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Chapter 11
A systematic review on pharmacogenetics in cardiovascular disease
Is it ready for clinical application?

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ABSTRACT

Pharmacogenetics is the search for heritable genetic polymorphisms that influence responses to drug therapy. The most important application of pharmacogenetics is to guide choosing agents with the greatest potential of efficacy and smallest risk of adverse drug reactions. Many studies focussing on drug-gene interactions have been published in recent years, some of which led to adaptation of FDA recommendations, indicating that we are on the verge of the clinical application of genetic information in drug therapy. This systematic review provides a comprehensive overview of the current knowledge on pharmacogenetics of all major drug classes currently used in the treatment of cardiovascular diseases.
INTRODUCTION

Pharmacogenetic studies search for heritable genetic polymorphisms that influence responses to drug therapy. Pharmacogenetics has many possible applications in cardiovascular pharmacotherapy including screening for polymorphisms to choose agents with the greatest potential for efficacy and least risk of toxicity. Pharmacogenetics also informs dose adaptations for specific drugs in patients with aberrant metabolism.

Each year an estimated 785 000 Americans suffer a myocardial infarction (MI) and 610 000 people experience a new stroke. Despite advances in management, 470 000 recurrent MIs and 185 000 recurrent strokes occur annually. The numbers from Europe are not much better, indicating the importance of optimizing treatment strategies.

Although several reviews on pharmacogenetics in cardiology have been published, most focused on specific drug subgroups. This systematic review offers a comprehensive summary of the current knowledge of pharmacogenetics in cardiology, elaborates on progress towards individualized drug therapy, and incorporates conclusions made during the Colloquium “Pharmacogenetics of cardiovascular drugs: implications for a safer and more efficient drug therapy” held by the Royal Netherlands Academy of Arts and Sciences in October 2010. In particular, the review considers the most relevant and well studied polygenic markers for some pressing clinical issues: (i) statins: focusing on variability in efficacy and risk of myopathy; (ii) resistance to antiplatelet medication; (iii) dosing issues with oral anticoagulants; (iv) suboptimal responses to β-blockers; and (v) the variable response in blood pressure and clinical events in patients taking angiotensin converting enzyme (ACE) inhibitors (Table 1).

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METHODS

Relevant articles were identified by searching MEDLINE using the following keywords and Medical Subject Headings (MeSH) terms: pharmacogenetics, genetic heterogeneity, genetic polymorphism, single nucleotide polymorphism (SNP), haplotypes, treatment outcome, adverse effects, drug therapy, cardiovascular diseases (CVDs), and/or coronary disease. We screened the title and abstract of possibly relevant citations and retrieved, if potentially interesting, full reports or abstracts. We checked the references of retrieved publications for additional relevant papers and included all available literature until May 2011. We excluded reviews, editorials and articles in languages other than English.

STATINS: VARIABILITY IN EFFICACY AND RISK OF MYOPATHY

Statins, or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are widely prescribed to lower plasma cholesterol levels, thereby reducing cardiovascular risk. Despite the clinical effectiveness of statins, proven in large randomized controlled trials, inter-individual variation in response means that many patients fail to achieve adequate reduction of cholesterol levels even after multiple dose adjustments. Genetic factors are thought to be partly responsible for this inter-individual variation. Indeed, more than 40 candidate genes have been described with respect to the differential effect of statins in decreasing the risk of clinical endpoints including cardiovascular death, MI and lipid lowering abilities (see Supplementary material online, Table S1).

Variability in lipid lowering

Several pharmacogenetic studies examined the gene encoding the cholesterol ester transfer protein (CETP) involved in cholesterol metabolism and, in particular, the TaqIB variant (rs708272). Initial studies associated the B2B2 genotype with lower CETP levels, higher high density lipoprotein cholesterol (HDL-C) levels and lower risk of progression of coronary artery disease (CAD), compared with the B1B1 genotype. However, on statin treatment B1B1 patients showed lower progression of CAD than B2B2 carriers. A meta-analysis confirmed the firm association between TaqIB and HDL-C levels and CAD risk, while no significant interaction between this polymorphism and pravastatin treatment could be demonstrated. This meta-analysis included studies that enrolled patients with and without a history of CAD. In contrast, the original study included only patients with significant CAD. Furthermore, long-term results from the Regression Growth Evaluation Statin Study (REGRESS), the first study to report the possible pharmacogenetic interactions between the CETP polymorphism and statin treatment, also contrast with the meta-analysis. REGRESS demonstrated significantly higher 10-
year mortality in statin-treated male patients carrying the B2 allele, compared with the B1B1 genotype. Therefore, although untreated B2B2 patients have a lower risk of CAD progression, statin treatment is more beneficial in patients with the B1B1 genotype, negating the initial advantage of the B2 allele in CAD.

The extensive studies of apolipoprotein E (APOE), and in particular the ε2/ε3/ε4 variants defined by combinations of two polymorphisms (Cys112Arg, rs429358 and Arg158Cys, rs7412) are equivocal. Several studies report an association between ε2 carriers and an increased lipid lowering response to statins or reduced cardiovascular outcomes, such as nonfatal MI and cardiovascular-related mortality, whereas other studies did not find significant associations. Although the first genome-wide association study (GWAS) on statin effects showed a significant association between the three APOE polymorphisms and the low-density lipoprotein cholesterol (LDL-C) response, a recent meta-analysis did not confirm this association. Therefore, it seems unlikely that a true association exists between APOE polymorphisms and the lipid lowering response to statin treatment.

Since most studies discussed so far had insufficient power to detect the small effect produced by individual genetic loci, Barber et al. combined three statin GWASs encompassing 3936 patients. Carriers of a polymorphism (rs8014194) in the calmin (CLMN) gene on chromosome 14 had a significantly greater reduction of 3% in total cholesterol compared to non-carriers. The exact function of calmin is unknown, but it contains a calponin-domain expected to have actin-binding activity and is strongly expressed by liver and adipose tissue. Nevertheless, this polymorphism explained only 1% of the variability in statin response. A SNP near APOE, in the APOC1 gene (rs4420638), showed moderate evidence for being associated with statin-induced LDL-C changes. Most other putative associations appeared to be independent of statin treatment and were in loci previously associated with lipid or lipoprotein traits.

**Kinesin-like protein 6 and variability in clinical outcome**

Next, carriers of the arginine-coding allele (rs20455) of kinesin-like protein 6 (KIF6) showed a substantially higher benefit with pravastatin treatment compared with non-carriers in three large clinical trials, which appeared to be independent from lipid and C-reactive protein-lowering effects. Kinesin-like protein 6 is a member of the molecular motor super family involved in the intracellular transport of several important molecules, including mRNA. A recent meta-analysis of 19 studies (in total 17 000 CAD cases and 39 369 controls) concluded that the SNP in question was not associated with the risk of CAD. This study was, however, unable to explore the effect of statins, since this information was not available of all included studies, but the probability of a strong influence on the effect of statin decreases with this publication. Nevertheless, further (functional) studies are needed to explore the role of KIF6.
Risk of myopathy

Genetic variations are also associated with an altered risk of adverse drug reactions (ADRs), especially statin-induced myopathy. The incidences of myopathy and rhabdomyolysis are estimated at 11.0 and 3.4 per 100 000 person-years respectively. The case mortality rate with rhabdomyolysis is 10%.27

The polypeptide organic anion transporter P1B1 (OATP1B1) encoded by the SLCO1B1 gene is involved in the hepatic uptake of statins.28,29 Two common polymorphisms (521T>C, rs4149056 and 388A>G, rs2306283) influence the transporter function of OATP1B1,30 markedly altering statin pharmacokinetics.31 A GWAS showed a strong association between a non-coding polymorphism (rs4363657) and statin-induced myopathy in patients treated with high-dose simvastatin.32 This polymorphism is in almost complete linkage disequilibrium ($R^2=0.97$) with the 521T>C polymorphism and was expressed by more than 60% of patients who developed myopathy with an odds ratio of 4.7 per copy of the C allele.32 A subsequent study confirmed this pharmacogenetic effect in simvastatin-induced myopathy,33 but not with either atorvastatin or pravastatin,33 probably indicating a simvastatin specific effect.34

In conclusion, the reports on genetic variability and response to statin treatment are difficult to interpret. Variability in study designs and endpoint definitions probably contribute to these mixed results. Since statins’ full pharmacodynamic spectrum, besides their lipid-lowering properties, remains largely unknown, it is not surprising that clear cut pharmacogenetic results are lacking. It is likely that future research will further elucidate statins’ exact mechanisms of action and help explain the variability in response. Newly identified loci influencing lipid concentration identified in recent GWAS, might also prove to have pharmacogenetic associations with statin treatment.35,36 Pharmacogenetic data on ADRs of statin is limited and more research dedicated to this problem is needed before concrete advises can be given on this notion.

RESISTANCE TO ANTIPLATELET MEDICATION

Blood platelets maintain appropriate hemostasis upon vascular injury, but also contribute to the pathophysiology of thrombosis. Thrombosis of atherosclerotic plaques is the most important underlying mechanism of CAD and embolic stroke, and consequently antiplatelet therapy is the cornerstone of secondary prevention in modern CVD treatment.37-40 Inter-patient variations in platelet reactivity and responses to antithrombotic treatment result in marked differences in the benefits derived from antiplatelet treatment. Indeed, up to 25% of patients continue to experience new thrombotic events despite guideline therapy.41,42 The term ‘resistance’ is widely used in such cases, although a clear-cut definition is lacking.43 The current clinical definition regards resistance as
recurrent cardiovascular events despite supposed adequate therapy. Biochemical definitions which are based on residual platelet activity during treatment measured ex-vivo by several laboratory tests, are however also clinically relevant. Trenk et al.\textsuperscript{44} for example, found a three-fold increase (95\% confidence interval (CI) 1.4-6.8, $P = 0.004$) in the 1-year incidence of death and MI among patients with high residual platelet activity at discharge after percutaneous coronary intervention (PCI) despite clopidogrel. However, although it is likely that biochemically non-responsive patients are also clinically non-responsive, biochemical ‘resistance’ is neither constant in an individual nor over time.\textsuperscript{42} Furthermore, there is an evident lack of concordance between laboratory tests used to express resistance.\textsuperscript{45-47}

Against this background, recent research suggests that genetic variation makes an important contribution to variation in responses to the three main groups of anti-platelet therapy: aspirin (see Supplementary material online, Table S2); thienopyridine derivates [clopidogrel (see Supplementary material online, Table S3), prasugrel (see Supplementary material online, Table S4), and the $P_{2Y12}$ receptor antagonist ticagrelor]; and GP IIb/IIIa receptor inhibitors (orbofiban and abciximab) (see Supplementary material online, Table S5).

**Aspirin**

The antiplatelet effects of aspirin (acetylsalicylic acid) arise from irreversible acetylation of cyclooxygenase (COX)-1, which catalyzes the formation of thromboxane A2, prostaglandin and related metabolites from arachidonic acid.\textsuperscript{48,49} Blocking COX-1 decreases the formation of thromboxane A2, thereby reducing platelet aggregation.\textsuperscript{50} Aspirin resistance is poorly defined: a patient can be a non-responder in one test and a normal responder in another. Because of this lack of uniformity, the reported prevalence of aspirin resistance differs widely between studies. A recent systematic review reported a mean prevalence of aspirin resistance of 24\% with a range between 0\% and 57\%.\textsuperscript{51}

**Conflicting results on aspirin efficacy**

Of the many pharmacogenetic studies on aspirin, Hanlushka et al.\textsuperscript{49} were the first to describe an effect of two polymorphisms in the COX-1 gene, -842A>G (rs10306114) and 50C>T (rs3842787), that are in complete linkage disequilibrium. Healthy individuals heterozygous for these polymorphisms showed greater inhibition of platelet aggregation compared to homozygotes of the wild-type allele. Two later studies confirmed this relationship in CAD patients.\textsuperscript{52,53} However, many other studies failed to find significant associations between variation of the COX-1 gene and residual platelet activity\textsuperscript{44,55} or clinical events, including non-fatal MI or cardiac death.\textsuperscript{56}

Platelet glycoprotein (GP) IIb/IIIa receptors bind fibrinogen, thereby cross-linking platelets and von Willebrand factor (vWF).\textsuperscript{57} Studies that focused on the 1565T>C
polymorphism (rs5918; PlA1/A2) of the *GP IIIa* (integrin β3) gene produced highly variable conclusions (see Supplementary material online, Table S2). A meta-analysis that included most of these studies concluded that the PlA1/A2 variant was significantly associated with aspirin resistance, but only in healthy individuals.\(^45\) Co-medications used by CVD patients potentially obscured aspirin resistance, while differences in laboratory techniques may have also influenced outcome.

The meta-analysis also revealed that variations of four other genes [*GP Ia* (807C>T, rs1126643), *COX-1* (-842A>G/50C>T), *P2Y12* (H1/H2, constituted by rs10935838, rs2046934, rs5853517 and rs6809699) and *P2Y1* (1622A>G, rs701265)] were not associated with aspirin resistance.\(^45\) However, a later relatively large study, including 200 high-risk atherosclerosis patients, found a strong association between aspirin resistance and the T allele of the 807C>T polymorphism of the *GP Ia* gene.\(^58\) A further study ruled out any influence of the -842A>G/50C>T polymorphism of *COX-1* and PlA1/A2 of *GP IIIa* on platelet response in a healthy Chinese population, in which these sites were non-polymorphic. However, the study showed attenuated antiplatelet effect during aspirin treatment in the presence of the *P2Y1* 893CC genotype (rs1065776).\(^55\) This last association contrasts with results from 469 Caucasian patients with a history of MI. Here, carriers of the 893T allele had an almost three-fold increased risk of aspirin resistance compared to the CC genotype.\(^59\)

**Complications of aspirin therapy**

A small number of studies focused on genes involved in aspirin-related ADRs. Park et al.\(^60,61\) reported that the promoter polymorphisms -863C>A (rs1800630) and -1031T>C (rs1799964) of *TNF-α* and -509C>T (rs1800469) of *TGF-β1* may contribute to aspirin-induced urticaria. Piazuelo et al.\(^62\) related the carriage of the A allele of the 27 bp variable number tandem repeats of *eNOS* with a decreased risk of upper GI bleeding. Further pharmacogenetic reports on ADRs of aspirin are necessary given the widespread prescription of aspirin.

In conclusion, the precise role of genetic variation in the variability of response to aspirin treatment has yet to be defined. The only consistent association is that of the PlA2 variant of the *GP IIIa* gene and aspirin resistance in healthy individuals. Standardization of techniques to measure and define response is a prerequisite for future studies, and to establish genetic variation’s contribution to aspirin resistance.

**Thienopyridine derivates/P2Y\(_{12}\) receptor inhibitors**

Clopidogrel resistance is common: the incidence was 21% (95% CI 17-25%) in a meta-analysis of patients after PCI.\(^42\) Estimates of the incidence vary widely between studies, mainly due to differences in the time of measurement and dose of clopidogrel. However,
the technique used to assess clopidogrel responses did not result in a different prevalence of clopidogrel resistance between studies.\textsuperscript{42}

Clopidogrel is an orally administered prodrug. Intestinal absorption is limited by P-GP, an efflux pump encoded by the \textit{ABCB1} gene. Most of the bioavailable fraction undergoes hydrolysis to an inactive metabolite. Approximately 15% is oxidized by the hepatic cytochrome (CYP) P450 system in two sequential steps to generate an active metabolite,\textsuperscript{63} which binds to, and irreversibly inhibits, the platelet ADP receptor P\textsubscript{2Y\_12}. By blocking this receptor, clopidogrel prevents platelet degranulation and inhibits conversion of the GP Ib/IIa receptor to the form that binds fibrogen and links platelets, thereby inhibiting platelet aggregation.\textsuperscript{64-67}

\textbf{Metabolism and resistance}

Several studies examined the influence of genetic variation in one or more enzymes of the CYP450 system on clopidogrel resistance. The most consistent finding is that the *2 allele of the \textit{CYP2C19} gene (rs4244285) (involved in both hepatic oxidative steps) leads to the formation of an inactive enzyme and, therefore, decreased platelet responsiveness to clopidogrel\textsuperscript{68} (\textit{Figure 1}). After the first report,\textsuperscript{68} which enrolled healthy male volunteers, studies replicated the reduced platelet responsiveness \textit{in vitro} and established an increased risk of cardiovascular events and death during clopidogrel treatment in different patient populations (see Supplementary material online, \textit{Table S3}). Other loss-of-functions alleles (*3, rs4986893; *4, rs28399504 and *5, rs56337013) have been associated with clopidogrel resistance although not as strongly and as consistently as the *2 allele, probably reflecting their lower allele frequencies.\textsuperscript{69-72} Furthermore, recent studies associated a gain-of-function allele (*17, rs1248560) and a higher platelet inhibition by clopidogrel and, as a consequence, increased bleeding risk.\textsuperscript{73,74}

The CYP2C9 loss-of-function allele *3 (rs1057910) has been associated with increased residual platelet activity and poor responder status after a 300 mg clopidogrel loading dosage in healthy subjects\textsuperscript{69} and CVD patients.\textsuperscript{76} Surprisingly, the latter study could not detect a similar effect of the risk allele during clopidogrel maintenance therapy. The authors hypothesized that since this enzyme is involved in the second metabolization step, the reduced enzyme capacity of the *3 allele becomes critical only with higher clopidogrel plasma concentrations.\textsuperscript{63}

Studies on genetic variation in other involved CYP450 enzymes, especially CYP3A4 and CYP3A5, did not show a significant influence on platelet responsiveness after clopidogrel treatment (see Supplementary material online, \textit{Table S3}). From a clinical point of view, an important finding came from Gladding \textit{et al.},\textsuperscript{77} who demonstrated that increasing the clopidogrel dose in patients with non-responder CYP2C19 polymorphisms can increase the antiplatelet response, indicating that personalizing doses using pharmacogenetics could potentially optimize treatment.
Drug transport and receptors

Although CYP2C19 polymorphisms probably account for most of the variability in clopidogrel response, variations in the function of intestinal efflux transporters might also influence clopidogrel bioavailability. For instance, the 3435C>T SNP of ABCB1 (rs1045642) was shown to influence P-glycoprotein transporter expression, resulting in two- to four-fold lower peak concentrations of clopidogrel and its active metabolite in TT carriers after oral administration. Two recent studies confirmed these findings and a recent GWAS could not reproduce the
association between this polymorphism and clopidogrel pharmacodynamics in healthy volunteers.\textsuperscript{80}

Another candidate gene encodes the P\textsubscript{2}Y\textsubscript{12} receptor, which is essential for platelet inhibition exerted by clopidogrel, prasugrel and ticagrelor.\textsuperscript{81-83} Ziegler et al.\textsuperscript{84} reported a four-fold increased risk for cerebrovascular events in patients that carried the 34C>T (rs6809699) variant of the P\textsubscript{2}Y\textsubscript{12} receptor receiving clopidogrel for peripheral artery disease. Moreover, Staritz et al.\textsuperscript{85} associated another polymorphism (52G>T, rs6785930) to clopidogrel resistance in patients receiving dual antiplatelet therapy (clopidogrel and aspirin) after PCI. However, other studies could not confirm these findings with respect to platelet response or cardiovascular events.\textsuperscript{70,86,87}

Finally, T allele carriers of the GP I\textalpha gene 807C>T (rs1126643) polymorphism show increased platelet aggregation during clopidogrel treatment than non-carriers, increasing their thrombotic risk.\textsuperscript{88} The same group confirmed these results in patients receiving dual antiplatelet therapy.\textsuperscript{89} However, another study could not confirm these results in 600 acute coronary syndrome (ACS) patients on dual antiplatelet therapy.\textsuperscript{90} The PlA polymorphism of the platelet GP III\alpha gene (rs5918) probably does not influence the effect of thienopyridine derivates, although small studies report positive as well as negative effects.\textsuperscript{91,92} Simon et al.\textsuperscript{70} did not find a significant association of the PlA polymorphism in a population consisting of 2208 patients with acute MI treated with clopidogrel and the rate of cardiovascular events (225 deaths and 94 non-fatal MIs or strokes).

In conclusion, resistance to clopidogrel is a major problem, and prediction of the problem could be of great value. The CYP2C19 loss-of-function allele *2 shows the most promise in predicting clopidogrel resistance and subsequent treatment failure, although this was not demonstrated by all studies.\textsuperscript{93} Many other polymorphisms in theoretically promising target genes do not produce consistent results. Furthermore, proton pump inhibitors, especially omeprazol, show high affinity for CYP2C19 and might inhibit clopidogrel metabolism, raising concerns about concomitant use.\textsuperscript{94} Clinical evidence of this adverse drug-drug interaction, however, derives mainly from observational studies. A recent review concluded that although definite evidence is lacking and further studies are needed, clinicians should keep in mind this possibly clinically relevant interaction when considering prescribing this combination.\textsuperscript{94}

**Prasugrel**

The pharmacogenetics of prasugrel, a newer thienopyridine, has not been as comprehensively investigated as clopidogrel. Nevertheless, several studies indicate that the main contributor to clopidogrel resistance, CYP2C19*2, does not affect prasugrel’s efficacy.\textsuperscript{69,95} In a large study, no significant attenuation of the prasugrel response was found in carriers of at least one of the known loss-of-function alleles of the tested CYP 450 genes. Furthermore, in ACS patients none of the genotypes was associated with clinical
cardiovascular events. Prasugrel's metabolic pathway explains this lack of association; CYP2C19 makes only a minor contribution to prasugrel's metabolism. CYP3A4 and CYP2B6 are largely responsible for converting prasugrel to its active form. CYP3A4 appears not to be very polymorphic and no clear association emerged between CYP2B6 variants and prasugrel function. Prasugrel's strong antiplatelet effect leads to a highly reduced residual platelet activity, making any effect of genetic variation difficult to determine. Prasugrel inhibits platelets sufficiently to reduce clinical endpoints despite decreased efficacy arising from the polymorphisms.

**Ticagrelor**

The newest P2Y12 receptor antagonist, ticagrelor, appears to be superior to clopidogrel and has similar potency as prasugrel. A genetic sub study of the largest ticagrelor trial to date, the PLATelet inhibition and patient Outcomes (PLATO) trial, genotyped 10,285 patients randomized to ticagrelor or clopidogrel treatment. No interaction between ticagrelor and any loss-of-function CYP2C19 allele (*2 till *8), gain-of-function CYP2C19*17 allele or ABCB1 3435C>T genotype emerged with regard to the either primary efficacy endpoint (composite of cardiovascular death, MI or stroke) or any type of major bleeding after up to 12 months treatment.

**Glycoprotein IIb/IIIa antagonists**

Glycoprotein IIb/IIIa antagonists are mechanistically different from aspirin, thienopyridine derivates and other P2Y12 inhibitors. Glycoprotein IIb/IIIa antagonists act primarily outside the platelet by competitively inhibiting the receptor essential for platelet bridging and aggregation. Several studies have focused on polymorphisms in the GP IIIa gene and antiplatelet effect, but the results appear inconclusive. One study on orbofiban found a relationship between the PlA2 allele with reduced platelet inhibition and increased cardiovascular event rate. However, other studies did not replicate this association. In addition, Shields et al. developed a model to predict recurrent events during orbofiban treatment that included 10 candidate genes: platelet receptors GP IIa, PlA1/A2 (rs5918), GP la 807C>T (rs1126643), GP Iba, -5C>T (rs2243093); platelet ligands β-fibrinogen, -455G>A (rs1800790) and vWF, -1051G>A; interleukins IL-1RN*2, and IL-6, -174G>C (rs1800795); adhesion proteins E-selectin, 128A>C (rs5361) and P-selectin, 715A>C; and metalloproteinase MMP-9, -1562C>T. In 924 ACS patients an association emerged between a combination of genetic variants and both recurrent MIs and bleeding outcomes during orbofiban treatment. Since the influence of individual genetic polymorphisms is usually small, combining multiple variants in a model might be useful. However, interpreting the results may be more difficult.
DOSING ISSUES WITH ORAL ANTICOAGULANTS

Coumarin anticoagulants (vitamin K antagonists), are widely used to treat and prevent venous and arterial thromboembolisms. However, anticoagulants’ narrow therapeutic range necessitates frequent monitoring of the INR (International Normalized Ratio) coagulation status, which is expensive for health care systems and inconvenient for patients. The large individual variability in drug response with all three of the most frequently prescribed anticoagulants (warfarin, acenocoumarol and phenprocoumon) complicates therapy and makes defining a standard fixed dose unachievable. Besides several patient-related (amongst others age, weight, body surface area and diet) and clinical factors (hepatic or renal disease, anemia, co-medication), pharmacogenetics explains a large part of the variability in dose requirements.\textsuperscript{105-107} (see Supplementary material online, Table S6).

Increased metabolism

Furuya \textit{et al.}\textsuperscript{108} first reported the influence of polymorphisms in CYP2C9, the primary enzyme involved in warfarin metabolism, on dose requirement in vivo. Carriers of the *2 allele (rs1799853) required a 20% lower warfarin dose to maintain a target INR between 2 and 4. Later studies associated the *3 (rs1057910) allele with an increased sensitivity to warfarin.\textsuperscript{109} In vitro, the *2 and *3 genotypes decrease CYP2C9 activity by 30% and 80% respectively.\textsuperscript{110,111} A large meta-analysis of 39 studies encompassing 7907 patients\textsuperscript{112} concluded that, compared to the wild type CYP2C9*1/*1 genotype, all variant genotypes (*1/*2, *1/*3, *2/*2, *2/*3 and *3/*3) required lower maintenance doses of warfarin. For instance, maintenance doses in the *2 and *3 homozygotes were 36% and 78.1% lower respectively.

However, using this pharmacogenetic knowledge in clinical practice is difficult. A meta-analysis investigating the safety and efficacy of genotype-guided dosing of warfarin in reducing the occurrence of major bleeding events and over-anticoagulation found a non-significant trend towards more rapid achievement of a stable dose in the genotype-guided dosing arm.\textsuperscript{113} Several ongoing randomized controlled trials, which are expected to encompass more than 2500 patients will help elucidate the role of genetic testing in warfarin management.\textsuperscript{113}

A second contributor

Another extensively studied gene is that encoding VKORC1 (vitamin K epoxide reductase complex subunit 1), which converts the oxidized, inactive form of vitamin K to the reduced, active form. Coumarins inhibit VKORC1, thereby inhibiting activation of the vitamin K dependent clotting factors II, VII, IX and X.\textsuperscript{114} A recent meta-analysis analyzed the relationship between mean maintenance dose of warfarin and three \textit{VKORC1}
polymorphisms (1173T>C, rs9934438; 3730G>A, rs7294 and -1639A>G, rs9923231). Carriers of 1173T>C and -1639A>G needed higher maintenance warfarin doses.

Genetic variation in several other genes—including APOE, glutamyl carboxylase (GGCX), calumenin (CALU), CYP4F2, epoxide hydrolase 1 (EPHX1) and factor VII (F7)—may also influence warfarin dosage requirements. However, although some studies found significant associations, results were inconsistent. Finally, recent GWASs identified CYP2C9 and VKORC1 only as major hotspots, indicating that polymorphisms in other genes probably only have minor influence on warfarin dosing.

Although warfarin is usually the anticoagulant of choice, acenocoumarol and phenprocoumon are used in most European countries. All three drugs are metabolized by CYP2C9. However, phenprocoumon appears to be less influenced by CYP2C9 polymorphisms due to significant metabolism by the non-polymorphic CYP3A4. The effect of the VKORC1 polymorphism is similar for all three drugs.

Dosing advice with genetic guidance
In conclusion, the association between warfarin dose and polymorphisms of the CYP2C9 and VKORC1 genes seems unquestionable, as demonstrated by numerous studies and several meta-analyses. Polymorphisms in CYP2C9 and VKORC1 together with additional clinical factors including co-medication, account for 50-60% of the inter-individual warfarin variability. Based on this evidence, a free web site is available to help clinicians estimate the therapeutic dose in patients new to warfarin (http://www.warfarindosing.org) (Figure 2). Furthermore, the U.S. Food and Drug Administration (FDA) now recommends considering lower initiation doses of warfarin for patients with certain polymorphisms in CYP2C9 and VKORC1.

SUBOPTIMAL RESPONSES TO B-BLOCKERS

β-blockers competitively antagonize β-adrenergic receptors (β-AR). Chronic β1-AR activation contributes to the pathogenesis of heart failure and β-AR blockade improves survival, remodeling and left ventricular ejection fraction (LVEF). Other major indications for β-blocker therapy are hypertension, angina pectoris and MI. As with most other drugs, not all patients benefit from β-blockers to the same extent, possibly due to genetic variation (see Supplementary material online, Table S7).

Receptors and treatment efficacy
The primary focus of studies examining pharmacogenetic determinants of β-blocker response has been the β-AR genes and two polymorphisms - Ser49Gly (rs1801252) and Arg389Gly (rs1801253) - in the beta-1 adrenergic receptor (ADRB1) gene in particular.
Ser49Gly and Arg389Gly are in linkage disequilibrium and 65% of the population carry the Ser49/Arg389 haplotype. Not all studies reported consistent results, but a meta-analysis combining 3 studies (encompassing 504 heart failure patients), concluded that Arg389 homozygote individuals showed significantly greater improvement of LVEF when treated with β-blockers, compared to Gly389 carriers. Other more recent studies drew the same conclusion. However, two large studies reported inconsistent results on mortality and heart failure related hospitalization. In the BEST study, the combination of Arg389 homozygote genotype and bucindolol treatment was associated with decreased mortality and hospitalization. However, no association emerged in MERIT-HT. Furthermore, several, but not all, studies associated Arg389 homozygotes with an increased effect of β-blockers in decreasing systolic and diastolic blood pressure or heart rate. Furthermore, the Gly allele of Ser49Gly was more commonly associated with a favorable outcome or improved response to β-blockers. However, many studies did not find any association.

Most studies assessing two common polymorphisms in ARDB2 (Gly16Arg, rs104213 and Gln27Glu, rs1042714) did not observe any association. However, two small studies associated the Glu27 allele with increased LVEF after β-blocker treatment compared...
Part III – Pharmacogenetics of cardiovascular diseases

Dosing problems of β-blockers

Many β-blockers are metabolized predominately by hepatic CYP2D6,152 which is highly polymorphic with many alleles having a decreased or absent function.153 Several studies report that CYP2D6 genotype profoundly affects β-blocker concentrations and the effect seems especially pronounced with metoprolol.154-157 Poor metabolizing genotypes increase metoprolol plasma concentrations, which results in greater reductions of heart rate and blood pressure,158-160 and an increased risk of ADRs.160 Although the evidence is derived from a few studies, dose adjustments should be considered in patients vulnerable to complications, such as heart failure patients.161

The use of different β-blockers between, and even within, studies complicates the evaluation of the evidence on the pharmacogenetics of β-blocker therapy. Differences in receptor specificity of the individual β-blockers could explain this heterogeneity. For instance, metoprolol and bisoprolol are β1-AR selective, while carvedilol and bucindolol are non-selective.162 In summary, the most convincing evidence suggests that the variable benefit from β-blocker treatment is influenced by the Arg389Gly polymorphism of the ADRB1 gene. Furthermore, indisputable evidence shows that polymorphisms of the CYP2D6 gene influence β-blocker metabolism and several reports indicate that poor metabolizers have increased efficacy and a greater risk of side effects. The data on other possible related polymorphisms is too thin to draw definite conclusions. Combining different risk alleles could provide more answers.163

THE VARIABLE RESPONSE IN PATIENTS TAKING ANGIOTENSIN-CONVERTING ENZYME-INHIBITORS

The angiotensin-converting enzyme gene insertion/deletion polymorphism

Most studies examining genetic factors influencing responses to ACE-inhibitor treatment evaluated the ACE gene insertion/deletion (I/D) polymorphism (rs4646994) (see Supplementary material online, Table S8), which correlates strongly with ACE plasma concentrations.164,165 Nevertheless, initial results were inconsistent, partly due to limited sample size. Recently, however, the Genetics of Hypertension-Associated Treatment (GenHAT) study did not find an association between the I/D ACE polymorphism and the 6-year non-fatal cardiac events.166 Similarly, the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) did not find an association between this polymorphism
and 4-year risk of cardiac events, mortality, neurologic events or decline or with blood pressure reduction during perindopril treatment. In contrast, in the population-based Rotterdam Study, hypertensive patients on ACE-inhibitors had an increased 10-year mortality risk when carrying the DD genotype compared to the II genotype, with the ID genotype in an intermediate position. However, the Rotterdam Study’s observational nature represents a limitation.

In conclusion, although the \textit{ACE} gene I/D polymorphism was a prime candidate, an association with therapeutic response seems unlikely. Indeed, since Cambien et al. classified this polymorphism as a potent risk factor for coronary heart disease, many predominantly small positive trials reported similar observations. But a meta-analysis, examining the ACE I/D polymorphism with regard to restenosis, showed that small positive studies were much more likely to be published than negative ones. This publication bias distorted the actual association.

\textbf{Other targets in the renin–angiotensin–aldosteronesystem}

Angiotensinogen (\textit{AGT}) is another candidate gene in pharmacogenetic research on ACE-inhibitors. In another cohort of the Rotterdam study, 4097 subjects were analyzed for the Met235Thr (rs699) polymorphism of \textit{AGT}. The Thr allele increased the risk of MI and stroke in ACE-inhibitor users. No significant association was found with \( \beta \)-blockers. The same authors published a paper on a different cohort of 2216 subjects from the Rotterdam study investigating whether the Met235Thr AGT polymorphism modified the risk of atherosclerosis associated with \( \beta \)-blocker or ACE-inhibitor therapy. They also examined the possible relation with the I/D ACE polymorphism and the angiotensin II receptor type 1 (\textit{AGTR1}) 573C>T (rs5182) polymorphism. None of the candidate polymorphisms strongly modified the risk of atherosclerosis, measured by arterial disease, carotid atherosclerosis and aortic atherosclerosis in hypertensives taking a \( \beta \)-blocker or ACE-inhibitor.

Furthermore, Su et al. genotyped a Chinese population of 1447 hypertensive patients from a benazepril trial for several polymorphisms in AGT, AGTR1 and AGTR2. No association with blood pressure response emerged for the Met235Thr AGT polymorphism. However, subjects with the AA genotype of the rs7079 polymorphism of the AGT gene showed an enhanced antihypertensive effect. Although mortality risk seems to be higher among 235Thr allele carriers of the AGT gene receiving an ACE-inhibitor based regimen, the risk of atherosclerosis or blood pressure reduction does not seem to be increased. Further studies examining the influence of this AGT polymorphism might better characterize this relationship. Moreover, common haplotypes composed of 7 polymorphisms in the AGTR1 gene were also associated with a greater antihypertensive effect on ACE-inhibitor therapy.
Prediction of benefit

Finally, the first results of the Perindopril Genetic Association study (PERGENE) were recently published.\textsuperscript{174} PERGENE included 8907 subjects and was designed to assess the feasibility of pharmacogenetic profiling of treatment benefit of ACE-inhibitors in patients with stable CAD. PERGENE investigated 52 haplotype-tagging polymorphisms in 12 candidate genes within the pharmacodynamic pathway of ACE-inhibitors and their relation with cardiovascular mortality, non-fatal MI, and resuscitated cardiac arrest during 4.2 years of follow-up. Two polymorphisms located in the AGTR1 gene (rs275651 and rs5182) and one in the bradykinin type I receptor (rs12050217) gene were significantly associated with perindopril’s treatment benefit. Combining these 3 polymorphisms in a pharmacogenetic score identified patients who did not benefit from perindopril (26.5%) (Figure 3). The event rate increased significantly in patients allocated to perindopril (6.3 to 10.4%, P\textsubscript{interaction} < 0.0001). The authors replicated this pharmacogenetic score in a subgroup of the PROGRESS trial\textsuperscript{174}. Five polymorphisms of the ACE gene (one in complete

\textbf{Figure 3} Pharmacogenetic score proposed by the PERGENE study for prediction outcome of treatment with angiotensin-converting enzyme-inhibitors.

Results of the PERGENE study indicated that carriage of 3 or more minor alleles (risk alleles) of rs275651 A>T, rs5182 C>T and rs12050217 A>G corresponded with an increased risk of cardiovascular mortality, myocardial infarction or resuscitated cardiac arrest during treatment with perindopril compared to placebo. Clinical consequences of these findings, whether indeed patients with this specific risk profile should not receive angiotensin-converting enzyme-inhibitor treatment, remain to be prospectively studied. Modified from Brugts et al.\textsuperscript{174}
linkage disequilibrium with the I/D polymorphism) were shown not to be associated with the endpoints, again stressing that the ACE I/D gene variation does not seem to carry clinical value. The PERGENE trial takes an important step closer to realizing individualized therapy for ACE-inhibitors, but requires replication.

Angiotensin receptor blockers (ARB) inhibit the renin-angiotensin-aldosteron system through a different mechanism than ACE-inhibitors. Although some small studies investigated pharmacogenetics of ARBs, replication in larger populations is necessary before drawing conclusions (see Supplementary material online, Table S8).

**FUTURE PERSPECTIVES AND CONCLUSION**

During the Colloquium, mentioned in the introduction, all above discussed subjects were covered and the following conclusions were made. Elucidating genetic variation in patients with CVD may enable tailored therapy, thereby optimizing efficacy while minimizing costs and adverse effects. Nevertheless, despite the vast amount of available publications, pharmacogenetics of CVD is not yet established in clinical practice. Indeed, studies of most polymorphisms produced inconsistent results due to the complex systems underlying CVD, which means variation in a single gene usually produces only a small effect on clinical phenotypes. Very large patient cohorts are needed to convincingly detect these subtle differences. Large studies designed to detect pharmacogenetic association, (e.g. large GWAS) are ongoing and will probably be of great value in unraveling the current inconsistencies as well as elucidating associations of genetic variation in other target genes. Moreover, new approaches like sequencing might result in new targets for subsequent research.

The relevance and usefulness of GWAS have recently been reviewed by Daly (Table 2) and commercially available arrays, like the Drug Metabolizing Enzymes and Transporters (DMET) platform, offer systematic exploration of potential relevant pathways. However, pharmacogenetics needs evidence other than GWAS to convince cardiologists of its clinical utility. In particular, large prospective studies should investigate the effect of genetic testing on clinical outcomes and stratify patients to determine groups for which genetic testing is relevant. For example, the JUPITER trial showed that statin treatment is more beneficial in subjects with high C-reactive protein. Development of risk models combining clinical characteristics (age, gender, and history), patient characteristics (for instance blood pressure, weight, and echocardiographic parameters), biomarkers and genetics will be most useful and informative.

Besides the genetic variation depicted in Table 3, the evidence for most other pharmacogenetic associations is not strong enough for clinical application. Nevertheless, currently several guidelines on drug prescription already contain pharmacogenetic infor-
Very recently Swen et al.\textsuperscript{178} published an update of the current Dutch guidelines implementing pharmacogenetics in several therapeutic recommendations. Also, over 60 FDA-approved drug labels contain pharmacogenetic information.\textsuperscript{179} For instance, in 2007, the FDA recommended initiation warfarin doses for carriers of certain CYP2C9 and VKORC1 polymorphisms. Moreover, in 2009 the FDA added a warning to the clopidogrel prescribing information to highlight the impact of the poor metabolizer genotypes of CYP2C19. Finally, the pharmacogenetic score developed in the PERGENE trial seems to be able to identify patients that will not benefit from ACE-inhibitor treatment and the clinical consequences of this knowledge, whether indeed patients with this specific risk profile should not receive ACE-inhibitor treatment, should be prospectively tested.

Ongoing large clinical trials, primarily designed as pharmacogenetic studies, and GWAS with large study populations promise to provide more convincing evidence.

\textbf{Table 2} GWAS on pharmacogenetics of cardiovascular drugs

<table>
<thead>
<tr>
<th>Study name</th>
<th>Drug</th>
<th>Outcome measure</th>
<th>Population size (disc./repl.)</th>
<th>Lowest P-value</th>
<th>Associated gene(s)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT</td>
<td>Atorvastatin</td>
<td>Lipid lowering response</td>
<td>1984/3761</td>
<td>5.5E-30</td>
<td>APOE, PCSK9, HMGCR</td>
<td>(17)</td>
</tr>
<tr>
<td>TNT, CAP and PRINCE</td>
<td>Statin</td>
<td>Lipid lowering response</td>
<td>3936\textsuperscript{a}</td>
<td>1.9E-08</td>
<td>CLMN, APOC1</td>
<td>(19)</td>
</tr>
<tr>
<td>SEARCH and HPS</td>
<td>Simvastatin</td>
<td>Myopathy</td>
<td>85[90]/21</td>
<td>16643\textsuperscript{b}</td>
<td>4.00E-09</td>
<td>SLCO1B1</td>
</tr>
<tr>
<td>PAPI</td>
<td>Clopidogrel</td>
<td>Platelet aggregation</td>
<td>429/227</td>
<td>1.5E-13</td>
<td>CYP2C19</td>
<td>(79)</td>
</tr>
<tr>
<td>-</td>
<td>Warfarin</td>
<td>Maintenance dose</td>
<td>181/374</td>
<td>2.6E-13</td>
<td>VKORC1, CYP2C9</td>
<td>(120)</td>
</tr>
<tr>
<td>WARG</td>
<td>Warfarin</td>
<td>Maintenance dose</td>
<td>1053/588</td>
<td>5.4E-78</td>
<td>VKORC1, CYP2C9, CYP4F2</td>
<td>(116)</td>
</tr>
<tr>
<td>Rotterdam Study</td>
<td>Acenocoumarol</td>
<td>Maintenance dose</td>
<td>1451/287</td>
<td>2.0E-123</td>
<td>STX4A (VKORC1), CYP2C9</td>
<td>(124)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Combined number of patients included in the meta-analysis. \textsuperscript{b} Case|control. TNT, Treating to New Targets study; CAP, Cholesterol And Pharmacogenetics study ; PRINCE, PRavastatin/Inflammation CRP Evaluation study; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; HPS, Heart Protection Study; PAPI, the Amish Pharmacogenomics of Anti-Platelet Intervention study; WARG, WARfarin Genetics study; disc., discovery cohort; repl., replication cohort

\textbf{Table 3} Best explored single nucleotide polymorphisms in field of cardiovascular pharmacogenetics

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Drug</th>
<th>Effect of variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19</td>
<td>*2 (rs4244285)</td>
<td>Clopidogrel</td>
<td>Decreased effect</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>*2 (rs1799853)</td>
<td>Warfarin</td>
<td>Increased effect</td>
</tr>
<tr>
<td></td>
<td>*3 (rs1057910)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VKORC1</td>
<td>-1639A&gt;G (rs9923231)</td>
<td>Warfarin</td>
<td>Decreased effect</td>
</tr>
<tr>
<td></td>
<td>1173T&gt;C (rs9934438)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>I/D (rs4646994)</td>
<td>ACE-inhibitors</td>
<td>No effect</td>
</tr>
</tbody>
</table>
Overall, there are enough reasons to remain optimistic that, even though we are taking small steps at a time, we are heading to an era where we can finally use pharmacogenetics in clinical practice to optimize treatment for the individual patient.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at European Heart Journal online.

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