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Future of Pharmacogenetics in Cardiovascular Diseases

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1. Introduction

Pharmacogenetics is the study of variations in DNA sequence as related to drug response (European Medicines Agency [EMA], 2007). Several gene-drug interactions have been discovered in the field of cardiovascular diseases (CVDs). These gene-drug interactions can help to identify nonresponse to drugs, estimate dose requirements or identify an increased risk of developing adverse drug reactions. An individualized approach based on pharmacogenetic testing will provide physicians and pharmacists with tools for decision making about pharmacotherapy. While pharmacogenetic testing is already part of everyday practice in oncology, it is not widely implemented in the field of CVDs. However, in the near future, pharmacogenetics will probably also play a valuable role in this field as well.

1.1 Complexity of pharmacogenetics of CVDs

Prophylaxis and treatment of CVD is complex. Patients often have more than one cardiovascular risk factor (e.g. hypertension and hypercholesterolemia) and/or CVD, or other comorbidities such as diabetes mellitus. Frequently, more than one drug is used by the patient and this may potentially lead to serious drug interactions with adverse health outcomes. Therefore, not only the comorbidities but also the interaction between co-medications should be taken into account if a pharmacogenetics based dosing strategy is developed.

1.2 The aim of this book chapter

The aim of this book chapter is to describe and explore several examples of gene-drug interactions in CVD, the factors that affect the implementation in clinical practice, the cost-effectiveness analysis of pharmacogenetic testing, and the development of new technologies that could improve research of pharmacogenetic interactions in CVD.

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2. Examples of pharmacogenetics for cardiovascular diseases

Cardiovascular drugs are widely used for prevention or treatment of CVD. Gene-drug interactions were demonstrated in the treatment with platelet inhibitors, anticoagulants, antihypertensive drugs and statins. The findings of the many studies that have been conducted on pharmacogenetics of antihypertensive drugs, are not suitable for clinical implementation, often because the results could not be replicated or the clinical relevance was low (Arnett & Claas, 2009). The most commonly prescribed drugs in the management of CVD with important gene-drug interactions are statins, clopidogrel and coumarin derivatives. These three drugs are candidates for pharmacogenetic testing in everyday practice and will be discussed in more detail below.

2.1 Statins

Patients with hypercholesterolemia have an increased risk of CVD. Statins are widely used to treat hypercholesterolemia and prevent CVD. This treatment, often accompanied by lifestyle changes, has been proven to be effective and safe, but the efficacy varies among patients (Pearson et al., 2000). The effect of statins depends on the statin concentration at the site of action, the liver. This concentration can be altered by several factors, like diet and concomitant medication (Romaine et al., 2010). Muscle symptoms are a common problem during statin use ranging from mild myalgia to severe rhabdomyolysis (Law & Rudnicka, 2006). Although muscle symptoms are generally not life-threatening, they can negatively affect the patient's quality of life and also his or her adherence to statin therapy (Peters et al., 2009).

Several transporters play a role in the access of statins in the liver. Multiple studies have demonstrated a role for statin transportation by the organic anion transporter polypeptide 1B1 (OATP1B1) (Niemi, 2007; Pasanen et al., 2006), which is encoded by the *SLCO1B1* gene. An impaired hepatic uptake of several statins has been shown for patients with a specific single nucleotide polymorphism (SNP) in this gene, namely the *SLCO1B1* c.521T>C SNP. A decreased effect of statins is therefore seen in patients with this variant allele. This effect was shown in users of atorvastatin, pitavastatin, pravastatin and rosuvastatin in some studies, while others could not find a significant or clinically relevant effect (SEARCH Collaborative Group, 2008; Voora et al., 2009).

The impaired hepatic uptake causes an increased plasma concentration of statins, which probably causes a higher rate of adverse events. Carriers of a c.521C allele show an increased risk of developing myopathy after simvastatin use. Because this SNP does not seem to influence plasma concentration of fluvastatin, this could be an alternative for patients at risk of simvastatin induced muscle symptoms (Niemi et al., 2006). Genotyping before starting statin therapy might help to choose the right statin. Carriers of a variant allele could then be identified and treated with a *SLCO1B1* genotype independent statin, for example fluvastatin. In this way, genotyping for this *SLCO1B1* SNP may increase the safety of statin therapy. This approach of determining the most optimal therapy has not yet been investigated in a clinical trial.

2.2 Clopidogrel

Clopidogrel is a platelet inhibitor (PI), used together with aspirin to treat patients after percutaneous coronary interventions. This dual antiplatelet therapy reduces the risk of stent

thrombosis, myocardial infarction, stroke and cardiovascular death. Clopidogrel monotherapy may be used for secondary prevention of atherosclerotic complications, in case aspirin can not be used, for example due to allergy (Anderson et al., 2010).

Clopidogrel is administered to patients as a prodrug. It needs to be metabolized by several hepatic cytochrome P450 (CYP) enzymes in order to form the active platelet aggregation inhibiting metabolite. This is done in two steps. During the first step, the intermediate 2-oxo-clopidogrel metabolite is formed. In this step three isoenzymes (CYP1A2, CYP2B6 and CYP2C19) are involved. During the second step this metabolite is hydrolyzed into the active thiol derivative R-130964, which blocks the ADP P2Y12 receptors on the platelet surface, causing inhibition of platelet aggregation. This step is catalyzed by four isoenzymes (CYP2B6, CYP2C9, CYP2C19 and CYP3A4) (Yukhanyan et al., 2011).

Although the effectiveness of clopidogrel has been demonstrated in many trials, variation in response is still an issue. Some patients experience cardiovascular events despite dual antiplatelet therapy (Yukhanyan et al., 2011). This difference in risk of cardiovascular events is genetically determined. In addition, response-variability is also caused by a genetically determined difference in platelet aggregation (Harmsze et al., 2010a). The interindividual variability in response to clopidogrel can be explained by multiple genetic and environmental factors. Variation in response to clopidogrel related to genetic variability in the *CYP2C19* gene has been investigated thoroughly, as the CYP2C19 enzyme plays an important role in both metabolizing steps (Anderson et al., 2010). In several studies a relationship between carriage of a loss-of-function allele in the *CYP2C19* gene and the occurrence of adverse cardiovascular events has been demonstrated. Up to now, more than 33 alleles of the *CYP2C19* gene have been identified. Most of these are rare in the general population. The most common allele in the European population is *CYP2C19**1. The enzyme encoded by this allele enables extensive metabolizing of clopidogrel into the active metabolite. A common variant allele is the *2 allele. Patients carrying at least one of this variant allele have a decreased activity of the CYP2C19 enzyme. This leads to a reduced plasma concentration of the active metabolite and possibly to an increased risk of recurrent cardiovascular events. Knowledge of the *CYP2C19**2 genotype can explain approximately 12% of the variation in response to clopidogrel. An increased risk of stent thrombosis has been demonstrated in carriers of a *CYP2C19**3 allele. Both carriers of a *2 of a *3 allele have a decreased enzyme activity, resulting in a lower amount of active metabolite (Harmsze et al., 2010b). The *CYP2C19**17 allele however, encodes for a more active enzyme. Carriers of this allele therefore have an increased antiplatelet response to clopidogrel. This might be associated with an increased risk of bleeding (Yukhanyan et al., 2011; Zabalza et al., 2011).

Pharmacogenetic testing for the *2 or *3 variant alleles could identify patients that are less likely to respond to clopidogrel and who might benefit more from treatment with an alternative, more expensive PI such as prasugrel or ticagrelor. Prasugrel and ticagrelor have less variability in response than clopidogrel, mainly due to a smaller influence of genetic variations. However, patients using prasugrel or ticagrelor have an increased risk of bleeding compared to patients using clopidogrel (Jakubowski et al., 2011; Collet & O'Connor, 2011). At the moment, randomized controlled trials (RCTs) are ongoing to evaluate the (cost) effectiveness of pre-treatment genotyping (Crespin et al., 2011). Based on the results of these trials, physicians can decide whether or not to prescribe clopidogrel or another PI on the patient's genotype.

2.3 Coumarin derivatives

Oral anticoagulants of the coumarin type are used to treat and prevent thromboembolic events in patients with different conditions, including venous thromboembolism and atrial fibrillation (Ansell et al., 2008). The effect is monitored by the International Normalized Ratio (INR), which should be kept within a certain range (for example, the range for atrial fibrillation is between 2.0 and 3.0). Wide interpatient variability in dose requirement means that the dosage is difficult to predict and frequent monitoring of the INR is necessary. INR values below the therapeutic range increase the risk of thromboembolic events while a supratherapeutic INR leads to an increased risk of bleeding events. These bleeding events can range from minor bleedings to major, life-threatening bleedings such as intracranial hemorrhage (James et al., 1992).

The wide variability in dose requirement is caused by several factors. Dietary intake of Vitamin K, comorbidities (e.g. altered thyroid function), concomitant medication, sex, age, height and weight all influence the required coumarin dose. Also genetic factors are shown to have an important role (Custodio das Dores et al., 2007; Penning-van Beest et al., 2001; Torn et al., 2005). First the influence of the *CYP2C9* gene, encoding the main metabolizing enzyme, cytochrome P450 2C9 (CYP2C9) was discovered. Carriers of a *2 or *3 allele require a lower dose and have an increased risk of overanticoagulation, which is associated with an increased risk of bleedings (Schalekamp, 2004). A few years later was discovered that with the *VKORC1* gene, encoding the target enzyme vitamin K epoxide reductase multiprotein complex 1, even a larger part of dose requirement variability could be explained. *CYP2C9* and *VKORC1* together explain approximately half of the variation in coumarin dose requirement (Schalekamp & de Boer, 2010; van Schie et al., 2011).

Currently, most patients receive an identical initial coumarin dosage. After a few days, the response is evaluated by INR measurement. The dose can then be adapted to the patient's needs. If patients are genotyped before starting coumarin therapy, they can receive a genotype-guided dose from day 1 on. This is suggested to prevent overanticoagulation in carriers of a variant allele and to reach a stable dose earlier. RCTs are currently ongoing to provide evidence for the (cost) effectiveness of pre-treatment genotyping for coumarin derivatives (van Schie et al., 2009; French et al., 2010).

In addition to the three mentioned examples, we expect more pharmacogenetic interactions will be found to be clinically relevant in CVD therapy.

3. Pharmacogenetic testing

Pharmacogenetic testing is thought to increase the efficacy and safety of drugs. However, for CVD, pharmacogenetic testing is not yet established in daily practice. Currently ongoing RCTs will hopefully provide evidence to implement pharmacogenetic testing in daily practice. However, implementation of a pharmacogenetic approach of a treatment depends on many different factors that extend beyond the outcomes of RCTs. These factors will be discussed in this paragraph.

3.1 Clinical trials to provide evidence

At this moment, a pharmacogenetic approach to determine the appropriate therapy for an individual patient is not yet widely used. There are currently only a few therapies where

genotyping is used to establish the right dose or make a decision about which drug to use. Pharmacogenetic testing has not yet been used extensively since physicians are still hesitant about genotyping. Although physicians are willing to customize the therapy for an individual patient based on the patient's genetic profile, their capacity to do so is limited by their time and complexity of the procedure (Levy & Young, 2008). However, genotyping may provide physicians with tools for optimizing drug treatment for the individual patient. In other words, it could provide the physician with information on the individual reaction of the patient to the medication or the dose, comparable to what liver- and kidney function tests provide them with. These function tests were implemented in clinical practice without evidence for their added value from clinical trials. However, it is unlikely that pharmacogenetic tests would be implemented without RCTs, because of considerable uncertainty surrounding their efficacy and overall health outcome impact. These RCTs are therefore needed to convince physicians of the added value of genotyping the patient.

Currently, a number of RCTs are underway to hopefully provide evidence of improved efficacy and safety by genotyping the patient and using this information to individualize the treatment. Use of the search term "pharmacogenetics" on clinicaltrials.gov, a website where clinical trials are registered, produced a list of 361 studies (performed on 15 August 2011). Of these studies, 117 studies were interventional studies seeking new volunteers. In contrast, use of the term "cardiovascular" produced a list of 20,123 studies. However, only 61 studies were found after combining the search term "pharmacogenetics" with "cardiovascular". Of those 61 studies, 4 were being performed for statins, 4 for clopidogrel and 18 for the coumarin derivatives. This suggests that pharmacogenetics is currently only a minor research field in clinical trials and that most of the activity in that field is on coumarin derivatives.

Although thorough research is currently being performed to investigate the added value of genotyping on the efficacy and safety of drugs, it is not feasible to conduct a clinical trial for each newly found gene-drug interaction. There are several reasons for this. The first reason is that it is not always ethical to perform a clinical trial, for example in a situation in which observational studies have already shown that patients will be at a risk for an adverse event if they have a certain genotype (Peters, 2010). Secondly, costs and resource use would be prohibitive (e.g. study personnel, insurance). Thirdly, clinical trials are time-consuming. The length of the actual follow-up period is only one factor here; clinical trials take substantial time to initiate (e.g. writing the protocol, instructing study personnel), perform and analyse. For obvious gene-drug interactions, it is not ethical to waste money and time for performing clinical trials instead of implementing them directly. This would mean that we expect, in the future, that some observational studies should provide sufficient evidence to implement the findings in clinical practice. However, replication of the results in observational pharmacogenetic studies is often not obtained. Therefore, strict guidelines should be developed to define which evidence is necessary to implement the investigated pharmacogenetic interaction into clinical practice. Factors to consider are:

- Have the results been replicated in different studies with independent researchers?
- Are the results valid for various countries and ethnicities?
- Is the estimated improvement large enough?
- Is the estimated improvement cost-effective (see also paragraph 4)?
- Is it feasible to implement it in clinical practice? For example:
    - Are the genotyping results available in time?

- Are all facilities available?
- Are the parties involved trained to perform the implementation?

3.2 Parties involved in implementation

Once studies have shown that a pharmacogenetic approach of determining the optimal treatment for a patient is superior to the conventional therapy, it can be implemented in clinical practice. There are multiple parties involved in the implementation of pharmacogenetic based therapies in everyday clinical situations. In this paragraph, we will discuss all different parties involved and their rationales.

3.2.1 Patients

Successful implementation of pharmacogenetic testing in everyday practice heavily depends on patient attitudes. Without the cooperation of patients, development of new pharmacogenetic strategies or guidelines is futile. Fortunately, research has shown that this group is willing to provide a sample for genotyping. Van Wieren-De Wijer e*t al*. examined the reasons for non-response in a pharmacogenetic case-control study. They approached 1871 myocardial infarction cases and 14,102 controls of which 794 and 4997 responded, respectively. Only 1.1% of the non-responding participants were unwilling to provide a DNA sample (van Wieren et al., 2009). Moreover, since this study used a case-control design where all cardiovascular events had occurred before testing, the participants could not benefit from the test outcome. In case their drug treatment would be personalized by their genetic profile, this percentage is expected to decrease.

3.2.2 Health care professionals

The attitude of health care providers towards pharmacogenetic guided therapies is important in making their decision about the treatment the patient will receive. Although the FDA updated the warfarin label already in 2008 (Teichert et al., 2009a; Food and Drug Administration [FDA], 2007), genotyping preceding the anticoagulation therapy with coumarin derivatives is not commonly performed. Currently, health care professionals' attitudes are reserved towards pharmacogenetic dosing. Not many therapies need pharmacogenetic testing at the moment, so health care professionals need to get familiarized with the idea of genetic testing, like they are familiarized with performing liver and kidney function tests. Different approaches are thought to help with familiarizing health care professionals with pharmacogenetic testing:

- Clinical trials are needed to convince the health care professional and make genetic testing as normal as liver and kidney function tests.
- Recommendations in guidelines and drug labels of pharmacogenetic testing to improve treatment quality are required, such as the FDA did for warfarin.
- Education of the health care profession on how to perform and use the pharmacogenetic tests is desired.
- Favourable experiences will stimulate the health care professional to use pharmacogenetic testing in everyday clinical practice.
- Facilities for genotyping need to be available at the right place and time.
- Consistency and standards for pharmacogenetic testing are needed.

The focus of the process should not only be on the physician but also pharmacists should be involved. To enhance the implementation of pharmacogenetic testing, the Royal Dutch Association for the Advancement of Pharmacy developed pharmacogenetic-based therapeutic (dose) recommendations (Swen et al., 2011; Wilffert et al., 2010). In addition, the pharmacist could be involved in genotyping the patient with easy to use point-of-care tests that will be available soon. The results of, for example, CYP-enzymes, could be used for decision making in multiple therapies. The pharmacist is not the only candidate to genotype the patient; others such the GP or a nurse in the hospital could also genotype the patient. Therefore, dissemination of the genotyping results (e.g., by means of electronic dossiers) is important.

3.2.3 Regulatory authorities

Regulatory authorities will also play an important role in the implementation of pharmacogenetic guided therapies in daily practice. They have the power to develop guidelines which health care professionals are obligated to follow. They can also adjust the label information of the medication.

In order to harmonize approaches to drug regulation, a guideline was developed to ensure that consistent definitions of terminology are applied across all constituents of the International Conference on Harmonisation (ICH) (EMA, 2007; FDA, 2008). This guideline contains nonbinding recommendations. The Committee for Human Medicinal Products (CHMP) facilitated an informal process of sharing scientific and technical information between applicants and regulators by releasing a concept paper on "Briefing Meetings on Pharmacogenetics". The Pharmacogenetics Working Party was set up to support discussions regarding the implementation of pharmacogenetic testing. In April 2006, a guideline on Pharmacogenetics Briefing Meetings was adopted by the CHMP. This guideline provides guidance for starting the discussion with the Pharmacogenetics Working Party and provides considerations on the submission of pharmacogenetic data in informal regulatory submissions. Briefing meetings take place when new pharmacogenetic information becomes available during the development of a new medicinal product or when a new indication is explored based on recent developments in pharmacogenetics (EMA, 2006). The Food and Drug Administration (FDA) developed a guideline called "Guidance for Industry, Pharmacogenomic Data Submissions". This guideline facilitates the scientific pharmacogenomics process and the use of pharmacogenomic data in drug development (U.S. Department of Health and Human Services et al., 2005). The FDA and European Medicinal Agency (EMA) have joint Voluntary Genomic Data Submissions (VGDSs). This is not part of the regulatory decision-making process, but gains an understanding of genomic data and provides options for sponsors to have joint FDA-EMA briefing meetings (Goodsaid, 2006). A consistent regulatory environment is also helpful in encouraging industry to develop pharmacogenetic products, and for consumers (including patients and physicians) to use the product.

3.2.4 Health insurance companies

Implementation of pharmacogenetic guided approaches to plan therapy will depend on whether it is reimbursed by health insurance companies. If the patient needs to pay for the genotyping kit, it is less likely that pharmacogenetic testing will be implemented in clinical practice than when health insurance companies will pay for it. However, these companies

will likely only pay for genetic tests if their use leads to more cost-effective care. Health insurers would be very interested in genotyping if it improved treatment effectiveness but also reduced total health care costs (including the cost of genotyping). There are different ways in which genotyping results could lead to lower health care costs, for example:

- Fewer visits to the GP or hospital for therapy adjustments, i.e. improved patient response or efficacy
- Better prophylaxis resulting in lower costs
- Fewer side effects, especially serious side effects resulting in expensive hospital admissions.

In some cases, health insurers may reimburse genotyping even if it is believed to increase overall costs. For example, if the genotyping approach is more costly and more effective compared to the non-genotyping approach, the health insurer could consider the greater effectiveness worth the extra cost. All in all, this means that pharmaco-economic evaluations are of importance in pharmacogenetic studies. See also paragraph 4 on cost-effectiveness analysis.

3.2.5 Researchers (public and private industry)

Sound scientific research is needed to develop new strategies of pharmacogenetic guided therapies. Without research, new ideas of pharmacogenetic guided therapies will not arise. Both the public as well as the private industry could perform this research. There are different focus points that researchers could have. First, they could investigate new pharmacogenetic interactions. Interactions could be of different value. They could look for common SNPs that have a small effect, but since the SNPs are common, many patients might benefit. On the other hand, they could investigate rare SNPs that might cause major effects, in which case there could be a huge benefit for relatively few patients. However, this last area of research would require big sample sizes to have enough power to investigate the effect of a rare SNP. Second, studies to develop better and faster genotyping methods will be required if pharmacogenomic testing is to be used just as extensive as liver and kidney function tests. An example of a user-friendly and quick genotyping system is Optisense's Genie 1 with HyBeacon® assays (Howard et al., 2011). See also paragraph 5. Third, the industry could develop new drug therapies for a subpopulation. For example, a new drug that does not have the desired effect in the whole population might benefit patients with a certain genotype. Although only for this subpopulation, this new medication could then still enter the market. Forth, scientists should aim to develop genotype guided therapies that do not require large and time-consuming clinical trials. Currently, clinical trials are needed to convince health care professionals, but in the future, cohort studies could be used for the implementation of pharmacogenetic testing. It is important that the results are replicated in various external datasets before being implemented in clinical practice. After implementation, it remains important to validate the process and, if necessary, adjust the pharmacogenetic based guidelines if it does not seem to be working satisfactorily.

3.3 Facilities

Several facilities should be in place before pharmacogenetic testing can be implemented in clinical practice.

3.3.1 Availability of genotyping results

Genotyping results should be available quickly. If results are available before the therapy starts, they are of greater value than when they become available after treatment start. However, in the current clinical situation, health care professionals need to collect blood samples from a number of patients to be able to genotype a batch of samples. Therefore, it can sometimes take a few weeks before the genotype is known. Currently, new techniques are being developed, and will continue to be developed in the coming years, to make genotyping results more rapidly available (Howard et al., 2011). The need to collect samples from many patients will diminish, since one assay can be run using a Point-Of-Care Test (POCT) for a single patient. By increasing the number of tests needed, the availability of POCTs will increase (Huang, 2008) and the price per POCT assay will probably decrease (see also paragraph 5).

3.3.2 Authority guidelines

The authorities can assist in implementing pharmacogenetic testing in clinical practice by developing guidelines and ensuring that health care professionals follow them. In 2008, the FDA updated the warfarin label (Teichert et al., 2009a; Food and Drug Administration [FDA], 2007) and advised pharmacogenetic testing before the coumarin therapy starts. However, at that time no guidelines were provided as to how the dosages should be changed based on the genetic profile of the patient. This illustrates that guidelines should contain information on how to adjust drug therapy based on genotype. It also underlines the importance that different parties work closely together.

4. Cost-effectiveness analysis of pharmacogenetic testing

Many would argue that clinical practice guidelines should just focus on whether pharmacogenetic testing improves effectiveness and ignore cost considerations. However, decision making about the widespread use of genotyping also depends on its cost-effectiveness. This means that even if authorities were to recommend genotyping patients prior to cardiovascular therapy based on proof of effectiveness, the recommendation might not easily be implemented without the support of other stakeholders. One important stakeholder is the payer, such as a health insurance company and its attitude can be an instrumental factor in the successful implementation of pharmacogenetic testing. Health insurance companies may require proof of cost-effectiveness - and some estimates of budget impact - before considering reimbursement.

A cost-effectiveness analysis (CEA) compares the total costs and effectiveness of two or more different treatment strategies. All sorts of costs must be considered here, including not just the cost of genotyping, but also the cost of monitoring and the cost of cardiovascular events that occur later in time. While costs are all expressed in the same way (money!), effectiveness can be defined in different ways. The definition of effectiveness determines how cost-effectiveness is expressed. For example, effectiveness can focus on the risk of an adverse event and the difference in effectiveness between two treatments can be expressed as the absolute reduction in risk of an event. The cost-effectiveness of one treatment versus another will then be expressed as the extra cost to avoid one adverse event (calculated by dividing the difference in costs by the reduction in risk). However, since this expression of

cost-effectiveness is very disease-specific, it is difficult, if not impossible, to compare the cost-effectiveness of different treatments for different diseases with each other and this comparability is valuable when making budget allocation decisions. For this reason, some authorities or health insurance companies require a cost-utility analysis. In a cost-utility analysis (CUA), the health gains acquired by a new treatment are expressed in Quality Adjusted Life Years (QALYs), which can be compared more easily with other treatments, also in other diseases, than the cost per adverse event avoided.

Several economic evaluations (such as CEAs and CUAs) have been performed for coumarin derivatives. The problem with these analyses is that no robust data on the effectiveness of genotyping are available yet; the large RCTs that can provide this data are still ongoing (van Schie et al., 2009; French et al., 2010). This current lack of evidence results in a wide variability in cost-effectiveness ratios among the studies that have been done, ranging from dominance (where use of genotyping reduces costs and increases health) to a very high incremental cost of $347,000 per QALY gained (Verhoef et al., 2010). The costs of genotyping are also not clear yet. In literature, the estimated cost of genotyping for *CYP2C9* ranges from $67 to $350 and the estimated cost of genotyping both *CYP2C9* and *VKORC1* ranges from $175 to $575. Recently, a Point-Of-Care Test (POCT) for genotyping *CYP2C9* and *VKORC1* has been developed. With this test, the patient's genotype can be determined in the physician's office within 2 hours and this is estimated to cost less than $50 per patient for both *CYP2C9* and *VKORC1* (Howard et al., 2011). The costs of genotyping are expected to decrease even further, with increased usage. This will also influence the chance that pharmacogenetic testing is cost-effective.

Decisions about whether or not to implement pharmacogenetic testing in clinical practice will differ among different countries. This difference can be caused by several factors. Firstly, the amount of money society is willing to pay varies among different countries. For example, this 'willingness to pay' is approximately $50,000 per QALY gained in the US or £20,000–30,000 (approximately $33,000-50,000) per QALY gained in the UK (National Institute for Health and Clinical Excellence [NICE], 2008). Secondly, the costs, not only of genotyping but also of the consequences like bleeding events, are not identical in all countries. Next to this, the effectiveness of genotyping can also be higher in one country than in another. This is for example possibly the case with coumarin derivatives. In some countries the standard care is already of very high quality, with specialized anticoagulation clinics to monitor the effect of the drug, while in other countries this is not the case and there is still room for further improvement.

As mentioned before, the use of pharmacogenetics in treatment with a certain drug can only be recommended if information on effectiveness and costs of genotyping is available, although it is not clear what level of evidence is needed for a valid decision. Obviously, it is impossible to obtain perfect evidence. Therefore, value of information (VOI) analyses could be performed to establish the cost–effectiveness of further research on the efficiency of the strategy. If the costs of performing this research are greater than the benefits of the additional information, then it would not be worthwhile to conduct this research (Sculpher & Claxton, 2005). The parameters that have the greatest influence on the uncertainty regarding the cost–effectiveness of genotyping should be the main focus of future studies in this area. The costs of conducting these studies should also be considered. However, this will also depend upon the regulatory environment, and VOI forms only a part of the picture.

5. Pharmacogenetic developments

Until now, only the most obvious gene-drug interactions have been detected since these are least complicated to detect when researchers are looking for causal SNPs. However, rare SNPs with large effects might as well be of importance, but it is a challenge to find large numbers of cases that are required to obtain enough power in pharmacogenetic studies when looking at smaller effects or lower allele frequencies (Daly, 2010). A trend is observed that larger studies are being performed and meta-analyses are carried out to investigate these less frequent genetic profiles. Several techniques are further developed and might lead to new insights in the pharmacogenetic research field. We will discuss them in this paragraph.

5.1 Candidate-gene studies

This type of study investigates the association between drug response and previously identified candidate genes. These candidate genes might play a relevant role in the pharmacokinetics or pharmacodynamics of the drug and might therefore be, for example, the metabolizing enzyme or the target protein. An example is the use of candidate gene approaches for the understanding of the overall drug response to coumarins. (Daly, 2010). In 1992, Rettie *et al*. indicated *CYP2C9* as main metabolizing enzyme of warfarin (Rettie et al., 1992). A few years later, Furuya *et al.* first reported that SNPs in this gene affect the stable coumarin maintenance dose (Furuya et al., 1995). A decade later, VKORC1 was identified as the target enzyme of the coumarins (Rost et al., 2004; Li et al., 2004) and studies confirming the association between *VKORC1* genotypes and stable coumarin maintenance dose followed. Another example is the role of the *CYP2C19* genotype on the clopidogrel (Hulot, 2006) therapy response and how the treatment with tamoxifen is influenced by the *CYP2D6* genotype (Hoskins, 2009).

5.2 Genome-wide association studies

Since 2007, genonome wide association (GWA) studies have become more frequently applied in the pharmacogenetics field. This resulted in novel identified associations between drug response and variations in DNA (Daly, 2010). In CVD, GWA studies resulted in confirmation of the already available knowledge, rather than in newly identified interactions. For clopidogrel, the influence of *CYP2C19* was confirmed (Schuldiner et al., 2009) and for statin induced muscle symptoms an association with *SLCO1B1* was found (SEARCH Collaborative Group, 2008) in a GWA study. In a GWA study on acenocoumarol maintenance dose, an additional effect was found for polymorphisms in *CYP4F2* and *CYP2C18* (Teichert et al., 2009b). These GWA studies led to more knowledge about several drug-gene interactions, but the causality of the relationship is not always clear in these studies. Another difficulty with this type of analyses is the need of large patient numbers because of the correction for multiple testing.

5.3 Sequencing

DNA sequencing is the determination of the nucleotide bases in DNA. In contrast to GWA studies, where tag SNPs are used to cover as much of the variation within the gene as possible, this technique will determine the exact order of nucleotides in DNA. Instead of tag SNPs that are usually markers for the causal SNP - and thereby introduce noise because they

are not always in complete linkage disequilibrium - the causal SNPs can be identified. Therefore, this technique might provide new insights in associations between drug response and pharmacogenetic parameters that are not observed when performing a candidate-gene study or a GWA study. It is possible to sequence a whole genome or whole exome. In addition, there is an option 'targeted sequencing' which means that a candidate gene is sequenced. This technique is relatively new and gaining interest in the last few years, but the same issues (i.e. causality of the relationship is not always clear and large patient numbers are needed) as with the GWA studies occur with sequencing. This warrants that the functionality of the SNP should be studied (Sadee, 2011).

5.4 Point-of-care testing

As discussed earlier, point-of-care tests can be used as mobile genotyping instruments in different settings, including the pharmacy, anticoagulation clinic and physician's office. It avoids the need to collect multiple samples and the genotyping results are available within 2 hours. This technique might be used to genotype the patient before the start of the therapy. However, the applicability of a point-of-care test may be different from centralized laboratory testing because of different sensitivity and specificity parameters. Also, it is not attractive to use such a test in research where large patient groups are needed to find a pharmacogenetic interaction, since that would be very labor intensive.

6. Conclusion

There is considerable potential for pharmacogenetic based drug dosing in CVD, but at the moment, these are not widely implemented in clinical practice. Convincing evidence was found for several CVD drugs. Carriers of a variant allele of the *SLCO1B1* gene could be treated with a *SLCO1B1* independent statin to increase the safety of the treatment. Clopidogrel is less metabolized into its active form by patients carrying a variant allele of *CYP2C19*, resulting in a less effective therapy. Information about the patient's *VKORC1* and *CYP2C9* genotype could be used when defining the appropriate dose during the anticoagulation therapy with coumarins to enhance the efficacy and increase the safety of the treatment. However, implementation of this knowledge is challenging and depends on multiple factors. First, clinical trials are needed to provide evidence for and enhance the implementation of pharmacogenetic testing. However, it is not feasible to perform a clinical trial for every newly found gene-drug interaction. Therefore it is desirable to develop guidelines to which observational studies should apply before implementing the gene-drug interaction in clinical practice. Secondly, multiple parties are involved, such as patients, health care professionals, regulatory authorities, health insurance companies and researchers. We discussed the different parties involved and their rationales. Thirdly, several facilities should be in place before pharmacogenetic testing can be implemented in clinical practice, such as availability of genotyping results and authority guidelines. Lastly, before it comes to implementation, the cost-effectiveness of the pharmacogenetic approach should be investigated. Health insurance companies may require proof of cost-effectiveness before considering reimbursement and therefore implementation of pharmacogenetic testing.

For the coming years, researchers will continue to develop the different genotyping methods. Larger studies will be performed and meta-analyses will be carried out to

investigate less frequent genetic profiles. Analysis of GWA studies and sequencing is challenging due to the enormous amount of data obtained by this technique.

In the field of oncology, pharmacogenetic testing already is part of daily practice. We expect that pharmacogenetic testing will also be implemented in CVD in the near future.

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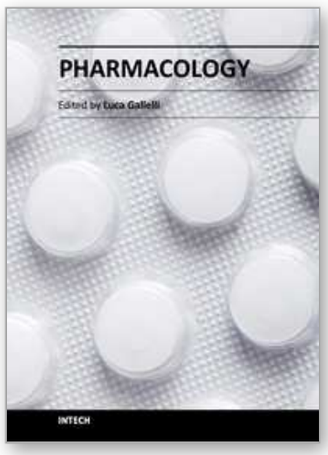

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The history of pharmacology travels together to history of scientific method and the latest frontiers of pharmacology open a new world in the search of drugs. New technologies and continuing progress in the field of pharmacology has also changed radically the way of designing a new drug. In fact, modern drug discovery is based on deep knowledge of the disease and of both cellular and molecular mechanisms involved in its development. The purpose of this book was to give a new idea from the beginning of the pharmacology, starting from pharmacodynamic and reaching the new field of pharmacogenetic and ethnopharmacology.

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