Pharmacogenetics

To the Minister of Health, Welfare and Sport

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Dear Minister,

The assignment of the Health Council of the Netherlands is, besides giving advice about questions of ministers, to draw attention to problems and developments which are important for the policies of the government. Within that framework, I present the advisory report entitled 'Pharmocogenetics'. It has been prepared after consultation of experts in the field, and the Boards of the Council on Genetics and Ethics & Law.

In this report the Council indicates the rapid growth of pharmacogenetic knowledge, which offers the possibility to prescibe some drugs more efficiently. It regards the adjustment of doses to the genetic constitution as well as the avoidance of adverse effects.

Pharmacogenetical investigation can, as is the case with other genetic examinations, result in problems with regard to insurances and appointments. Therefore, it is increasingly important to monitor the legal regulations of such investigations.

To avoid misunderstanding about pharmacogenetic examinations, the Council indicates that adequate information for patients is required.

Yours faithfully,
(Signed)

Prof dr JJ Sixma

Pharmacogenetics

to

The Minister of Public Health, Welfare and Sport

No. 2000/19E, The Hague, 31 August 2000

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Executive summary

Pharmacogenetics deals with the influence of genetic variation on the effects of medicines. Therapeutic effects as well as side effects of a drug can vary due to the genetic make-up of the recipients. Genetic variation in non-human organisms is not considered in this report.

Genetic differences can result in considerable variation in the rate at which a given medicine is broken down within a patient's body. The metabolism may take longer than anticipated, thereby increasing the risk of side effects. In case of high metabolic rates the therapeutic effect may be diminished or absent. Metabolic rates depend mainly on cytochrome P-450 and N-acetyltransferase enzymes. Patients may be classified as fast or slow metabolizers, depending on the activity levels of these enzymes. The best known of the cytochrome P-450 enzymes is CYP2D6, which plays a part in the metabolism of beta-blockers, antidepressants and other drugs. About 8 per cent of the Dutch population have a low CYP2D6 activity. Other cytochrome P-450 subtypes also result in delayed metabolism of certain medicines. Slow N-acetyltransferase forms are found in a majority of the population. These enzymes play an important role in the metabolism of various drugs, for example isoniazid, which is used to combat tuberculosis. Probably, tests will be available in the near future to determine patient's genotypes for metabolic conversion, i.e. whether they are fast or slow metabolizers.

Pharmacogenetic effects can be caused by differences in enzymatic conversion rates, but also by inter-individual variations in the proteins to which the drugs are targeted (target proteins), or by genetically determined unintended interference with normal physiological processes. For example, asthma drugs such as salbutamol attach

them themselves to a particular receptor. Genetic differences in this receptor mean that the efficacy of such drugs varies from patient to patient. Interference by genetically determined factors can, for example, occur with anti-malarial drugs, which may result in serious forms of anaemia.

The development of pharmacogenetics began in the 1950s. Today, the discipline is the focus of increasing attention for various reasons. Considerable momentum is obtained from the rapid expansion of scientific knowledge regarding the human genome, which has shed light on genetically determined factors affecting the action of drugs. In addition, ever more epidemiological data is available on the incidence of side effects of drugs. Meta-analyses indicate that serious side effects are more common, even with correctly prescribed medicines, than was until recently supposed. Data from the USA suggests that 7 per cent of hospital patients receiving medication experience serious side effects. Although such problems are not attributable to genetic factors alone, the application of pharmacogenetic principles could reduce their incidence.

Interest in pharmacogenetics has also been fuelled by the increasing emphasis on efficiency in health care. By taking more account of individual genetic make-up, better use can be made of medicines. In the future, the dosages prescribed for individual patients may in certain cases be adjusted on the basis of DNA test results, thereby hastening recovery and reducing side effects. A more rapid recovery is, for example, possible if the metabolic conversion rate is known when antidepressants or antipsychotics are initially administered. Furthermore, pharmacogenetic information about individual sensitivity would reduce the incidence of side effects when antidepressants, antipsychotics and other medicines, such as anti-tumour drugs, are prescribed. Cost-savings are therefore possible, especially where hospital admissions can be prevented. However, savings in drug costs are unlikely, as an increased dose may be just as desirable as a decreased dose.

Pharmacogenetic information is potentially significant in relation to insurance and employment. DNA tests could indicate, for example, that an individual was more likely to become ill and thus to incur expenses for the insurer/employer. It is open to question whether the Medical Examinations Act provides sufficient clarity regarding the use of genetic data, or to what extent the provisions of the Act are adhered to in practice. The developments outlined above require revision of the regulations on DNA testing (presently confined to special clinical genetics centres).

As pharmacogenetic knowledge develops, it can be increasingly used for the development of new medicines. This may result, on the one hand, in drugs that exhibit less variation in their metabolic conversion rates and, on the other, in drugs for which pre-prescription DNA or enzyme testing is desirable. Most recently released drugs have been developed with metabolic variations involving the main cytochrome P-450

enzymes in mind. In the years ahead, the number of potential pharmaceutical research topics will grow rapidly as a result of increasing (pharmaco-) genetic knowledge (in the literature, this research area is generally referred to as *pharmacogenomics*). This is likely to lead to the development of many more pharmaceutical products. In turn, the availability of new drugs will have implications for the health budget and necessitate greater emphasis on the efficacy of the products prescribed.

Where pharmacogenetic tests are carried out, it is important that the individuals concerned are properly informed, partly because misapprehensions regarding genetic testing are commonplace. The purpose and scope of such tests should be made clear and steps taken to ensure the confidentiality of the results obtained. These principles apply equally to (pharmaco-) genetic scientific research into associations between genotypes and the effects of drugs or the prevalence/risk of disease. In that case, allowance should also be made for the fact that the implications of such research are not always entirely clear. Furthermore, interpretations by researchers may in a later stage shown to be wrong. The advisability of informing the patients concerned about scientific research findings therefore warrants careful consideration.

Chapter 1

Introduction

The term “pharmacogenetics” is the name of a specialist scientific area, which focuses on the connections between genetic variations and differences in the effects of medicines.

1.1 Examples

A striking pharmacogenetic example is the product, pravastatin, which decreases the cholesterol concentration in blood and is used to reduce the chances of atherosclerosis of the coronary arteries (Kui98). Depending on a genetic disposition (the presence of different alleles of the cholesterylester transferase gene), atherosclerosis will or will not be inhibited in patients who use the statin. As a result, it is estimated that for 16% of the Dutch population, the remedy does not have the desired effect. According to researchers, this means that it is possible to improve the cost-effectiveness of the treatment for vascular coronary disorders (Kui98, Mol99). Other genetic differences could also be important for the effectiveness of statins (Maa99).

This example applies for the absence of an effect, and therefore the unnecessary use of the medicine concerned for certain patients. In other cases, there is a variation in the genetic predisposition which causes side effects of medicines. Some classical examples include the breakdown of red blood cells after the use of medicines (haemolytic anaemia) and severe fever reactions in anaesthesia (malignant hypothermia). Haemolytic anaemia occurs with the use of remedies against malaria and other medicines in the case of a certain hereditary absence of an enzyme involved in

the conversion of glucose (glucose-6-phosphatedehydrogenase). The enzyme is lacking in the black population in approximately 10% of men and 1% of women (Hoc52, Web97).

Pharmacogenetic phenomena are not restricted to enzymes, but can also affect other proteins. An example is the above-mentioned malignant hyperthermia, a rare but serious complication with the use of halothane and succinylcholine for anaesthesia (Den62). One of the causes of this complication is a variant in a gene which codes for a protein in the muscle membranes (Fuj91, Gli91). This sort of protein is called a target protein if the medicine concerned targets it.

Side effects can occur as a result of a variation in target proteins, but also as a result of variation in the conversion of medicines in the body. For example, the breakdown of the anti-epileptic drug phenytoin can be very slow in some patients as a result of genetic variation, leading to high and possibly toxic levels. On the other hand, codeine only works after it has been converted into an active product by enzymes. As a result of genetic differences this conversion is slow in some people. For the desired effect (for certain forms of pain) a higher dose or a different remedy will be required (Des91, Sin93, Pou96).

These examples show that genetic variation can lead to differences in the effects of medicines in a variety of ways. In the international literature, these differences are covered by the field of pharmacogenetics, sometimes known as pharmacogenetics, sometimes as pharmacogenomics (Col99b). However, most researchers use the last term to cover all the knowledge of the genome which is relevant for pharmacotherapy (Rai98). As genetic variation which leads to differences in the pharmacological effects of drugs, also causes different reactions to other foreign bodies, there is an overlap between pharmacogenetics and the toxicological approach to genetic differences in sensitivity (Ruij96). It goes without saying that research into the genetic predisposition to diseases and into gene therapy is not covered by the term pharmacogenetics.

1.2 Growing interest

In recent years, there has been a marked increase in the interest in pharmacogenetics. One of the reasons for this is that the extent of the damage caused by the use of medicines is considerably greater than was assumed until recently (Laz98). According to a meta-analysis of 39 studies in the US, the percentage of serious, medicine-related incidents amongst patients admitted to hospital is 6.7 (95% reliability interval 5.2-8.2). For 0.3% of the patients, these were fatal (interval 0.23-0.41). These data concern drugs which were presumed to have been prescribed correctly, and did not concern abuse or overdoses resulting from errors (Laz98). A concern about the extent of the

side effects of medicines appears justified on the basis of the analysis. The media also fairly regularly devote attention to this problem, for example, following research into thrombosis amongst users of oral contraceptives (Mar98, Her99). A quote from these studies:

> Perhaps women who take the pill should eventually be genetically screened beforehand. However, there is a great deal of dispute about this (NRC Handelsblad, 12-7-1999).

The second cause of the increase in interest in pharmacogenetics is the rapid growth of knowledge about genetic polymorphisms. There is a polymorphism when different forms of a particular gene occur in a population (for rare forms these are known as variants). The most common polymorphisms in the human genome are differences between single nucleotides, internationally referred to as single nucleotide polymorphisms or SNPs. SNPs are very common and can be detected with computerized methods quickly and relatively cheaply (Col97). It has been predicted that in the near future it will be possible to verify 100,000 SNPs in a few hours, for a few hundred dollars (Sti98). The SNPs are very important for association research which examines the influence of genetic variation on the predisposition to various complaints. Partly for this reason, one of the objectives of the human genome project for the period 1998-2003 is to examine 100,000 SNPs (Col98). The research of SNPs has a strong influence on pharmacogenetics, because it will examine associations with effects of medicines, as well as associations with diseases.

A third important reason is the idea that medicines could be used in a much more rational way (Hou98). In other words, pharmacogenetics readily falls under the objective of “managed care”. This could lead to an improvement in the relationship between costs and returns. If a particular medicine does not work, or if only low dosages are required as a result of a genetic disposition, this could certainly lead to savings. On the other hand, it may become clear that higher doses are required, or different and possibly more expensive medicines. In these cases the relationship between costs and returns can also improve, though with a higher expenditure on drugs. Probably the greatest health benefits and the greatest savings can be achieved by reducing various side effects (Wol00).

For an evaluation of the possible positive effects which a pharmacogenetic study could have – either for an individual patient or for particular medicines – it is important that the variation in the reactions to medicines are determined by a number of different factors. The age and sex are important factors, but so are the patient’s condition and the possible use of other substances, particularly other medicines. Therefore, when medicines are prescribed, it will be necessary to take into account a variable response, even if a pharmacogenetic profile has been determined for the patient. If the response

variation is much larger in practice than that which can be explained by genetic differences, drawing up a DNA profile becomes considerably less significant.

Up to now, relatively little attention has been devoted in the Netherlands to pharmacogenetics. However, the importance of the new developments has been pointed out in a number of scientific journals such as the Pharmaceutisch Weekblad and the Tijdschrift voor Psychiatrie (Wei97, Tou98b). The Ziekenfondsraad (health insurance funds council) has placed the subject "genotyping (research into genetic material) for the prescription of antidepressants" on the prioritisation list of "cost-effectiveness subjects". Pharmacogenetics has also been mentioned in the "DNA diagnostics" advice of the Health Council: the development of these medication-linked diagnostics appears to be possible in the short term (GR98). This advice also notes that, partly because of the large financial interests, the application will probably be extremely extensive.

1.3 The structure of this report

This report deals concisely with the genetic basis of differences in the effects of medicines in chapters 2 and 3. Consecutive subjects are the differences based on metabolic rates, the differences which occur as a result of the genetic variation in target proteins of drugs and the interferences of medicines resulting from genetic differences. These chapters serve to explain the nature and extent of the genetic variation. No attempt has been made to summarize all of the present knowledge; for this, reference is made to the scientific literature. Annex B contains further details regarding the genetic differences in the rate at which medicines are metabolized. Annex C is a register of the medicines referred to in this advice.

The importance of pharmacogenetics varies for different specialist areas of medicine. Chapter 4 gives some examples of important implications in the field of psychiatry and oncology. For psychiatric complaints, pharmacogenetics is particularly relevant because the effects of many medicines take a long time to become apparent. Reliable predictions about these effects could lead to improvements in the use of medicines. For oncological complaints, the importance lies mainly in the serious nature of the side effects of many medicines. Some of the side effects are the result of genetic characteristics, and could possibly be avoided with the use of pharmacogenetic research.

In addition to the direct consequences for the prescription of medicines to particular groups of patients, pharmacogenetics could be significant for taking out insurance and for appointments to particular jobs, as indicated in chapter 5.

The enormous increase in knowledge on the human genome will result in many new pharmacogenetic insights, as well as a large number of contact points for the development of new medicines. Chapter 6 is devoted to this.

Patients should be informed about pharmacogenetic discoveries which are made with a view to the prescription of medicines. The final chapter deals with possible misunderstandings with regard to information based on these discoveries, and the required confidentiality.

Chapter **2**

The metabolism of medicines in the body

Genetic variation which is expressed in the enzymes which metabolize medicines can lead to various differences in the effects of those medicines. As a result of rapid metabolism, the medicine will leave the body sooner and have less or no effect. In the case of slow metabolism, the effective concentration is achieved sooner, but the chance of side effects is also greater. The best known enzymes which influence the metabolic rate are those of the type cytochrome P450 and some transferases. There are variants of these enzymes which can lead to rapid metabolism as well as variants which cause delays (Hod98).

Genetic variation can be determined by establishing the genotype or phenotype. In the first case, the measurements are taken at the DNA level. The form of a polymorphism (see chapter 1.2) can be determined in a patient by means of a DNA analysis. For some genes it is known to what extent polymorphisms are present in certain populations. Determining the genotype has the disadvantage that not all the genetic variation is usually detected. Analysis of the phenotype is generally understood to be the measurement or estimate of the activity of an enzyme, for example, by measuring the concentration of the medicine or a related metabolic product in blood. The phenotype can be influenced by factors other than genetic variation. For example, some enzymes are known to lead to induction (increased production) or inhibition, as a result of various substances.

In the metabolism of drugs a distinction is made between phase I and phase II enzymes. Oxidation, reduction or hydrolysis takes place under the influence of phase I enzymes, while phase II enzymes lead to the attachment of the medicine or the oxidized

product to another component (usually a substance in the body). In general, the conversions lead to a product which is more soluble than the medicine itself, so that it can be removed from the body faster.

2.1 Phase I enzymes

Most phase I enzymes are of the cytochrome type P450 (CYP). There are about sixty of these enzymes in humans (Nel96, Cha99, Wei99). The CYP superfamily is involved in a large number of metabolic processes of substances both in the body and foreign to the body. The enzymes of this superfamily are very important to render toxic food components harmless (Neb97). Polymorphisms of the genes which code for these enzymes can cause differences in the metabolism. For pharmacogenetics, it is important whether these differences result in a (strong) variation in the concentration of medicines in the blood (Ber98). For another enzyme in this group, CYP2D6, there are polymorphisms which lead to the slowing down of the metabolic rate in 8% of the Dutch population (Sch86, Tam99). The pharmacogenetic importance of CYP2D6 was first discovered in the examination of debrisoquine which reduces blood pressure. It showed that there can be a strong variation in the metabolism of debrisoquine (differences of a factor of 20 in the concentration of the breakdown product 4-hydroxydebrisoquine in the urine). The researchers then traced the genetic background of this variation (Idl79).

Oral legend has it that this polymorphism was discovered when the head of the pharmacology unit that was testing debrisoquine as an antihypertensive agent collapsed with vascular hypotension on taking a trial dose of the new drug. He was found to be a poor metabolizer (Omi99a).

Apart from forms of CYP2D6 which slow down the metabolism of various medicines, there are also forms with a greatly increased level of activity. In a study of psychiatric patients in the Netherlands, it was found that more than 3% were ultra rapid metabolizers (Ste98).

The metabolisms of various types of medicines is influenced by the enzyme CYP2D6, including anti-arythmic drugs, antidepressants, beta-blockers, and anti-psychotic drugs (Ber98, Tou98a, Eva99).

For the enzymes CYP2C9 and CYP2C19, there are also polymorphisms which are important for the rate at which medicines are metabolized (Lin97, Neb99, Wei99). Enzymes from the CYP superfamily, in particular CYP3A4, are also important for interactions between medicines (Bae99).

Approximately 3% of the population has 2 alleles of *CYP2C9* which code for an enzyme with a low level of activity (the slow metabolizers, Stu96, Yas99). This enzyme is important, inter alia, for the metabolism of tolbutamide and glipizide (medicines for the treatment of patients with non-insulin dependant diabetes). In fact, the genotype is not determined for the use of these medicines but the effect on the glucose levels determines any adjustment in the medication.

CYP3C19 is involved in the metabolism, for example of the anti-malaria drug proguanil (War91), and the stomach acid inhibitor omeprazole (And92). There are non-active variants of the enzyme; this means that 13-23% of Asians and 2-5% of Europeans are slow metabolizers (Kal86, Wil89, Mor94). While this leads to an insufficient effect with proguanil, omeprazole works better (against infections of Helicobacter pylori) in slow metabolizers (Fur98). More details about the medicines for which the metabolism is influenced by the enzymes of the CYP superfamily, such as phenytoin and diclofenac, are included in annex B.

The most important forms of the CYP superfamily can be determined with commercially available DNA chips. These chips could start to play a role in the near future, for example, in the policy on medication in hospitals. In some cases, this will make it possible to achieve the best dosages more quickly, or a non-effective medicine can be abandoned. However, in many cases, for example, in the case of anticoagulants and anti-diabetic medicines, determining the genotype is not particularly important because, as indicated above, the phenotype (concentration in the blood or clinical condition) also depends on environmental factors.

Enzymes other than the CYP enzymes also belong to the phase type, such as the NAD(P)H-quinonoxidase, an enzyme needed, inter alia, for anti-mitotic agents such as mitomycin to work (see chapter 4).

2.2 Phase II enzymes

The phase II enzymes carry out attachment reactions between various substances (endogenous as well as exogenous compounds), resulting on the whole, in more soluble products. Important enzymes for this connection include the N-acetyltransferases (NAT), which metabolize various medicines by combining an acetyl group with an amino group. Several forms are known (Cri91, Mey97, Bro00). NAT variants of type 2, which are very common in the population, have different levels of activity. Isoniazid, a medicine to combat tuberculosis, is an example of a medicine which can result in excessively high concentrations in the blood with a low degree of activity (Eva60). In order to oppose the side effects which occur as a result, pyridoxine is added for the treatment with isoniazid.

A second example is procainamide, which is used for heart attacks and has an increased chance of various serious side effects in slow metabolizers (Far00).

The effect of sulfasalazine for patients with systemic lupus erythematosus (an auto-immune disease) is possibly also dependent on the NAT genotype (Sab97). Various associations with cancer have been described for the polymorphisms of *NAT*, for example, with cancer of the colon. However, the significance of these associations is still insufficiently clear (Bad95, Bel95, Bro00).

Another enzyme which is important for phase II metabolism is the thiopurinemethyl transferase (TPMT). This is involved in the metabolism, amongst other things in anti-mitotic agents, such as mercaptopurine (see chapter 4.2) and the immunosuppressive drug, azathioprine. There are not (yet) any substances known in the body which are metabolized by the enzyme. Some forms of TPMT have been described which have a reduced level of activity. In Caucasians, almost 10% have a reduced activity, and the enzyme is inactive in 0.3% (Wei80, Col99a). The lack of activity is not associated with disease.

Azathioprine is used for transplants, immune diseases, or rheumatism (if other anti-rheumatic medicines are ineffective). Its immune suppressive effect is dependent on the metabolic product, thioguanine. However, the effect of the TPMT is to form the methylderivate instead of this. In the 0.3% of "inactives" referred to above this can therefore result in serious side effects, such as the suppression of bone marrow and disorders of the gastro-intestinal tract.

In addition to TPMT, there are also other methyltransferases which result in pharmacogenetic symptoms (Wei99). For catechol-O-methyltransferase there is a genetic polymorphism which results in the activity of the enzyme being three to four times lower than average in approximately one in four Caucasians. The level of the anti-Parkinson drug L-dopa, amongst others, is higher as a result (Wei77, Lac96).

More details on the pharmacogenetics of the NAT enzymes are given in annex B.

Phase II enzymes other than the transferases mentioned above are also important from the point of view of pharmacogenetics. The superfamily of UDP glycosyltransferases (UGTs) is composed of the UGT1 and UGT2 families (Mac97). These enzymes combine glucuronic acid and other sugars to various substances, both in the body and foreign to the body. One particular form of UGT1 is involved in the metabolism of the topo-isomerase inhibitor irinotecan (see chapter 4). Paracetamol is metabolized by various UGTs. There is a great deal of genetic variation in the UGT superfamily (Omi99d, Wil99), including the promoter of the *UTG1A1* (Hal99), and certain forms can lead to more or less severe hyperbilirubinemia (Rit92, Bos95).

Various glutathion-S-transferases and sulphotransferases could also be important for the metabolism of medicines. Polymorphisms have been described of these enzymes of which the significance is not yet apparent (Eva99, Wor99).

2.3 Transport proteins

Apart from the metabolisms by phase I and phase II enzymes, the availability of medicines in the body can vary as a result of genetic differences in transport mechanisms (Eva99). An example of a protein involved in this sort of transport is the serotonin transport protein (5-hydroxyltryptamine transporter, 5-HTT). Genetic variation which results in differences in the expression of this protein can influence the effect of certain antidepressants (see chapter 4.1).

Research is being conducted into the P-glycoprotein and other proteins which can remove cytostatics from cells. However, the significance of genetic variation is still not sufficiently clear.

It is probable that the knowledge on transport proteins will increase significantly as a result of genome research and targeted experimental research, for example, into ABC proteins (ATP-binding-cassette), which are necessary for a variety of transport processes. This knowledge could also result in more possibilities for the optimal use of medicines.

Chapter **3**

Target proteins and interferences by medicines

The variation described in chapter 2 concerns the rate at which different medicines are metabolized, which can result in differences in effectiveness and in side effects. A second type of genetic variation which can have this result concerns other proteins (for example receptors) which can be affected by medicines.

A variant is known of a receptor in the muscle cell which can lead to malignant hyperthermia. After administering an anaesthetic such as halothane, there is a strong rise in the patient's temperature, accompanied by stiffness in the muscles. In the past, most of the patients affected by this complication died; however, if adequate measures are taken to counter this, the patient usually survives (Den98). Malignant hyperthermia is the result of genetic mutation in the muscular membranes. When a mutation in a particular gene (the gene which codes for the ryanodine receptor in the muscle) was found for malignant hyperthermia in an animal, a variant of that gene was also traced in human patients (Fuj91, Gil91). The ryanodine receptor is known as the target protein of the medicine halothane. However, malignant hypothermia can also occur in people with variants in other genes (Den98, Rob97). In the Netherlands, a frequency is reported of 1 in 200,000 anaesthetics, which means that this complication occurs approximately five times a year (Sno97). Although it is possible to test for the sensitivity to malignant hyperthermia (a contraction test on the patient's muscle fibre), this test is only carried out in exceptional cases because of the low frequency in the population as a whole and its complicated nature (Sno97).

Another example of a target protein is the beta2-adrenergic receptor. Research into the effectiveness of salbutamol (albuterol) and formoterol, in patients suffering from

asthma led to the conclusion that polymorphisms in the gene for the beta2-adrenergic receptor play a role (Hal95, Lig00). The effect of these medicines, determined on the basis of the volume of forced exhaled air, is considerably higher in patients with the so-called “arg16” form than in other patients (Tan97 Lim99). Further research into this form is important, also in connection with the effect of other beta2-antagonists. In anti-asthma drugs such as zileuton, the effect of which is based on inhibiting the leukotriene synthesis, 5-lipoxygenase is the target protein; the effect depends on the genotype of that enzyme (Sil98, Dra99).

In other cases, the variation in the effect is not produced by a target protein but by unintentional interference. Genetic differences between proteins can result in a medicine disrupting a normal physiological process. An example of this is haemolytic anaemia (anaemia resulting from the loss of red blood cells) which can occur in people with variants of the glucose-6-phosphate dehydrogenase when they take anti-malaria drugs (Hoc52 Web57). Interference with the production of energy by mitochondria influences the chance of deafness induced by aminoglycoside in people with particular variants in the mitochondrial DNA (Pre93, Tor99). With this sort of variant, there is also an increased chance of deafness without the use of aminoglycoside; this chance is increased with the use of the medicine. It is not possible to draw a clear line between the pharmacogenetic effect and the occurrence of a hereditary disease.

The enzymes which are responsible for the metabolism and the proteins on which medicines can have an effect were discussed separately above. However, in the presence of polymorphisms, an undesirable effect can result in particular from the use of drugs if the patient has a combination of a slow metabolism and sensitivity to side effects (Eva99). While patients with only a slow metabolism build up an effective concentration of the medicine in the blood sooner than others, this can actually be a disadvantage for a patient who also suffers from such sensitivity. Therefore some researchers expect that in the near future, the administration of some drugs will take place only after various relevant DNA polymorphisms have been determined (Eva99, Goo99).

Chapter **4**

Implications for psychiatry and oncology

Pharmacogenetic knowledge is important for virtually every category of patients, but plays a particularly important role in psychiatry and oncology. In psychiatry, many medicines are administered for which it only becomes clear whether they benefit the patient concerned after some considerable time. In some cases, such as with anti-psychotic drugs, the chance of side effects is fairly large. Diagnostics often provide few indications for predicting the effect of a medicine in an individual case. Therefore, for psychiatry, it would be quite significant if it were possible to estimate the effects better on the basis of a genetic profile. In oncology the severity of the side effects of various cytostatics is a good reason for carrying out pharmacogenetic analysis. Many medicines are extremely toxic for tumour cells but are not very specific. This means that with a slow metabolism, high concentrations can build up in the body, causing a great deal of damage to normal cells.

In fact, there are relevant pharmacogenetic examples in virtually every specialist area of medicine. For example, genetic variations are important for the use of the medicines to treat asthma, salbutamol and zileuton, the inhibitors, enalapril, lisinopril and captopril of the angiotensin-converting enzyme (ACE), and the anti-diabetic drug tolbutamide (Ess96, Eva99). The effectiveness of salbutamol for asthma can vary because of differences in the target protein; this means that different doses are needed to achieve the same effect (Lim99). The use of zileuton leads to liver damage in 3% of the patients treated; currently, research is being carried out into the genetic basis of this side effect (Sti98). The pharmocogenetics of ACE inhibitors is being studied in order to optimize anti-hypertensive therapy (Kle97, Mat98, Oka99, Pin99). The concentration in

the blood of the anti-diabetic drug, tolbutamide, can vary between individuals because of genetic differences in the metabolic rate (Bha97, Kid99).

4.1 Antidepressants and antipsychotic drugs

Antidepressants and antipsychotic drugs are widely used medicines, partly because the disorders concerned are often of a chronic nature. Antidepressants can be divided into tricyclic drugs, serotonin-uptake inhibitors and other remedies. The effectiveness of these medicines is relatively similar, though there are differences in the side effects. The antipsychotic drugs can be divided into the conventional and the atypical drugs. There are several similarities between antidepressants and antipsychotic drugs. For patients suffering from severe depression, it is recommended to start with the tricyclic drugs, and for non-affective psychoses, with traditional antipsychotic drugs (NHG94, NVP98). If the therapy is not successful, the dosage should be increased. As indicated in 2.1, genetic variation in the enzymes CYP2D6 and CYP2C19 leads to differences in the metabolic rate of various tricyclical antidepressants. The former enzyme is also important for the metabolism of conventional psychotic drugs (Tou98a, Fan99, Fjo99, Som99). If the desired effect is not achieved with an increased dosage either, it is possible to transfer to other drugs (serotonin-uptake inhibitors or atypical antipsychotic drugs respectively). The same happens if certain side effects occur, such as tardive dyskinesis (a motor disorder) with the use of antipsychotic drugs. However, for some patients also other drugs are not effective. In addition, it is possible that various side effects will occur again. For the serotonin-uptake inhibitors, the above-mentioned serotonin transport protein is important for its effect. Because of the difference in the promotor of the relevant gene, there is a difference in the response to treatment (Sme98, Kim00). For the atypical antipsychotic drugs, the success of the therapy depends also on the genotype of the serotonin receptor 5HT2A (Arr98, Joo99). The side effect of tardive dyskinesis occurs particularly in patients with a particular form of the dopamine D3 receptor (Ste97). Agranulocytosis (the loss of white blood cells) is a potentially life-threatening side effect of the atypical antipsychotic drug, clozapine. The rate at which for example clozapine is metabolized can vary considerably (Ben98). Therefore regular blood tests are necessary for the use of this medicine. The chance of agranulocytosis is associated with a still unknown gene in the HLA area (Cor95). Determining a genetic profile before starting to administer antidepressants or antipsychotic drugs to a patient could therefore speed up the appropriate treatment in a patient, and prevent some of the side effects. For an analysis of the cost-effectiveness, it is important to know how much time can be gained, and how much this can influence the quality of life, apart from the price of the DNA test and the difference in price between the medicines concerned.

Other medicines used in psychiatry have also been subjected to pharmacogenetic research. For example, an analysis of the use of methylphenidate for patients with attention deficit hyperactivity disorder (ADHD), revealed a link between a certain polymorphism in the gene for the dopamine transporter and the response to the drug (Win99). Presently, the number of patients who were examined is too small to draw any definitive conclusions in this respect.

In the press, the possible results of pharmacogenetic research have been presented in a variety of ways. For example, the often optimistic description could be as follows (Sch98b):

> The doctor diagnoses severe depression and says reassuringly, "Now we'll just test your DNA to see what meds will work best for you," as he reaches over and plucks a hair from her head. "When we get the results this afternoon, I'll call in a prescription, and you'll soon be feeling better." For the first in a long time, she smiles hopefully.

However, even with the best results, pharmacotherapy with antidepressants and antipsychotic drugs is not effective for every patient. Therefore in addition to improvements with the use of current pharmacogenetic knowledge, the development of new pharmaceutical products is also desirable.

4.2 Anti-mitotic drugs

Side effects dependent on a genetic predisposition occur frequently with the use of oncolytics (cycostatics). In addition, new DNA mutations can develop in tumour tissue which are important for the choice of medicines to be used.

A classical pharmogenetic example in oncology concerns the enzyme thiopurinemethyl transferase (TPMT). As indicated in chapter 3, there are variants of TPMT with a reduced level of activity. Mercaptopurine, which is used for certain leukemias, is metabolized by TPMT. As with the use of azathioprine and other thiopurine derivatives, a low level of activity of the enzyme leads to a high concentration of the toxic thioguanine, which can lead to bone marrow suppression, amongst other things (Lar98, Iye98a). Therefore with the use of mercaptopurine, it is recommended to check the concentration of the metabolic products in the blood (Kry99). In the Mayo Clinic, approximately a thousand tests are carried out every year to determine the type of TPMT.

A more recent example is the cytostaticum irinotecan. It is metabolized by esterases into the active product SN-38, the effect of which is based on the inhibition of the topo-isomerase I. SN-38 is metabolized by the attachment of a glucuronic acid group by the phase II enzyme UGT1A1 (see chapter 3). The various genetic variants of

UGT1A1 lead to considerable differences in the metabolic rate of SN-38 (Iye98b, Iye99). These differences are caused, inter alia, by the variation in the promotor of the gene, as in Gilbert's syndrome, which occurs in approximately 6% of the population (Bos95, Hal99). The patients concerned have an increased chance of toxic effects from SN-38.

Mitomycin is an oncolyticum which is used for patients with cancer of the bladder. The enzyme NAD(P)H-quinonoxidase (a phase I enzyme, see chapter 3) is necessary to activate this anti-tumour medicine. An inactive allele of the enzyme occurs with frequencies of 0.16 and 0.49 in Caucasians and Chinese respectively (Gae98). On this basis, 2.5 and 24% homozygotes (carriers of two of the same, in this case, inactive alleles) can be expected in these groups respectively, for which the therapy with mitomycin is not effective.

Dihydropyrimidine dehydrogenase is an enzyme which is important for treatment using the antimetabolite fluorouracil. It is administered for carcinoma in the colon and rectum, and the toxicity of the medicine depends to a great extent on the activity of this enzyme. In the complete absence of activity, as is the case in a certain genetic metabolic disease, even low dosages produce serious side effects (Dia88). Therefore the great differences in the side effects of fluorouracil appear to be partly dependent on the considerable variation of the activity of the enzyme concerned (Iye98a, Luz93). Research is being carried out into the possible genetic causes of these differences in activity (Sti98). There is also research into drugs with a similar effect, but without the toxicity of the fluorouracil (Dia99). Another possibility of countering the effect of fluorouracil consists of the development of antimetabolites which inhibit thymidylate synthase (Dan99). However, for this enzyme also there are genetic polymorphisms (Mar99a).

Various other genes are also of interest to pharmacogenetic researchers, for example, aromatase (an enzyme in the CYP family), which is inhibited by various medicines for beast cancer, and the gene for the androgene receptor, in connection with the anti-androgenous effect of, for example, bicalutamide, for the treatment of prostate cancer.

It appears to be increasingly possible to predict the effects and side effects of oncolytics. It is expected that unravelling the human genome will result in these predictions becoming routine (Col99c):

> Drugs such as those for cancer will routinely be matched to a patient's likely response, as predicted by molecular fingerprinting.

The predictors appear to be thinking, in the first place, of the inherited DNA. However, another type of research, which is incidentally related to pharmacogenetics, is probably

more important. This concerns various genes in the tumour tissue, in which – sometimes considerable – changes occur. These changes are important for the nature of the tumour, and therefore the treatment and the prognosis. One example is the possible amplification in breast cancer tissue of the gene for the HER2 receptor. If this amplification has occurred, there may be a favourable effect with a treatment with antibodies against that receptor. Differences in the effect can also occur with inhibitors of the topo-isomerase I, such as camptothecin and the above- mentioned irinotecan (Tam91, Pon99). Changes in a series of genes can be examined for the prognosis of carcinoma in the colon and rectum, and in some cases the therapy can be adapted (Mcl99, Mid00).

It is probable that the intensive research into the changes in the DNA with the occurrence of tumours will stimulate the development of oncolytic drugs. The individual variation in the inherited genetic material is of less direct importance. Though the development of tumours is influenced by a genetic predisposition to a considerable extent, the choice of therapy will be determined primarily by the great differences in the tumour tissue.

The above suggests that greater knowledge about the genes which are important for the differences in the side effects and of the genetic changes in tumours will lead to the development of new cytostatics and to an increase in the number of DNA tests for the suitability of these drugs in individual cases.

Chapter **5**

Consequences for insurance and appointments

One important social issue arises from the fact that pharmacogenetic information could have consequences for insurance and for appointments in certain jobs. This information could indicate a subclinical problem, or an increased sensitivity to certain substances. It is part of the general question regarding the use and the privacy of genetic data.

With regard to data which are used to discover whether a medicine to be prescribed will have side effects, it seems unnecessary to take special precautionary measures. By determining the genetic variation concerned, the medication can be administered more effectively, and in general the data concerned have fewer implications than the predictions about monogenetic diseases. However, there are situations in which these data could give rise to a problem. One possibility is that the information could show that a patient requires expensive medicine. If this concerns chronic use, there could be considerable financial consequences. It is important to ensure that the possibility of taking out insurance does not change as a result, particularly when the insurance companies gain more influence on the medication to be administered.

Another possibility is that some data can be used in future for the prediction of risks of multifactor disorders. Examples of these possibilities are available: variants of *NAT1*, *NAT2*, *CYP2* and the gene for glutathion transferase have been related by some researchers to particular forms of cancer (Rob96, Neb97, Har97, Sch98a, Hen99, Omi99c, Bro00). For a polymorphism of the NADP(H)-quinonoxidase, mentioned in chapter 4.2 in connection with the anti-tumour medicine mitomycin, there is also an increased chance of leukemias and a particular type of kidney stones (Sch98c, Lar99).

For the beta2-adrenergic receptor, there are polymorphisms which influence the effectiveness of anti-asthma drugs (Hal95, Lig00). However, these polymorphisms can also be associated with other phenomena, such as obesity (Lar97). The determination of a genotype, for optimum treatment, can reveal the increased chance of a disease in these situations.

Further research will probably produce far more of these associations. The enzymes which metabolize medicines are also involved in the metabolism of other foreign substances. These also include toxic compounds, in which differences in metabolism produce different health risks. Proteins targeted by a particular medicine have a variety of functions. A variation in these proteins can result in side effects of medicines, but obviously also in a sensitivity to certain diseases. For example, the receptors targeted by antidepressants and antipsychotic drugs could be important for the chance of psychiatric disorders (Pro95, Odo99, Sch99). Therefore, clear rules governing the use of (pharmaco)genetic data are important.

In several countries, the regulations on the place of genetic data for taking out insurance are inadequate. Predictions on the basis of (pharmaco)genetic data can lead to conflicts of interest between the insured parties and the insurance companies and between insured parties themselves. For example, there have been warnings with regard to pharmacogenetic research about the possibility that insurance companies would only insure people with a particular DNA profile under special conditions (Sti98). A difficult situation which arises with regard to genetic data for insurance exists in the United Kingdom. Although it was assumed that there was a moratorium on the use of data from DNA research, insurance companies asked for extra premiums on the basis of these data (Dic99). In fact, in anticipation of the proposals of the Genetics and Insurance Committee, these additional premiums will be refunded for the time being.

There does not appear to be an easy solution for this conflict of interests. On the one hand, it is important to prevent the unjust exclusion of people on the basis of their genetic constitution (Wer99):

> In this context, an adequate protection of personal medical genetic data in social interaction is important, particularly for taking out private life insurance, individual disability and pension insurances and for the access to work.

On the other hand, insurance companies consider that selection is undesirable, i.e., for people to take out insurance on the basis of increased risks, which are known only to the persons to be insured. For example, a genetic profile could reveal the chance of early disability. If the person concerned then took out an extra insurance against this, this could lead to a problematical situation. The interests of other insured parties could

also be jeopardized. This conflict of interests is not resolved by merely providing adequate protection for genetic data.

The Council of Europe has adopted a convention on human rights and medicine containing the provisions (Cou96, Chapter IV, Article 12):

> Tests which are predictive of genetic diseases or which serve either to identify the subject as a carrier of a gene responsible for a disease or to detect a genetic predisposition or susceptibility to a disease may be performed only for health purposes or for scientific research linked to health purposes, and subject to appropriate genetic counselling.

The Genetic Non-discrimination in Health Insurance and Employment Act applies in the United States of America. On the basis of this act, genetic information may not be used for taking out sickness insurance or for the appointment, promotion or dismissal of personnel.

In the Netherlands, restrictions have been imposed by law on medical tests for appointments, and disability, pension and life insurances (Wmk97). Data from research into untreatable, severe disease which has not yet manifested itself, may not be used by law for these tests. There is also a moratorium on the use of data which have been obtained from genetic research (Poo99). This concerns research by or through a doctor at the chromosome or DNA level into hereditary characteristics. Co-operation with such research is not a condition for taking out an insurance policy. The moratorium applies for an indeterminate period, with a period of notice of two years. Exceptions have been made in the law and in the moratorium for insurances with high payments. However, the question is whether the law on medical tests is adequately observed in practice (Hel99). Research has been carried out on a small scale into the possibilities of taking out insurance for people with hereditary hypercholesterolemia. In approximately one in three people who were tested, problems arose which were virtually all related to life insurances for sums under the legal limit (Maa00). The situation can be unclear, for example, if a genetic characteristic is determined both at the level of DNA and on the basis of a gene product (a protein or metabolite).

Furthermore, medical tests are permitted by law if special medical requirements apply with regard to a particular job. This is relevant for pharmacogenetic research because of the possibility that people with a particular DNA profile could have an increased chance of becoming ill in certain jobs, such as paint firms or the chemical industry. Simply prohibiting the use of genetic data is not appropriate, because the use of this knowledge could prevent damage to health. It is not clear what should be done when an increased chance of illness is shown by DNA tests in employees who are already in employment. Further regulations are necessary with regard to the

above-mentioned problems in relation to insurance and appointments, for example, introduced by an independent commission such as the Genetics and Insurance Committee in the United Kingdom.

The recommendations of the Health Council have already argued the case for further regulations with regard to genetic research (GR89, GR94, GR98, GR99). The 1989 recommendation about the possible consequences for access to the labour market, has been partly incorporated into the legislation on medical tests. The recommendations on (non-)somatic DNA research – either in the form of screening or in the context of patient-related diagnostics – noted that such research can only be carried out under the current regulations by the clinical-genetic centres. According to the Health Council, DNA analysis should only fall under the responsibility of these centres if it is carried out for the purpose of genetic counselling (GR98, GR99). The increase in DNA research – not only with regard to pharmacogenetics, but also with regard to a variety of diagnostics, unrelated to genetic counselling – means that it is necessary for further regulations to be drawn up with regard to this research.

Chapter **6**

The development of new medicines

The increase in pharmacogenetic knowledge has important implications for the development of new medicines. It will be necessary to take into account the individual differences between patients to a far greater extent than has been done up to now (Kle98, Emi00). There are more and more possibilities to test (new) medicines for the metabolism by various enzyme systems and the interaction with polymorphous proteins. The pharmaceutical industry systematically tests the effects that can be expected with new medicines for a limited number of genetic differences. Computerized procedures have been developed, in particular for polymorphic enzymes, such as those in the CYP superfamily (Moo99). If it is to be expected, on the basis of pharmacogenetic data, that patients will present with a variety of reactions to a medicine being developed, it can be decided at an early stage to change to different substances. The laboratory costs of obtaining these data are slight in comparison with the total costs of the introduction of a medicine. Therefore some researchers expect that it will be possible to reduce both the length of time and the costs of the development of new medicines (Zuh98). In other words (Sch98b):

"If we could identify who will strongly benefit (from a drug), we could promote it to a defined segment of the population; that should also make it easier to show it's safe and effective," says Brian Spear, director of pharmacogenetics at Abbott Laboratories in North Chicago.

The possibility to investigate polymorphic proteins is increasing because there is a strong increase in the number of known polymorphisms. The most widely examined

polymorphisms are the single nucleotide polymorphisms or SNPs, a several thousands of which are now known. As indicated in chapter 1, approximately 100,000 SNPs will be determined with the help of a DNA bank of 450 different samples in the context of the Human Genome Project (Col97). At the same time, a consortium of ten large pharmaceutical industries will explore 300,000 SNPs with a subsidy from the Wellcome Trust, (about half of which will be localized in the genome, Mar99b). The great majority of SNPs are actually non-coding DNA; important because of the possibility of determining associations with diseases. The direct significance for pharmacogenetics lies in the polymorphisms which lead to change in the composition of the protein, which is the case for fewer than 5% of SNPs. Despite this low percentage, it is probable that there will be a marked increase in the number of known polymorphisms that are relevant for pharmacogenetics.

For the pharmaceutical industry it is also important that it will be possible to improve the position in the market for pharmaceutical products from the application of pharmacogenetic knowledge. This market is complex, and it is difficult to provide a new product with a clear identity; genetic profiling could increase its competitive position (Hou98). On the basis of these developments, some people expect a fairly far-reaching change; in an interview, an employee of a large pharmaceutical firm declared (Volkskrant, 6-2-99):

> Within ten years, the pharmaceutical industry will switch to selling medicines with a single general message on the sale of genetic information and genetic diagnostics, in combination with a suitable medicine for every patient.

It is difficult to predict how soon these developments will take place. In contrast to the optimistic predictions, there are also warnings that the development of new drugs will become less attractive (Lar98). Because of the application of pharmacogenetic knowledge, the population of patients in divided into increasingly small sectors, which could result in a large-scale orphan drug syndrome. The extra costs which would be incurred by testing the effects of genetic variation in the population of patients could also inhibit pharmaceutical development (Coh98). This is particularly the case if commissions for the evaluation of medicines – such as the Food and Drug Administration and the European Medicine Evaluation Agency – started to demand these tests on a large scale. In fact, this sort of demand could be restricted to the clinical trials, in which the medicine is tested. It seems likely however that the above-mentioned advantages could amply overcome these objections.

It is important to take into account also a very strong increase in the possibilities of developing new drugs in other ways. As a result of the application of combinatorial chemistry – methods for the synthesis of large series of substances – it is possible to

find out more quickly which substances are pharmacologically interesting (Eic95, Bur97). The determination of DNA sequences has produced a much larger number of reference points in this respect. Until recently, the composition of only a few hundred proteins was known. With the exploration of the human genome, this number has increased to tens of thousands.

Chapter **7**

Information for patients

Patients should be informed about (poli)clinical examinations carried out on them. Particular attention should be paid to this information if there is a danger of misunderstandings, which is often the case with regard to genetic aspects. The aim and scope of pharmocogenetic determination can be wrongly interpreted in various ways.

Publications in the media may give the impression that the cause of a disease almost always lies in a gene. The pharmocogenetic determination could serve to confirm this conception. This could give patients the idea that there is a sort of "gene passport", which would make it possible to predict all sorts of diseases with great certainty. Also, the misunderstanding could arise that the effects of a medicine can be predicted from the results of a DNA determination. In particular when new pharmacogenetic tests are introduced, attention should be devoted to the possibility of such misunderstandings arising.

With regard to the information given to patients, there is a difference between research in the context of patient care, and scientific research. With regard to patient care, pharmocogenetic research tackles questions such as "Which medicine will be most suitable in view of the genetic predisposition?" and "How much should be prescribed, bearing this predisposition in mind?" With a view to the above-mentioned misunderstandings, it is worth mentioning that the results cannot be used for the diagnosis and do not provide a definite answer about the prognosis (Ros00). In addition, the information should clearly indicate that the genetic data are confidential. With regard to the confidentiality of these data, it is often important to remember that they may also be relevant for family members. In some cases there should be consultation

with the patient and the clinical geneticist about the desirability of informing family members.

The above concerns (poli)clinical research: the information is obtained on the basis of a patient's request. However, in scientific genetic research the data are experimental and the clinical significance is not always clear. As not all the future implications of scientific research can be contained in the informed consent, some researchers argue that the results should not be simply included in the medical files of the patients concerned. The Privacy Workshop Planning Subcommittee of the National Action Plan on Breast Cancer in the US suggests assigning the genetic data from scientific research a separate legal status, and creating the possibility that participants in scientific research should not be informed of the results if the significance of the results is unclear, and the participants do not benefit from them (Ful99). It was considered that in some cases the researcher does not correctly assess the implications of his or her research either, and that the information could be an unnecessary burden for the subjects of the study. It is one of the tasks of the medical-ethical commissions which evaluate scientific research in the field of genetics in the Netherlands, to consider the question whether the (future) patient will benefit from a knowledge of the research results, and whether the inclusion of data in medical files has been adequately regulated.

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A Experts who were consulted

B Phase I and II enzymes

C Register of medicines which are discussed

Annexes

Annex **A**

Experts who were consulted

- dr PA Baede-van Dijk, pharmacochemist; Board for the Evaluation of Medicines, The Hague
- dr AF Cohen, professor of Medical Pharmacology; University of Leiden
- dr JJ Kastelein, internist; Academic Medical Centre, Amsterdam
- dr D Touw, hospital pharmacist and clinical pharmacologist; Academic Hospital of the Free University, Amsterdam
- dr J van der Weide, clinical chemist; Veldwijk Psychiatric Hospital, Ermelo, and St Jansdal Hospital, Harderwijk
- dr F de Wolff, professor of Clinical and Forensic Toxicology; University of Leiden

This report has been writen by dr PA Bolhuis, staff member of the Health Council of the Netherlands.

Annex **B**

Phase I and II enzymes

Phase I enzymes

Most phase I enzymes are of the type cytochrome P450 (Cha99, Wei99). This indication of the type is based on a band which is found in the spectrum at 450 nanometer after the binding of carbon monoxide to a characteristic group in the enzyme. The enzymes of this type form a so-called superfamily which comprises several families and subfamilies. In man, there are approximately sixty of these enzymes (Ne196). The nomenclature is based on the percentage of similarity between the enzymes. Within a family, there is more than a 40% similarity between the amino acids; in a subfamily, this is more than 55% similarity. Families are indicated with a number, subfamilies with a letter. The individual enzyme is also given a number, so that, for example, CYP2D6 is the sixth enzyme in the subfamily D, of the second family of the superfamily of cytochrome P450 (CYP). A gene which codes for a particular protein (for example, a particular enzyme) is shown in italics. Any forms of polymorphism and variants of a gene are indicated with a number after an asterisk.

The CYP superfamily is involved in a large number of metabolic processes involving both endogenous and exogenous substances. The enzymes of this superfamily are extremely important for rendering the toxic components of foodstuffs harmless (Neb97). Polymorphisms of the genes which code for these enzymes can cause differences in metabolism. For pharmacogenetics, it is important whether these differences lead to the occurrence of (greatly) divergent concentrations of medicines in the blood (Ber98). This is the case for various different enzymes, in particular for

CYP2D6, CYP2C9, and CYP2C19 (Lin97, Neb99, Wei99). Enzymes from the CYP superfamily are also important for the interactions between medicines, in particular, CYP3A4 (Bae99).

For the CYP2C subfamily, four genes have been localized in a cluster on chromosome 10, viz. *CYP2C8, CYP2C9, CYP2C19* and *CYP2C18* (Gra95, Omi99b). Various medicines are metabolized by enzymes of this subfamily, in particular by CYP2C19. Some examples include citalopram, indomethacine, papaverine, proguanil, teniposide, clomipramine, diazepam, hexobarbital, imipramine, moclobemide, omeprazole, phenytoin, propranolol, tolbutamide and warfarin (Ber98, Tou98a). The genetic variation in this subfamily was examined in detail for the enzymes CYP2C9 and CYP2C19. The enzymes which are coded by the alleles *CYP2C9*2* and *CYP2C9*3* have a lower activity. Almost four out of ten Caucasians have one of these alleles in combination with the normal allele *CYP2C9*1* (the intermediate type); approximately 3% have two alleles which code for an enzyme with low activity (the slow metabolizers, Stu96, Yas99). These alleles appear to occur less frequently in other populations (Wor99). The reduction in activity can be different for different medicines. For example, for the genotype *CYP2C9*3*, the metabolism of tolbutamide and phenytoin (diphenylhydantoin) is slowed down, but not that of diclofenac (Min98).

With users of warfarin, the chance of bleeding is significantly increased in the presence of this sort of allele of *CYP2C9* (Ait99). In addition, the introduction of therapy appears to lead to problems more often than with homozygotes with two alleles of the type *CYP2C9*1*. In the Netherlands, it is mainly the coumarin derivatives, acenocoumarol and phenprocoumon that are used as anti-coagulants. Phenprocoumon is also a substratum for the enzyme CYP2C9 (Hem99). As the effect of the anti-coagulants depends on several other factors such as nutrition and medication, the genotype is not determined, but the remaining coagulating activity is regularly checked.

The CYP2C9 is also important for the metabolism of the anti-diabetic drugs, tolbutamide and glipizide (medicines for the treatment of patients with non-insulin dependent diabetes). In this case the genotype is not determined either, but the effect on the glucose levels determines any modification of the medication.

CYP2C19 is involved in the metabolism of the anti-malarial drug proguanil (War91), and the gastric acid inhibitor omeprazole (And92), amongst other things. There are non-active forms of CYP2C19; as a result 13 to 23% of Asians and 2 to 5% of Europeans are slow metabolizers (Kal86, Wil89, Mor94). In Asia, 99% of these can be attributed to two deficient alleles; in Europe, this applies for 87% (Mor94). In addition, five other alleles are described as being inactive (Omi99b). In the Netherlands, the slow form of CYP2C19 is present in 2% of the population (Alv90, Tam99). The anti-malarial

drug proguanil must be metabolized as an active form (cycloguanil) by CYP2C19. It is likely that the treatment will have insufficient effect in slow metabolizers.

On the other hand, a course of omeprazole against infections of Helicobacter pylori appears to work better in slow metabolizers than in the intermediary type (with an active and an inactive gene), or the normal form (two active genes). Curing this condition by administering a mixture of omeprazole and amoxicillin for a particular period has resulted in success for these three types has resulted in success for respectively 100, 69 and 29% of the patients (Fur98).

More than one enzyme is important for the metabolism of some medicines. For example, for the above-mentioned anti-diabetic drug tolbutamide, it is not only CYP2C9, but also CYP2C19 that is important. For the metabolism of the anti-epileptic drug phenytoin, these two enzymes are also both involved (Mam98). Because the difference between an effective and a toxic concentration is very slight (and the link between the dose and the level is not linear), phenytoin is a medicine in which the level should be checked irrespective of the patient's genotype.

For another enzyme in this group, the CYP2D6, there are polymorphisms which lead to slower metabolism in 5 to 10% of the Caucasian population. In the Netherlands, this slow form is present in 8% of the population (Sch86, Tam99). A particular form of CYP2D6, for which the metabolism is slowed down, is found in 50% of Asians and much less in Caucasians (Hod98). The pharmocogenetic importance of CYP2D6 first became apparent from research into the medicine debrisoquine, which reduces blood pressure: there was a marked variation in the metabolism of debrisoquine (twentyfold differences in the concentration of the breakdown product 4-hydroxy debrisoquine in the urine. The researchers then checked the genetic background of this variation (Idl79).

In addition to forms of the CYP2D6 in which the metabolism of various drugs is slowed down, there are also forms of greatly increased activity. The increase is based on the duplication(s) of the gene (Mey94). On this basis, a distinction is made, depending on the nature of the CYP2D6 genes, between poor, extensive and ultrarapid metabolizers (Mey94). The frequency with which the various alleles are found was investigated in Germany and other places (Sac97). The most common allele has a frequency of 0.36, followed by an allele with a slightly reduced activity and a frequency of 0.32, and an allele without activity with a frequency of 0.21. Other alleles are found with frequencies lower than 0.02, including three duplicated alleles, two alleles with reduced activity, and various alleles with no activity. (Sac97). In a study of psychiatric patients in the Netherlands, it was found that more than 3% had a duplicated allele and were therefore ultra rapid metabolizers (Ste98).

The metabolism of various types of medicines is influenced by the enzyme CYP2D6, including anti-arhythmic drugs, antidepressants, beta-blockers and antipsychotic drugs (Ber98, Tou98, Eva99):

Table 1 Examples of drugs which are metabolized by the enzyme CYP2D6.

anti-arythmic drugs	propafeneon, encainide, flecainide, mexiteline
beta-blockers	alprenolol, metoprolol, propanolol
antipsychotics	perphenazine, thioridazine, flufenazine, haloperidol, zuclopentixol, risperidon
antidepressants	nortriptyline, desipramine, fluoxetine, paroxetine, sertralin, mianserin, maprotiline, imipramine, amitryptiline, clomipramine
analgetics	codeine

Phase II enzymes

The Phase II enzymes catalyse reactions between various substances, generally resulting in more soluble products. Important enzymes include the N-acetyltransferases 1 and 2 (NAT1 and NAT2), which metabolize various medicines by connecting an acetyl group to an amino group. There are various forms of both these enzymes (Cri91, Vat95, Mey97, Wor99). Although it is assumed that there are differences in the effects of medicines because of the polymorphism of *NAT1*, there is no clear information about the importance of this variation (Spi96, Gra97). However, some associations with certain forms of cancer have been described (Bad95, Bel95).

Dozens of forms of the enzyme NAT2 have been described (Mey97, Omi99c, Bro00). For example, for the use of isoniazid and hydralazine, it is important that there is a difference in the level of activity between forms of NAT2 which are very common in the population. Carriers of these forms are generally characterized as poor or rapid acetylators. Rapid acetylators are in the minority (30 to 40%) in Europeans, Africans and North Americans, but in the majority in Asians and Indians (80 to 90%), while South Americans fall in between (Cas95, Mey97). The poor acetylators are homozygotes, i.e., both their alleles of *NAT2* are of the slow type; on the other hand, the rapid form is achieved with both one and two alleles of the rapid type (Eva60). By determining the phenotype (the activity of the enzyme in relation to a particular substratum), it is relatively easy to establish whether a patient is a poor or a rapid acetylators (Cas95, Wor99).

Isoniazid is an example of a medicine which can reach an excessively high concentration in the blood because of the slow metabolism (Eva60). It is recommended as the basic therapy for tuberculosis in combination with other medicines (Far99). However, neurological disorders can occur as a side effect (drowsiness, concentration

disorders, affective disorders), and therefore patients in the Netherlands are almost always given pyridoxine.

Another example which is also well known is procainamid, which is used for heart attacks. Poor metabolizers have an increased chance of various serious side-effects (Far99).

In a study into the effect of sulfasalazine in patients with systemic lupus erythematosus (an auto-immune disease), considerable differences were found, depending on the genotype (Sab97). While seven out of eight patients who improved were rapid acetylators, the three in whom there was no progress proved to be slow metabolizers. Furthermore, there were more side effects in the latter group. Despite the small number of patients studied, the researchers consider that it is advisable to determine the genotype or phenotype of NAT2 before deciding to administer sulfasalazine. As for *NAT1*, associations with cancer have been described for *NAT2*, in particular with cancer of the colon. However, the number of patients studied was too low to draw any definitive conclusions (Bro00).

Another enzyme that is important for phase II metabolisms is thiopurinemethyl transferase (TPMT). This is involved in the metabolism of, amongst other things, anti-tumour drugs such as mercaptopurine and the immunosuppressant drug azathioprine. TPMT transfers a methyl group to various substances containing sulphur, including a number of medicines (Len83, Web97, Kry99). As yet, no substances in the body are known which are metabolized by the enzyme. It is possible that TPMT is involved in the metabolism of selenium and tellurium (Dor82, Cou98).

Some forms of TPMT have reduced activity. In Caucasians, three groups can be distinguished: more than 90% with normal activity, slightly less than 10% with reduced activity, and 3% in which the enzyme is inactive (Wei80, Col99a). In Asia, the normal form occurs in 95 to 98% of the population (Col99a). The absence of activity is not associated with any disease.

Azathioprine is administered in the case of transplants, immune disease and rheumatism (when other anti-rheumatic medicines are ineffective). The immune suppressive effect of azathioprine depends on the metabolic product thioguanine. However, the enzyme TPMT leads to the formation of the methyl derivative instead. As a result of the reduced activity of the enzyme, the same doses lead to a clearly higher concentration of thioguanine in the blood in approximately 10% of patients, and in a marked increase in 1 in 300 patients. These increases can lead to severe side effects, such as bone marrow suppression and disorders of the gastro-intestinal tract. Similar symptoms occur with the use of mercaptopurine (see chapter 4.2).

The most common polymorphisms of *TPMT* are relatively easy to determine with the help of a method based on the polymerase chain reaction (PCR). It is also possible to determine the phenotype by measuring the activity radiochemically, though more

laboratory facilities are required for this. Research into the genotype of patients who had to stop the therapy with azathioprine because of the side effects, led to the conclusion that a PCR analysis in advance incurs considerably lower costs than the analysis of concentrations in the blood and the treatments which had to be broken off (Bla98). In the 10% of patients with a reduced activity of the TPMT, a modified dose could be administered (Bla98).

As indicated in chapter 2.2, there are other methyltransferases apart from TPMT which also lead to pharmacogenetic effects (Wei99). For catechol-O-methyltransferase there is a genetic polymorphism, which means that in approximately one in four Caucasians the activity of the enzyme is three to four times lower than average. With the same dose, the lower activity means that the concentration, for example, of the anti-Parkinson drug, L-dopa, is higher (Wei77, Lac96).

Phase II enzymes other than those of the above-mentioned transferases are also important from the point of view of pharmacogenetics. The superfamily of UDP-glycosyl transferases (UGTs) comprises the UGT1 and UGT2 families (Mac97). These enzymes link glucuronic acid and other groups of sugars to various compounds. A particular form of UGT1 is involved in the metabolism of the topo-isomerase inhibitor, irinotecan (see chapter 4). Paracetamol is metabolized by various UGTs. There is a great deal of genetic variation in the UGT superfamily (Omi99d, Wil99), for example in the promotor of *UTG1A1* (Hal99), and certain forms can lead to more or less serious hyperbilirubinemia (Rit92, Bos95).

Various glutathion transferases and sulphotransferases could also be of importance for the metabolism of medicines. Polymorphisms have been described of these enzymes, although their significance is not yet clear (Eva99, Wor99).

Annex **C**

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