapine and other classes of antipsychotic medication (Cohen et al., 1999). Another example is the relation between vitamin D supplementation and the bone mineral density of the femoral neck. The genotype of the vitamin D receptor based on the presence (b) or absence (B) of the *Bsm*I restriction site is responsible for different responses to vitamin D supplementation. The mean increase in bone mineral density in the vitamin D group relative to the placebo group was significantly higher ($P = 0.03$) in people with the BB (increase 4.4%) and Bb genotype (increase 4.2%) than in people with the bb genotype (decrease 0.3%) (Graafmans et al., 1997).

3.3. Gene–drug interactions in the causal pathway of disease

There is a growing interest in genes that are in the causal pathway of diseases and are able to influence the drug response (Nebert, 1999; Nakagawa and Ishizaki, 2000). A complicating factor is that most diseases have a polygenetic origin and that therefore different genetic pathways may operate in patients with the same phenotype. These genetic differences may also lead to different responses to drug treatment.

Hypertension, for instance, is a complex phenotype that involves many regulatory systems (Nakagawa and Ishizaki, 2000). These different regulatory mechanisms underlie the different forms of essential or primary hypertension. Different pressor mechanisms that operate in a given patient can also be associated with his or her responsiveness to different therapies. A large variety of antihypertensive drugs are currently available, reflecting the difficulty to lower blood pressure effectively without producing side effects that affect patient compliance (Ferrari, 1998). Different polymorphisms are expected to change the response to different antihypertensive agents. A key concept here is the polymorphism for adducin in relation to treatment for hypertension (Cusi et al., 1997). Cusi et al. (1997) found a significant link between of the alpha-adducin locus and essential hypertension in that patients with the mutant allele had a greater sensitivity to changes in sodium balance. The adducin polymorphism upregulates the sodium–potassium pump and triggers a hormonal dysregulation (an increased production of an endogenous sodium–potassium pump inhibitor, the so-called ouabain-like factor (OLF)) (Ferrari, 1998). This results in a salt-sensitive form of essential hypertension. Cusi et al. (1997) suggest that the alpha-adducin polymorphism may be an important key for identifying hypertensive patients who will benefit from diuretic treatment. Other polymorphisms that might play a role in the response to antihypertensive

therapy are polymorphisms of angiotensinogen (Higorani et al., 1995; Dudley et al., 1996), angiotensin-converting enzyme (Iwai et al., 1998; Sasaki et al., 1996), angiotensin II type 1 receptor (Higorani et al., 1995; Benetos et al., 1996) and G-protein β_3 (Schorr et al., 2000). Several clinical and epidemiologic studies have evaluated possible interactions between pharmacotherapy and these polymorphisms. The results so far are not conclusive and much more research has to be done. The genetic pharmacoepidemiology of hypertensive therapy is a key to understanding the complex interaction between various genotypes and phenotypes in relation to drug response (Pinto and van Gilst, 1999).

A second example of a genotype that is in the causal pathway of a disease and able to influence the drug response is the apolipoprotein E genotype in Alzheimer disease. Apolipoprotein E is critical in the modulation of cholesterol and phospholipid transport between cells of different types. It plays a role in the compensatory response of neuronal systems to injury, by modulating the transport of cholesterol and phospholipids during the re-innervation process (Poirier, 1994). Human Apolipoprotein E is a polymorphic protein with three common alleles, Apolipoprotein E ε 2, Apolipoprotein E ε 3, and Apolipoprotein E ε 4. The latter is associated with sporadic and late-onset familial Alzheimer disease (Poirier et al., 1995). This association suggests that a dysfunction of the lipid transport system involved in compensatory sprouting and synaptic remodelling might be crucial to Alzheimer disease pathogenesis (Poirier, 1994). Tetrahydroaminoacridine, an anticholinesterase, was the first drug that alleviated dementia in a clinically significant way. Riekinnen et al. (1997) showed that tetrahydroaminoacridine only increased electroencephalogram arousal in Alzheimer patients without an Apolipoprotein E ε 4 allele. This result suggests that the Apolipoprotein E genotype may modulate the effect of anticholinesterase drugs on cortical electroencephalogram arousal in Alzheimer disease patients. The results of Poirier et al. (1995) also strongly support the concept that APOE ε 4 plays an important role in the poor response to therapy with acetyl cholinesterase inhibitors in Alzheimer disease patients. This was an important finding with regard to the development of drugs against Alzheimer disease. In the past, several potential drugs did not pass the development phase because only one-third of the patients reacted to the therapy, one-third was non-responder and one-third experienced a variety of adverse effects.

Exposure to estrogens can also play a role in the onset of Alzheimer disease. The use of estrogens in combination with the Apolipoprotein E ε 4 genotype gives a lower relative risk of developing Alzheimer disease (Slooter et al., 1999) (see Table 1). Slooter et al. conducted a case-control study with 109 patients and 119 controls. Each female patient with early-onset Alzheimer disease (before 65 years) was matched to a control by age and place of residence. After adjustment for age and educational level, Slooter et al. found an odds ratio of 0.34 (95% confidence interval 0.12–0.94). For subjects with the Apolipoprotein E ε 4 genotype the odds ratio was 0.37 (confidence interval 0.08–1.58). The Apolipoprotein E ε 2 genotype had an odds ratio of 0.25 (95% confidence interval 0.02–3.63) and the Apolipoprotein E ε 3 ε 3 genotype had an odds ratio of 0.60 (95% confidence interval 0.19–1.88). These findings suggest that estrogen use might be beneficial in the prevention of early-onset Alzheimer disease and that the efficacy of estrogens depends on the Apolipoprotein E genotype (Slooter et al., 1999).

A compelling case in recent pharmacogenetics is the association between the cholesteryl ester transfer protein (CETP) polymorphism and statin therapy. CETP has a central role in reverse cholesterol transport, the mechanism by which cholesterol is eliminated from the body. Pravastatin therapy slowed the progression of coronary arteriosclerosis in subjects with the wild genotype but not in subjects who were homozygous for the polymorphism. Sixteen percent of the population under study was homozygous for the polymorphism (the B2B2 genotype). This common polymorphism might be a reason not to treat subjects with statins (Kuivenhoven et al., 1998). The question is whether these subjects experience no beneficial effects of statins against clinical manifestations of atherosclerosis such as myocardial infarctions. B2B2 carriers have lower plasma levels of CETP and higher levels of HDL (high-density lipoprotein) cholesterol than subjects heterozygous or homozygous for B1. The progression of atherosclerosis is slower in these subjects. The relative risk on atherosclerosis is lower than 1 (Table 3). Statins slowed the progression of atherosclerosis in subjects without the B2 allele (direct exposure-outcome effect < 1.0), but had no effect in subjects with the B2 allele. The gene–exposure interaction gives a relative risk of atherosclerosis of 1 (Kuivenhoven et al., 1998).

The treatment of hypercholesterolaemia is one of the most challenging areas of modern pharmacotherapy, both in terms of evaluating effectiveness and safety, appropriate targetting of patients who may benefit, and the cost-effectiveness of treatment. Epidemiological research on the role of the CETP polymorphism in reducing the risk of cardiovascular events in daily practice is urgently needed (Kleyn and Vesell, 1998).

4. Future challenges

Genetic pharmacoepidemiology aims to explain the variability in drug response between individuals, and especially the proportion of variability, that is caused by the genetic profile (Khoury, 1998; Hunter, 1999). It also aims to determine the clinical relevance of polymorphisms that are known to modify drug response. Important factors of clinical relevance can be divided into two groups. The first

concerns clinical relevance from a patient's perspective. Is it always relevant to know the genotype before drug treatment is started? What is the clinical relevance for the entire population? How often does the polymorphism occur in the population, and what is the distribution of it in various subpopulations? What is the burden of avoidable adverse effects when subjects are needlessly treated? Is screening the patient's genotype before drug treatment is started cost effective? Before these questions can be answered some challenges and problems have to be solved.

The first question is how to track relevant polymorphisms. There is an enormous amount of literature on genetic polymorphisms related to drug actions, and therefore there is a clear need to select and target the most challenging ones (McCarthy and Hilfiker, 2000; Evans and Relling, 1999). In certain disorders (like hypertension) there are several different drugs available. Usually, patients do not react to all these therapies. After a while, most patients switch to another drug (Storm, 2000), and eventually they will get the drug that suits them best. The genotype is 'selecting' the drug that gives a response, and vice-versa. The genotypes of subjects who have switched to other drugs might provide useful information about the polymorphism responsible. These 'selection patterns' in combination with linkage analyses can provide new clues about polymorphisms active in changing the response to antihypertensive drug treatment. Cardiovascular pharmacotherapy is both a very promising but also a very challenging area for population-based pharmacogenetic research. There is an interaction between the polymorphic metabolism of cardiovascular drugs and the molecular genetics of cardiovascular diseases in relation to the genes determining the responsiveness to a given drug (Pinto and van Gilst, 1999; Nakagawa and Ishizaki, 2000).

If the variability in drug response cannot be explained by the known polymorphisms, pharmacoepidemiology can also contribute to the search for unknown genetic links (using single nucleotide polymorphisms (a single nucleotide polymorphism is a simple base-pair substitution that occurs within and outside genes), etc.) (McCarthy and Hilfiker, 2000). In the future, treatment outcome will be increasing linked to genetic variability. Because single nucleotide polymorphisms are found all over the genome, the genome can be scanned for polymorphisms that change a patient's response to certain drugs.

Determining the clinical relevance of a polymorphism is a major task for genetic pharmacoepidemiology (Evans and Relling, 1999; Roses, 2000). Several factors play a role. A good example of clinically relevant decisions that have to be made is the screening for Factor V Leiden of women using oral contraceptives. It would be possible to screen all women using 'the pill' or who intend to start using it. This would deny effective contraception to 3–6% of white women, while preventing only a small number of venous thromboembolism (Vandenbroucke et al., 1996). The conclusion of Vandenbroucke et al. (1996) is that there are certain situations in which it may be wise to determine the genotype. For example, if the woman has already had a venous thrombosis or if she has relatives who have had a venous thrombosis at an early age. Such women have a greater chance of being homozygous for the Factor V Leiden mutation. Homozygous carriers may have a more than hundred-fold increased risk of venous thromboembolism if they use oral contraceptives. This means that the clinical situation of the patient and her relatives will continue to direct the physician's advice, and not only the genotype.

A key factor determining the success of genetic pharmacoepidemiology will be the access to data on genotype and other relevant molecular markers (Sander, 2000). The ethical, legal and social implications of population based genotyping are still unresolved and much debated. It is important that distinctions are made between disease susceptibility gene polymorphisms which provide information about risks (of diseases), and pharmacogenetic profiles (Roses, 2000).

The results of genetic pharmacoepidemiological studies will be used by the pharmaceutical industry to develop new drugs (Mancinelli et al., 2000). New disease insights are generated when the variability in drug response can be explained by genetic profile. These insights create new leads for the search for future medicines and better-individualised treatment options (Evans and Relling, 1999). From a marketing perspective, industry is of course interested in the distribution of polymorphisms in the population. There is a risk that medicines only will be developed for the most common, commercially attractive, genotypes (Serono, 2000). Whether this will also lead to new 'orphan diseases' remains uncertain. While for the currently available drugs there is almost no information on genetic causes underlying individual variations in drug response known, it is expected that, in the near future, for new drugs there will be a large amount of knowledge on mutations in genes that will alter the drug response. New drugs may be marketed for subjects with a specific genotype. Clinical trials can be smaller and more efficient (Mancinelli et al., 2000). But what about population-based studies? Probably in the future drugs will be used differently in daily practice than in clinical trials, as is the case nowadays. Pharmacoepidemiology will continue to evaluate the cost–benefit ratio of drug treatment in the general population. The question is whether all variability can be explained by a subject's genotype. Experience from the past (for example, with the discovery of stereochemistry, pharmacokinetics, and others) has taught us that variability in drug-response is multifactorial. Pharmacogenetics will add new explanatory power to the pharmaceutical sciences, but will we be able to explain all the variability in drug response (Evans and Relling, 1999; Roses, 2000)? Probably not. In the future, we will have fewer drugs that suit all individuals. There will be an increase in compounds that are suitable for a relatively small genetically selective patient group. At

the interface between pharmacoepidemiology and pharmacogenetics, there is much challenging work to do.

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