The role of pharmacogenetics in drug-induced toxicity

Is there anybody today who does not use any medication? The total turnover of pharmaceutical care in the Netherlands in 2014 was 4.3 billion euros. Medication costs make up 9.8% of the total healthcare costs. The Dutch spend a mean of 253 euros on medication per person per year, the over 65-years of age category spending more than twice that much namely 604 euros. This article discusses some of the aspects of side-effects of drugs, and also presents a possible explanation why drugs sometimes fail to cure the disorders they are intended for.

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Doctors normally prescribe a standard dosage of a medication for a particular disease. However, they are frequently confronted with patients responding differently to medications in terms of effect and side-effects, which cannot be explained purely on the basis of differences in dosage. Many patients suffer from more than one disorder, requiring them to use several medications simultaneously. These may counteract each other, while in other cases they may reinforce each other's effects. Such interactions feature frequently in patient information leaflets and databases on drug characteristics.

In the context of lung diseases, 'drug-induced' toxic reactions in the lungs are a well-known phenomenon, see also www.pneumotox.com for specific drugs and possible consequences. Such reactions by the pulmonary tissue may make it harder for the lungs to absorb oxygen. So medications and toxic substances that are breathed in can have literally breath-taking effects on the lungs. One of the possible explanations for side-effects lies in the patient's genetic profile. Research to find out whether differences in the efficacy and side-effects can be explained by genetic profiles is the subject of the discipline of pharmacogenetics. Orally administered drugs are absorbed into the bloodstream in the intestinal epithelium. The body will then try to eliminate these substances, which are usually lipophilic (i.e. poorly dissolvable in water), as quickly as possible. It does this by converting them into hydrophilic compounds, which

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dissolve in water, a process involving so-called phase I and phase II reactions.1 Cytochrome P450 (CYP450) has a major role to play in this biotransformation by catalysing the phase I reactions. The best-known application of phase II reactions is in the genotyping of thiopurineS-methyltransferase (TPMT), in order to predict the metabolisation and hence the toxicity of azathioprine or 6-mercaptopurine. This article focuses on the cytochrome P450 system.

Classification

The CYP450 system encompasses 75 families (indicated by a number) each of which has its own subfamilies (indicated by a letter) and individual isoforms (indicated by a number). Specific alleles that provide clues to the presence or absence of a mutation are indicated by an asterisk and a number (sometimes in combination with a letter, for e.g. *1A). An example is CYP2D6*1/*3, where CYP stands for cytochrome P450; 2 means that it belongs to a particular family, in this case sharing a 40% homologous protein sequence; D is the subfamily; 6 is the isoenzyme and represents a single gene; and *1/*3 indicates the specific alleles (Figure 1). Usually, *1(A) indicates the 'wild type', but it is always the code for a fully functional allele.[2] Only 4 of the families are relevant within the human detoxification system: CYP1, CYP2, CYP3 and CYP4. The 4 isoforms which are involved in approximately 80% of the oxidation of exogenous compounds are CYP2C9, CYP2C19, CYP2D6 and CYP3A4.

Phenotype and genotype

A person's phenotype forms the expression of their genotype, and is determined by the combination of their genetic information and environmental factors. A person's genetic information in principle involves 2 alleles of each gene, one derived from each parent, in a Mendelian inheritance pattern. There may, however, be mutations that lead to multiple or different alleles. This then affects the functioning of the protein in which the gene is expressed. If genotypic differences are found in more than 1% of the population, the term polymorphism is used.

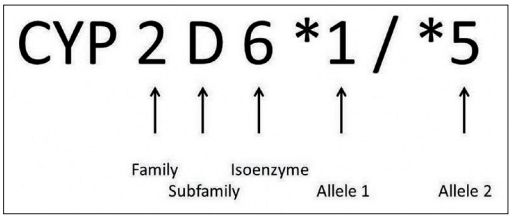

Figure 1. *Naming of CYP450 genes. The fully functional allele is indicated with *1, whereas the variant alleles are indicated with higher allele numbers and result in an aberrant functioning enzyme.*

Polymorphism in the CYP450 system

A polymorphism in the CYP450 system can lead to aberrant expression of the enzymatic catalysts. A drug that is taken by a patient will initially be metabolised by a phase I enzyme. For most medications, we now know which CYP450 system (or systems) converts them (see e.g. www. drug-interactions.com). Conversely, medication, but other substances as well, can also influence the action of various CYP450 systems. Examples of such other substances include stimulants like alcohol, nicotine, caffeine and marihuana, but also, for instance, grapefruit.[1,3,4] In recent years, newly developed molecular techniques, especially the

polymerase chain reaction (PCR), enable relatively simple DNA analyses for individual patients, in order to determine whether they might respond to certain drugs in an aberrant way (Figure 2). Polymorphisms in the CYP450 system can in principle lead to four different metabolising classifications, based on the functionality of the enzymes.

The most frequent manifestation or 'wild type' is usually an enzyme with normal activity, corresponding to a homozygous and functional allele presentation. A person sporting this form is called an 'extensive metabolizer' (EM), see also the above section on 'Classification'. A

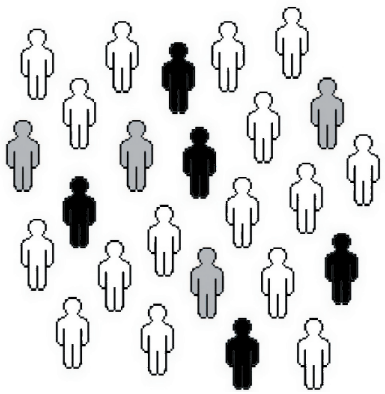

Figure 2. Population of different responders.Patients with the same diagnosis and treated with the same medication. Most patients react well (white), some of the patients do not respond to the therapy (grey), and some react with adverse reactions or toxicity (black).

heterozygous allele group, in which one allele is deficient due to a polymorphism while the other allele is normal, results in the enzyme's function being reduced but not absent, and a person presenting with this genotype is called an 'intermediate metabolizer' (IM). If two inactive DNA variants are inherited, however, this can result in a non-functioning system, and people with this defect are characterised as 'poor metabolizers' (PMs). Finally, genes may also be duplicated, resulting in very rapid enzyme action converting medications very quickly. These people are known as 'ultra-rapid metabolizers' (UM). An example of the difference in metabolic rates or concentration-time curves between the different metabolizers is shown in Figure 3.

Clinical practice

How long will it be before a patient enters a pharmacy and is asked to show their 'genetic medication passport'? Such a document would record things like the patient's

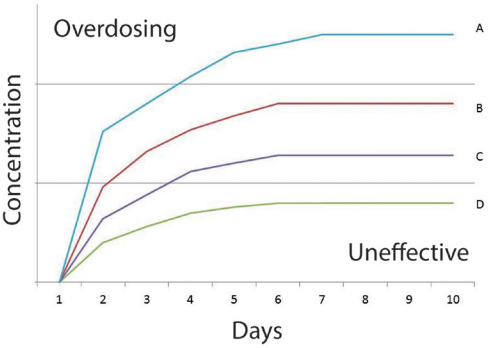

Figure 3. Simplified concentration-time curves of genetically different persons reacting to the same medicine dosage. A: poor metabolizer (PM), absent or severely reduced enzyme activity; B: intermediate metabolizer (IM), reduced enzyme activity; C: extensive metabolizer (EM), normal enzyme activity; D: ultra-rapid metabolizer (UM), enhanced enzyme activity.

polymorphisms, and would enable the pharmacist to decide on the best dosage and combination of medications for this patient, purely based on their genetic profile. Of course, the patient's age, physical condition, diet, etc. would also play a major role. It is perfectly clear that such a passport will sooner or later be introduced, the only question is when and for what purposes. Obviously, this will only be possible if the system is based on sufficient 'evidence-based' research.

Case reports

A 42-year-old woman was visiting the outpatient hepatology clinic because of progressive fatigue, dyspnoea, paraesthesia's of the fingers, hands and legs. She felt agitated and had an elevated heart rate in rest of 110 beats per minute. She was known with morbid obesity (BMI 46 kg/m^2), arterial hypertension, left ventricle hypertrophy with a decreased left ventricle ejection fraction (40% of predicted), depression and non-alcoholic steatohepatitis.

At that time haematological and biochemical laboratory investigations revealed no abnormalities. However, the chest X-ray showed discreet diffuse reticular abnormalities and lung function testing revealed a decreased diffusing capacity (61% of predicted).

Two month before presentation at the outpatient clinic, the psychiatrist started venlafaxine 75mg to treat her depression. As no mental improvement occurred, the venlafaxine dose was increased gradually to 225mg daily, but still no improvement was established, moreover the patient developed a worsening of her situation with increasing fatigue, paraesthesia's, dyspnoea, agitation and tachycardia. These adverse effects coincided the most with the side effects listed for venlafaxine, moreover, this drug is known to cause serotonin-syndrome in some cases (table1).[5] This prompted us to measure the serum venlafaxine level and its main active metabolite ODV which were 1300 µg/L and <100 µg/L, respectively, well above the recommended range of 195 to 400 µg/L.[6] At the same time, three common CYP450 polymorphisms (CYP2C9, CYP2D6 and CYP2C19) were genotyped, as described previously.[7] These tests were performed to rule out or establish a genetic component. Moreover, they were used to investigate whether any drug-drug interactions might be causing additional problems. The patient appeared to be a PM for CYP2D6 when the genotype CYP2D6*4/*4 was found, indicating that no functional CYP2D6 enzyme was present. Furthermore, CYP2C9*1/*2 was also found, making her an IM for CYP2C9. Mainly the fact that the patient appeared to be a PM of CYP2D6-substrates explained the high toxic serum levels of venlafaxine without the presence of its main active metabolite ODV. Venlafaxine was stopped immediately and the metoprolol she was on, also metabolized by CYP2D6, was stopped as well. Gradually, her complaints of agitation, dyspnoea and tachycardia disappeared. A control chest X-ray performed 6 weeks later did not show any of the earlier described abnormalities.[8,9]

A 34-year-old woman was admitted to our hospital. She was suffering from dyspnoea, malaise, hypoxia, coughing with episodes of a little haemoptysis and exacerbation of asthma. Her chest X-ray showed a discreet diffuse interstitial damage. She was an irregular cocaine user (again two weeks before the last admission). Physical examination revealed some crackles at auscultation, she was tachypnoeic (25 breaths/min) and her body temperature was 37.8°C. She had a hypoxemia (pO_2 7.2kPa, breathing room air), a serum C-reactive protein of 12 mg/L, a white blood cell count of 7.4 10^9/L and a normal number of eosinophils. The urinary test for Legionella pneumonia and Streptococcus pneumoniae was negative as well as the serology for common viruses, Mycoplasma, Rickettsia and Chlamydia. She has been HIV positive for three years now. She was diagnosed with cardiac failure and had a myocardial infarct (MI) due to cocaine-associated ischemic injury one month before this admission. After the MI she was prescribed aspirin in order to prevent clot or thrombocyte aggregation forming and methadone to control her addiction, both these drugs are known to influence the coagulation. In addition, relevant polymorphisms were profiled. This revealed that she appeared to be an IM for CYP2C9 (CYP2C9*1/*3) and VKORC1 heterozygote (GA/CT). She was treated with corticosteroids and her

clinical condition improved within a few days, her hypoxia normalized as well as the chest X-ray (Figure 4a and 4b). When triggered by (illicit) drugs as in this case, an over-anticoagulation and subsequent diffuse alveolar damage may occur. Moreover, in the presented case the influence on the vitamin K cycle was strengthened by the presence of CYP2C9 allelic variant combined with the CYP2C9 inhibition by cocaine use.[10-12]

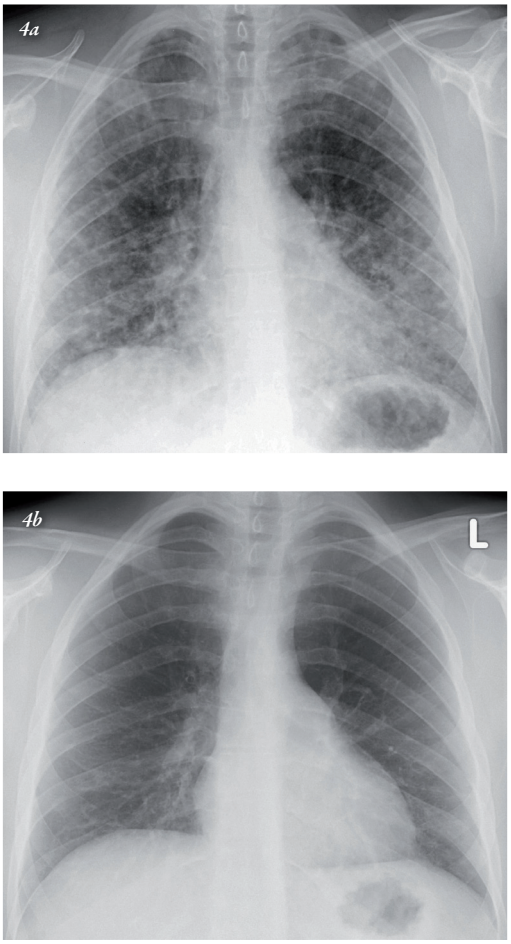

Figure 4a and 4b. *Chest x-ray of case 2.*

3a: Taken at presentation, showing a widespread nodular pattern with mid-zone and lower-zone predominance. 3b: Follow-up chest x-ray of case 2, taken 2 weeks after presentation, without the abnormalities found earlier.

These case histories illustrate that doctors should be careful when prescribing multiple medications that are metabolised by the same CYP450 system. The dosage should be reduced, or, if possible, a different drug should be selected. If not, a significant accumulation of the medication may arise, which may lead to toxic serum levels and serious side-effects.[13] An example: Of the top 100 of frequently prescribed drug in the Netherlands, 15 are metabolised by CYP2D6 and only 3 of them are not listed on the pneumotox.com website as a possible cause of drug-related lung injury. In general, checking CYP450 polymorphisms will only be considered if no explanation is found for the adverse side-effects.

Daily clinical practice

There are some situations, however, where polymorphism analysis is already a routine procedure in clinical practice. A well-known example is CYP2D6 in psychiatry, but CYP2C9 and VKORC1 assessments are also used for patients who have to have anticoagulant treatment with coumarins, and CYP2D6 genotype screening as an indicator for the treatment with tamoxifen is currently being introduced.[14] There is already a US website online about this subject (www.youscript.net by genelex.com), where the expected serum concentration of medications in individual patients can be estimated based on the patient's CYP450 polymorphism. In the Netherlands, the Royal Dutch Pharmacists' Association KNMP is currently developing a pharmacogenetic website, for which many interactions and polymorphisms have to be identified. After completion, this program should provide a major impetus to pharmacogenetics as a discipline in the Netherlands. Pharmacogenetics is currently a booming discipline. Not only is the number of scientific publications rising rapidly, but clinical applications are being developed.[13] In the case of drug-induced interstitial lung disorders, research has shown that patients with this condition have more CYP450 polymorphisms than the healthy population.[10]

Practice recommendations

Pharmacogenetic knowledge is of major clinical importance in determining individual patients' sensitivity to a particular medication, and is essential for anyone who is responsible for prescribing drugs. Polymorphisms of the CYP450 system and other systems can lead to unexplained drug reactions, side-effects or even toxic reactions.[15] If a doctor is unable to explain such a reaction, pharmacogenetics may be able to offer a solution. A number of specialised laboratories, especially in university medical centres, now offer the option of having patients genotyped for the most common CYP450 polymorphisms. Within the foreseeable future, this knowledge will also be used in general practice to provide personalised and tailored advice on medication, making 'individualised medicine' an indispensable part of medicine. A genetic medication passport will be able to prevent much drug-induced suffering in the future.

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