

Pharmacogenetics of cardiovascular drug therapy

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Summary

In developed countries cardiovascular disease is one of the leading causes of death. Cardiovascular drugs such as platelet aggregation inhibitors, oral anticoagulants, antihypertensives and cholesterol lowering drugs are abundantly prescribed to reduce risk of cardiovascular disease. Notable interindividual variation exists in the response to these pharmacotherapeutic interventions, which can be partially explained by factors such as gender, age, diet, concomitant drug use and environmental factors. Notwithstanding, a great part of this variability remains unknown. To a smaller or larger extent, genetic variability may contribute to the variability in response to these cardiovascular drugs. This review gives an overview of pharmacogenetic studies of genes that were reported to be associated with four commonly prescribed drugs/drug classes (platelet aggregation inhibitors, coumarins, antihypertensives and statins) and were studied at least 2 times with a similar outcome measure. In the field of cardiovascular drug therapy, polymorphisms in candidate genes such as the cycloxygenase-1, vitamin K reductase complex subunit 1, CYP2C9, alpha adducin and 3-hydroxy-3-methylglutaryl-CoA reductase have received a great amount of interest in the pharmacogenetics of aspirin, coumarins, antihypertensives and statins respectively. However, only variations in VKORC1 and CYP2C9 have consistently been associated with drug response (coumarins) and have clinical implications. Clinical trials should provide evidence for the effectiveness of genotyping before this procedure will be a part of every day anticoagulant therapy. In spite of the tremendous amount of publications in this field, there is no reason to advocate for genetic testing for any other drugs cardiovascular drug therapy yet.

Current approaches in pharmacogenetic research do not seem to lead to results that meet our expectations of individualized medicine. Therefore, new approaches are needed addressing issues and challenges such as the number of SNPs studied, study power, study design and application of new statistical methods in (pharmaco-)genetic analysis.

KEY WORDS: pharmacogenetics, antihypertensive drugs, statins, oral anticoagulants, aspirin.

Introduction

Cardiovascular disease is one of the leading causes of death, especially in developed countries (1). To reduce the risk on cardiovascular disease (CVD), cardiovascular drugs are abundantly prescribed all over the world for the treatment of conditions such as hypertension and hyperlipidemia. There is notable interindividual variation in response to these drugs, which is partially explained by factors such as gender, age, diet, concomitant drug use and environmental factors. Nevertheless, large part of this variability remains unknown. Genetic variability may contribute to the explanation of variability in response to these cardiovascular drugs (2). Pharmacogenetics focuses on genetic determinants of drug response and aims to optimize effectiveness and to minimize the risk of adverse drug reactions (ADR). Drug response can be modified by polymorphisms in genes that encode drug targets or components in the pathway through which a drug has its effect and are considered pharmacodynamic genes. Genes involved in the metabolism of a drug, such as cytochrome P450 (CYP) enzymes, are classified as pharmacokinetic genes. Finally, there are genes that are involved in the causal pathway of a disease and are able to modify the effect of a drug.

In the past decade, the pharmacogenetics field has enjoyed a tremendous surge in activity due to advances in technology. Many association studies have been published. This review gives an overview of the pharmacogenetics of the most commonly prescribed drugs in the management of cardiovascular disease such as platelet aggregation inhibitors, coumarins, antihypertensives and statins.

Methodology

We chose the four most commonly used drug classes in the field of cardiovascular treatment, i.e. platelet aggregation inhibitors (aspirin, clopidogrel), anticoagulants (warfarin, acenocoumarol, phenprocoumon), antihypertensive drugs (diuretics, beta blockers, ACE inhibitors, angiotensin 2 type 1 antagonists, and cholesterol lowering drugs (statins). We restricted our search to those polymorphisms that were reported to be associated with modified response to these drugs and were studied at least 2 times with a similar clinical relevant outcome measure, because replication of genetic association studies are highly recommended to warrant a true association (3). We therefore, excluded studies that only reported pharmacokinetic parameters. Studies on genetic polymorphisms in strong linkage with other polymorphisms were also considered as replication studies.

In our literature search strategy, we searched MEDLINE for recently published reviews of the pharmacogenetics of each drug (class) to list the pharmacogenetic studies that have been conducted. An additional search for studies that were not yet addressed by recent reviews was conducted using MEDLINE. Tables presented in the article give the name of the author of the initial association with a brief description of the findings. The last two columns of each table give the references of studies that have reported either similar or dissimilar results.

Platelet aggregation inhibitors

Pharmacotherapy with platelet aggregation inhibitors such as aspirin and clopidogrel is an important intervention in cardiovascular risk management. In high risk patients, its role in the primary and particularly in the secondary prevention of cardiovascular death, myocardial infarction and stroke is well established (4). However, part of the patients suffer a (recurrent) thromboembolic vascular event despite platelet aggregation inhibitor therapy (5). Several studies have been conducted, investigating the possible genetic contribution to treatment failure. These studies mainly use platelet function tests, including thromboxane (TX) levels (urine or serum), arachidonic acid (AA) tests, adenosine-diphosphate (ADP) tests and others which are described elsewhere (6), either as their main outcome or to define 'resistance' to platelet aggregation therapy. Only few studies on clinically relevant outcomes such as MI and stroke have been conducted in relation to the pharmacogenetics of aspirin or clopidogrel. Table I gives an overview of the genes that were studied *in vivo* more than once.

Aspirin irreversibly inhibits the cyclooxygenase-1 (COX-1) enzyme, ultimately resulting in a decreased amount of thromboxane A2 (TXA2). TXA2 is responsible for activation of platelet aggregation. Therefore, polymorphisms in the *COX-1* gene may affect response to aspirin therapy.

In 2005, Maree et al. reported an association between a polymorphism in the *COX-1* gene and platelet function in response to aspirin (7). Five common SNPs were genotyped in 144 patients with cardiovascular disease who were treated with aspirin for at least 2 weeks. Aspirin response, determined by serum TXB2 levels and AA-induced platelet aggregation, was associated with the A842G polymorphism. Patients carrying the -842G polymorphism were less sensitive to aspirin treatment (7). Lepantalo et al. reported similar results in 101 patients undergoing elective percutaneous coronary intervention (8). Gonzalez-Conejero et al. investigated the C50T polymorphism which was in complete linkage disequilibrium with the A842G polymorphism. Only the results of the TXB2 assay were similar to those reported in literature, whereas no drug gene interaction was shown using the AA-induced platelet aggregation (9). These results are generally consistent and show that the -842G allele (in linkage disequilibrium with -50T allele) is associated with reduced platelet sensitivity to aspirin. Only one small study including 38 healthy participants could not find any differences in the AA-induced platelet aggregation or TXB2 synthesis (10). The exact mechanism of the interaction between aspirin and the A842G and C50T polymorphisms has not been elucidated yet.

Another gene that has been investigated several times with re-

gard to the pharmacogenetics of both aspirin and clopidogrel is the gene coding for the platelet glycoprotein IIIa (ITGB3) subunit, part of the glycoprotein IIb/IIIa receptor which is present on the platelet surface. Most research focused on the P1A1/A2 polymorphism, in which the P1A1 is the wild-type variant (11). Undas et al. were first to report on the effect of this polymorphism on platelet functioning after *in vivo* exposure to aspirin, showing that subjects carrying the P1A1/P1A2 genotype were less sensitive to aspirin than homozygous P1A1 carriers (12). These findings have been replicated in small studies (13, 14), whereas other larger studies could not find such an association (15-17) or even showed opposite (18) findings corroborating results from earlier *in vitro* studies (19, 20). Inconsistent results have been reported for the association between the ITGB3 P1A1/P1A2 polymorphism and variability in response to clopidogrel as well (16, 21, 22). The contribution of the P1A1/P1A2 polymorphism to the pharmacogenetics of platelet aggregation inhibitors has not been elucidated yet.

In addition to genetic variability in *COX-1* and *ITGB3*, polymorphisms in *COX-2* and *P2Y12* have been associated with modified response to respectively aspirin (9) and clopidogrel (23), but were studied in a very small number of patients and larger studies did not replicate these results (16, 17, 24).

Clopidogrel is a prodrug and has to be activated by hepatic cytochrome P450 (CYP) isoenzymes in order to inhibit platelet aggregation. Among other CYP isoenzymes, clopidogrel is metabolized by CYP2C19 enzyme encoded by the *CYP2C19* gene. *CYP2C19*2* is a common genetic variant that encodes a deficient version of the enzyme. Hulot et al. showed in 28 healthy subjects that heterozygous carriers of the *2 allele had decreased platelet responsiveness to clopidogrel (25).

Two other studies including 74 (26) and 94 (27) healthy subjects also reported the *CYP2C19*2* allele to be associated with decreased platelet responsiveness to clopidogrel. In conclusion, the *CYP2C19*2* polymorphism seems to explain part of the interindividual response to clopidogrel, but larger studies are needed to elucidate the clinical implications of this drug gene interaction.

More comprehensive reviews that cover the pharmacogenetics of aspirin and clopidogrel can be found elsewhere (28, 29).

Anticoagulants

Coumarins – warfarin, phenprocoumon and acenocoumarol – are commonly used for the treatment and prevention of thrombotic diseases. Dosing of coumarins, however, is difficult as a result of a narrow therapeutic range and large interindividual differences in response to a certain dose (30).

Table I. Genetic association studies on response to platelet aggregation inhibitors.

Gene	Study	SNP	Findings	Similar	Dissimilar
<i>Aspirin</i>					
COX1	Maree et al. 2005 (7)	5 SNPs**	842G carriers less sensitive to aspirin treatment	(8, 9)*	(9, 10) *
COX2	Gonzalez-Conejero et al. 2005 (9)	G765C	765C increased sensitivity to aspirin		(17)
ITGB3	Undas et al. 1999 (12)	PIA1/PIA2	PIA2 less sensitive to aspirin	(13, 14)	(15-18)
<i>Clopidogrel</i>					
ITGB3	Angiolillo et al. 2004 (21)	PIA1/PIA2	P1A2 carriers lower antiplatelet effect compared to A1A1		(16, 22)
P2Y12	Bura et al. 2006 (23)	H1/H2	H2H2 carriers less responsive to clopidogrel		(16, 24, 122)
CYP2C19	Hulot et al. 2006 (25)	*1/*2	*1/*2 decreased platelet responsiveness	(26, 27)	

* Result replicated in TXB2 assay, result not replicated in AA induced platelet aggregation assay.

** A842G, C22T, G128A, C644A and C714A.

Consequently, an overdose will increase the risk of haemorrhage and an insufficient dose may lead to failure of prevention of thromboembolism. Patients' characteristics such as weight, diet, disease state and concomitant use of other medications have been shown to affect the response to coumarins (31). In addition, genetic variation seems to play a role in the explanation for the interindividual variation. To date, studies on the pharmacogenetics of coumarins have mainly focused on the *CYP2C9* and *VKORC1* polymorphisms and their modifying effect on mean daily dosage and the risk of bleeding, ultimately aiming to prevent bleedings and to predict dosage. Although the evidence for the efficacy of coumarins on secondary endpoints is well established (32), future pharmacogenetic research may also address the variation in the efficacy of coumarin in preventing thrombotic diseases.

The most extensively investigated variants of the *CYP2C9* gene encoding the CYP2C9 enzyme are *CYP2C9*2* (C430T) and *CYP2C9*3* (A1075C), in which the *2 and/or *3 variants encode enzymes with a decreased activity compared to the wild type, *CYP2C9*1*. Sanderson et al. reported in a systematic meta-analysis that lower maintenance doses of warfarin are required in subjects carrying either variant allele. They showed that *2 and *3 carriers have an increased risk of bleeding as a result of higher plasma levels of warfarin due to slow metabolism of warfarin by the CYP2C9 enzyme (30, 33). Also for acenocoumarol, the *CYP2C9*2* and *CYP2C9*3* variant have been associated with low dose requirements (34-37).

Moreover, the *CYP2C9*3* genotype has been associated with a decreased chance to achieve stable anticoagulation within 6 months, an increased risk for over anticoagulation, a higher initial INR after standard dose acenocoumarol (38) and an increased risk of major bleeding events (39).

Although research on phenprocoumon is sparse, the *CYP2C9*2* and/or *CYP2C9*3* allele have also been associated with lower dosage requirements, a decreased chance to achieve stability, an increased risk of over anticoagulation (40) and bleeding (41). In the Rotterdam study, no such interactions between the *CYP2C9* genotype and phenprocoumon treatment were found (37, 39), possibly due to power problems and use of different study design. Moreover, the contribution of the *CYP2C9* genotype to the variation in response to phenprocoumon may be smaller as acenocoumarol and warfarin are more substantially metabolised by CYP2C9 enzyme than phenprocoumon.

Coumarins exert their anticoagulant effect by inhibiting the vitamin K epoxide reductase complex (VKORC), preventing VKOR from converting vitamin K epoxide to reduced vitamin K (42), which is essential for functioning of several clotting factors such as factor II, VII, IX and X.

The T allele of the C1173T polymorphism in the *VKORC1* gene, coding for vitamin K epoxide reductase, has recently been associated with reduced dose needs of warfarin (30), acenocoumarol (43-45) and phenprocoumon (43, 44). In addition to this single SNP approach, five haplotype constructs of 10 SNPs in the *VKORC1* gene have been allocated to low and high dose warfarin haplotype groups A and B explaining the interindividual variability in response to warfarin partly (46). Furthermore, it has been reported that being a carrier of the T allele is associated with an increased risk of severe over anticoagulation (INR > 6.0) (47) and with a higher risk for bleeding when using phenprocoumon (44).

Polymorphisms in genes encoding vitamin K-dependent proteins such as clotting factors II and VII have also been studied. The T165M polymorphism of the *factor II* (*F2*) gene and the G-401T and G-402A polymorphisms of the *factor VII* (*F7*) gene have been part of pharmacogenetic research.

D'Ambrosio et al. reported that carriers of the M allele of the *F2* T165M polymorphism require lower doses of warfarin (48). Other studies did not confirm an association between the *F2*

T165M polymorphism and warfarin dose requirements (49, 50). Carriers of the A allele of the *F7* G-402A polymorphism (50) and T allele carriers of the *F7* G-401T (48) allele have been reported to require lower doses warfarin, but no other studies have confirmed these results yet (48, 50). To what extent these polymorphisms contribute to variability in response to warfarin remains to be determined.

Many articles present estimates of the contribution of various factors, including *VKORC1* and *CYP2C9* polymorphism, to the interindividual variation of coumarin response (up to 60%) and also propose algorithms for coumarin dosing (31). However, before we can implement this knowledge in the guidelines of anticoagulation therapy, a controlled clinical trial is needed to validate these dosing algorithms. A more comprehensive overview of the current status in the pharmacogenetics of coumarins can be found elsewhere (30, 31).

Antihypertensive drugs

The most prevalent indication in cardiovascular drug therapy is hypertension. Its high prevalence and the strong association with cardiovascular morbidity have given rise to the question of who to treat with which drug. In the past decades, the search for markers that can predict response to therapy has experienced a tremendous surge. The major drug classes available for the treatment of hypertension are diuretics, beta-blockers, ACE inhibitors and angiotensin 2 type 1 receptor antagonists. A complete review of the pharmacogenetics of antihypertensives can be found elsewhere (51).

Diuretics

Diuretics are considered the first line pharmacological intervention for most patients with hypertension (52). The long term beneficial effects of thiazides are thought to result from a reduction of peripheral vascular resistance, decreased blood vessel sensitivity to catecholamines and indirect activation of the renin-angiotensin-aldosterone system.

To date, several pharmacogenetic studies have been published on the pharmacogenetics of diuretics.

Polymorphisms in five genes were involved in more than 1 study, namely alpha adducin (*ADD1*) *G460W*, angiotensin converting enzyme (*ACE*) *I/D*, angiotensin (*AGT*) *-6A*, angiotensin receptor (*AGTR1*) *A1166C* and G-protein beta-3 subunit (*GNB3*) *C825T*.

The *ADD1* *G460W* polymorphism has been shown to affect renal proximal tubule sodium reabsorption in hypertension with increased reabsorption in patients carrying the *W* allele (53), possibly influencing the responsiveness to diuretics. Cusi et al. first reported on the *ADD1* *G460W* polymorphism and the association with altered response to hydrochlorothiazide (54). They found that heterozygous hypertensive patients experienced a greater fall in mean arterial pressure in response to 2 months' treatment with hydrochlorothiazide than wild-type homozygous hypertensive patients (54). Following this study, these results were replicated in 2 other studies also showing that carriers of the *ADD1* *W* allele respond better to hydrochlorothiazide (55, 56). Large scale studies with up to 36,000 patients (57) could not replicate these findings (57-60) and thereby rule out a major role for the *ADD1* *G460W* polymorphism in predicting blood pressure response to diuretics.

Additionally, some studies have associated the *ADD1* *W* allele with a better response to diuretics studying clinical outcomes like stroke or MI (61). Yet again, these results could not be replicated (57, 62).

Besides the *ADD1* gene, researchers have taken a great interest in the *I/D* polymorphism of the *ACE* gene encoding the angiotensin converting enzyme which has a pivotal role in the renin-angiotensin-aldosteron system (RAAS), converting angiotensin I into angiotensin II. *ACE* plays an important role in the regulation of the water sodium balance and in the maintenance of vascular tone. Judging from the studies listed in table II, nothing can be concluded about the effect of the *ACE I/D* polymorphism on blood pressure, as the direction of the effect of the genotype appears to go either way. In addition, no association has been found between the *ACE I/D* polymorphism and diuretic therapy on any clinical outcome (63). Other polymorphisms in the RAAS that have been studied are present in the *AGT* and *AGTR1* gene. The *AGT A(-6)G* and *M235T* polymorphism and *AGTR1 A1166C* polymorphism have been associated with modified response to diuretics in African American women only (64). These results were not replicated in the Doetinchem Cohort Study (59). Contrary to earlier findings (64), a smaller study in forty-five Chinese subjects showed that *G* allele carriers of the *AGT A(-6)G* polymorphism experienced greater blood pressure response to diuretics (65). Clearly, larger studies are needed to elucidate the possible role of these polymorphisms in the response to diuretics. Furthermore, an association with the *GNB3 C825T* polymorphism has been reported and replicated, both studies showing that subjects carrying the *TT* genotype would benefit more from diuretics in lowering blood pressure (59, 66). The mechanistic pathway of this possible gene-treatment interaction is that the *T* allele of the *GNB3 C825T* polymorphism has been associated with low plasma renin and an elevated aldosterone-renin ratio (67) which could influence the response to diuretics. A study by Schelleman et al. could not corroborate previous findings concerning the association between the *GNB3 C825T* polymorphism and clinical events (62).

Beta-blockers

The main target of β_1 -selective beta-blockers is the beta-1-adrenoreceptor encoded by *ADRB1*, in which the Arg389Gly polymorphism is a common variant located in the intracellular GTP binding domain. The *Arg389* variant has been shown to possess a three- to four-fold higher adenylyl cyclase activity that in turn may cause a modified response to beta blockers (68). Although O'Shaughnessy did not report an association of the *ADRB1* polymorphism and altered response to atenolol or bisoprolol (69), in the year 2003 three articles were published reporting an association of the *Arg* variant with better response to beta-blockers, either SBP (70-72), DBP (72, 73) or resting MBP (70, 72). This observation could not be confirmed by two other studies (74, 75).

Beta adrenergic receptors are G-protein coupled receptors. Therefore the *GNB3 C825T* polymorphism possibly modifies the blood pressure response to beta blockade. In females only, the *CC* genotype of the *GNB3 C825T* polymorphism has been associated with better blood pressure response to beta blockers (75), a finding that was not confirmed in the Doetinchem study (59). Also the *AGT A(-6)G* polymorphism has also been associated with the pharmacogenetics of beta blockers, but the association has only been reported once (76) whereas other studies failed to confirm this association (77, 78).

Larger scale studies should confirm these interactions because these studies are all underpowered.

ACE inhibitors

The *ACE* gene – coding for the drug target of ACE inhibitors – has been studied over 20 times.

Table II includes all studies that evaluated blood pressure response to ACE inhibitors according to *ACE I/D* genotype, including treatment duration ranging from single doses to several years, small scale studies as well as larger studies and studies in both healthy subject and patients. At first, smaller studies reported a more beneficial effect of ACE inhibitors on blood pressure in subjects carrying *I*. Large studies that have been published more recently (63, 79) could not confirm these results, suggesting that the *ACE I/D* polymorphism is not likely to be a strong modifier of blood pressure response to treatment with ACE inhibitors.

Another gene involved in the RAAS that has been associated with modified response to ACE inhibitors is the angiotensin (*AGT*) gene. Initially, a study in 125 subjects showed that the *T* allele of the *M235T* polymorphism was associated with better blood pressure lowering response to ACE inhibitors compared to homozygous *M* allele carriers (80). Several other studies, among which one included 1,447 patients, found no such association (Table II), concluding that there is no interaction between the *AGT M235T* polymorphism and the response to ACE inhibitors.

Angiotensin II antagonists

Another category of antihypertensives that act on the RAAS, are the angiotensin II type 1 receptor antagonists. Genes in the RAAS are considered candidate genes in the pharmacogenetics of angiotensin II type 1 receptor antagonists. Studies in the SILVHIA trial including almost 50 subjects taking irbesartan reported that the *ACE I/D* (77) and *CYP11B2 C-344T* polymorphism were associated with modified blood pressure response (77, 81). Subjects carrying the *ACE II* polymorphism (77) and subjects carrying the *CYP11B2 TT* variant (81) had a more pronounced blood pressure response to irbesartan. Redon et al. conducted a similar study including 206 subjects treated with telmisartan, but no significant associations were found in any of the RAAS gene polymorphisms (82). Contrary to the results in the SILVHIA trial, Ortlepp et al. reported the *C* allele of the *CYP11B2 C-344T* polymorphism to be associated with better response to candesartan (83). Currently, the role of polymorphisms in the RAAS in response to angiotensin II antagonists is unknown. Larger studies are needed to elucidate the pharmacogenetic contribution, as these studies are all underpowered.

Cholesterol lowering drugs

Statins primarily reduce the risk on coronary artery disease (CAD) by lowering blood cholesterol through inhibition of the HMG-CoA reductase enzyme. Although large clinical trials found a 27% average relative risk reduction of major coronary events (84), there is large variability in benefits from statin therapy. Many genes involved in the pharmacodynamic pathway of statins have been part of pharmacogenetic research in patients with primary hypercholesterolemia, with an emphasis on genes involved in the cholesterol pathway, although genes involved with possible pleiotropic effects of statins gain more and more interest.

The drug target of statins, encoded by the HMG-CoA reductase (*HMGCR*) gene was one of the candidate genes in the two studies addressing a considerable number of SNPs (85, 86). The PRINCE study found a pharmacogenetic association with two (SNP12 and SNP29) out of 33 SNPs investigated in the *HMGCR* gene on total cholesterol as well as on LDL cholesterol (85). SNP29 was also one of the four SNPs that was studied in the *HMGCR* gene in the ACCESS trial, showing no

Table II - Genetic association studies on antihypertensive response.

Gene	SNP	Outcome	Study	Findings	Similar	Dissimilar
<i>Diuretics</i>						
ADD1	G460W	Blood pressure	Cusi et al. (54)	W allele carriers respond better	(55, 56)	(57-60)
		MI/stroke	Psaty et al. (61)	W allele carriers respond better		(57, 62)
ACE	I/D	Blood pressure	Sciarrone et al. (56)	Better response in II carriers	(123)**	(123)**
AGT	A(-6)G*	Blood pressure	Frazier et al. (64)	-6A greater response (in AfrA women)		(59, 65)
AGTR1	A1166C	Blood pressure	Frazier et al. (64)	A allele better response (in AfrA women)		(59, 65)
GNB3	C825T	Blood pressure	Turner et al. (66)	Better response TT compared to CC	(59)	
<i>Beta Blockers</i>						
ADRB1	R389G	Blood pressure	Sofowora et al. (71)	AA greater response than GG	(70, 72, 73)	(69, 74, 75)
AGT	A(-6)G*	Blood pressure	Kurland et al. (76)	-6A allele greater response		(77, 78)
<i>ACE inhibitors</i>						
ACE	I/D	Blood pressure	Hingorani et al. (80)	No effect	(59, 63, 78, 79, 124-133)***	(133-137) (II > DD) (138-140) (DD>II)
AGT	M235T	Blood pressure	Hingorani et al. (80)	MT and TT greater response than MM		(59, 60, 140-142)
		Clinical event	Bis et al. (143)	TT less risk of stroke (not MI)		(62)
<i>Angiotensin2 type 1 receptor antagonists</i>						
ACE	I/D	Blood pressure	Kurland et al. (77)	II carriers greater response		(82, 83)
CYP11B2	C-344T	Blood pressure	Kurland et al. (81)	TT carriers greater response		(83)
AGTR1	A1166C	Blood pressure	Miller et al. (144)	C carriers greater response		(77, 82)

* In linkage disequilibrium with M235T polymorphism. ** Similar result for women, men more benefit with DD genotype. *** O'Toole et al. (133): Lisinopril no association, captopril associated. AfrA = African American descent.

such association (86). Both SNP 12 and SNP 29 are located in a non-coding region and further research should determine whether there is a molecular explanation for the results found in the PRINCE study.

The cholesteryl ester transfer protein (CETP) enzyme plays a central role in transport of cholesterol from peripheral tissues back to the liver, reverse cholesterol transport (RCT). Taq1B is a common polymorphism in the *CETP* gene and is associated with variations in lipid transfer activity and high density lipoprotein (HDL) levels (87) and may therefore alter response to statins. CETP concentrations are believed to be highest in homozygous *B1* carriers and lowest in CETP homozygous *B2* carriers. The effect of Taq1B on statin therapy was first investigated by Kuivenhoven et al, showing that pravastatin therapy slowed the progression of coronary atherosclerosis in *B1B1* (variant) carriers, whereas *B2B2* carriers did not benefit from pravastatin therapy (88). Several other studies investigated the *CETP* polymorphism as well and could not find an association of the Taq1B polymorphism with altered efficacy of statins in preventing cardiovascular diseases (89-92) or found the opposite (93). A large meta-analysis including over 13,000 patients found no gene treatment interaction between statins and the Taq1B polymorphism of the *CETP* gene (94). Similar to *CETP*, lipoprotein lipase (LPL) and hepatic lipase (LIPC) are genes that code for enzymes that transfer lipids between lipoproteins and mediate lipolysis. These genes have been associated with altered lipid response to statin therapy but no conclusive results have been published (86, 95, 96).

Apolipoprotein E (ApoE) is a genetically polymorphic protein defined by three alleles – *E2*, *E3* and *E4* – that influences hepatic cholesterol content. Lipoproteins with the *E4* isoform are taken up with greater affinity than those with the common *E3* isoform, which in turn are taken up more efficiently than those

with the *E2* isoform. Ordovas et al. reported that carriers of the *E2* genotype experience greatest LDL reduction in response to statin therapy in comparison to *E3* and *E4* carriers (97). With great interest, many similar studies were conducted trying to clarify the role of the *ApoE* polymorphism in the pharmacogenetics of statins (Table III). There is a reasonable body of evidence supporting the findings from Ordovas et al, among which are some large scale studies (86, 98, 99).

ApoE2 carriers seem to benefit most from statin therapy regarding lipid profile improvement, however a sub study of the Scandinavian Simvastatin Survival Study (S4) reported subjects carrying the *ApoE4* genotype to have the largest risk reduction of mortality (100). These results were not replicated (101) and the effect of the possible gene treatment interaction on clinical outcomes remains inconclusive. Besides *ApoE*, genetic variations in the genes coding for apolipoprotein B (*ApoB*) and apolipoprotein A1 (*APOA1*) have been part of pharmacogenetic research with inconclusive results (Table III). *PON1*, encoding the paraoxonase enzyme, is closely related to *ApoA1* and the transcriptional activity of the *PON1* gene has been shown to be increased by simvastatin (102). One small study including 51 patients reported the R allele of the *PON1 R192Q* polymorphism to be associated with better HDL response than those carrying the QQ genotype (103). Another study, did not confirm these results (104) and further research is needed to elucidate the role of this candidate gene.

Some functional mutations in the low density lipoprotein receptor (LDLR) cause familial hypercholesterolemia (FH), whereas more common genetic variations in the LDLR have been shown to modify the cholesterol lowering response to statins. Sterol regulatory elements binding factor 1 (SREBF1) and SREBF chaperone (SCAP) have been shown to regulate LDLR expression (105) and other enzymes in the lipid metabolism

Table III - Genetic association studies on response to cholesterol lowering drugs

Gene	SNP	Outcome	Study	Findings	Similar	Dissimilar
ApoE	E2/E3/E4	LDL reduction	Ordovas et al. (97)	Greatest LDLC reductions in E2 carriers	(86, 98, 99, 145-147)	(85, 148-153)
		HDL increase	Ballantyne et al. (154)	Enhanced HDL response E2	(152, 153)	
		Death	Gerdes et al. (100)	Attenuated mortality risk for E4 carriers		(101)
LDLR	Avall/Pvull	LDL reduction	Salazar et al. (155)	Attenuated LDL reduction A+ and P1 carriers		(85, 156)
ApoB	I/D	LDL reduction	Guzman et al. (157)	II carriers greatest LDL reduction		(85)
	Xba1	TC reduction	Ye et al. (145)	Greater TC reduction patients carrying X allele		(150)
APOA1	G-75A	HDL increase	Lahoz et al. (158)	Carriers GG genotype greater HDL response		(86, 159)
CETP	Taq1B	HDL increase	van Venrooy et al. (160)	B1 allele greater increase HDL-C		(85, 86, 161)
		Changes MSD/CE	Kuivenhoven et al. (88)	Decreased atherosclerosis progression in B1B1 carriers	(90-93, 162)	
HMGCR	33 SNPs	LDL reduction	Chasman et al. (85)	Reduced response heterozygous for SNP12, SNP29		(86)
ACE	I/D	LDL reduction	Marian et al. (107)	Enhanced response in DD subjects		(86)
		Angiogram/CE	Marian et al. (107)	Higher regression rate in DD subjects		(86, 108-110)
CYP7A1	A-204C	LDL reduction	Kajinami et al. (163)	C allele diminished LDL cholesterol response		(86, 147)
SREBP1	G-36del	HDL response	Salek et al. (164)	Reduced ApoA1 response in carriers del/del		(165)
SCAP	A2386G	TC/TG/LDL	Fiegenbaum et al. (165)	Better response in G allele carriers response		(164, 166)
LIPC	C-514T	HDL response	Lahoz et al. (96)	Greatest HDL response in T allele carriers		(86)
LPL	S447X	TC reduction	Thompson et al. (86)	Homozygous X greatest TC reduction		(95)
TLR4	D299Q	CE reduction	Boekholdt et al. (111)	Carriers G allele greatest response	(112)	
PON1	R192Q	HDL increase	Malin et al. (103)	Carriers R allele greatest response		(104)
SLCO1B1	T521C	TC reduction	Tachibana-limori et al. (113)	Better response in TT carriers		(86, 115, 116)
ABCB1	C3435T	LDL reduction	Kajinami et al. 2004 (117)	CC carriers of C3435T polymorphism experience smaller LDL reduction. No effect of G2677T/A		(118, 167)

CE = clinical events, MSD = mean segment diameter, TC = total cholesterol, TG = triglyceride.

(106) and have therefore also been investigated for genetic variation in relation with modified response. To date, replication of associations in pharmacogenetic studies of both the *LDLR* gene as well as the *SREBF1* and *SCAP* genes lack (table 3). Future studies may explain the role of genetic variations in these important genes in the lipid metabolism.

In addition to genes involved in the lipid lowering response to statins, polymorphisms in the *ACE* and toll like receptor 4 (*TLR4*) genes have been studied. The *ACE* I/D polymorphism was first studied in the *LCAS*, showing cholesterol reduction and atherosclerosis progression was most beneficial in subject carrying the DD genotype (107). Large studies in *GENHAT* (108) and *ACCESS* (86) could not confirm the gene treatment interaction from *LCAS* and studies in *CARE* (109) and in the Rotterdam study (110) even reported contradicting results. If there is an interaction between the *ACE* I/D and the effectiveness of statins, the interaction is small and its direction and molecular basis is unknown. The toll like receptor mediates innate and adaptive immunity. A functional polymorphism in the *TLR4* gene D299G has been studied in *REGRESS* and *SAS*, both including approximately 650 patients. Both studies showed that carriers of the G allele treated with statins had a lower risk of cardiovascular events than non carriers treated with statins (111, 112), suggesting that statins interact with inflammatory factors like the toll like receptor.

Polymorphisms in pharmacokinetic genes encoding the solute carrier organic transporter (*SCLO1B1*) involved in the hepatic uptake of statins and the adenosine triphosphate-binding cassette (ABC) B1 transporter involved in the hepatobiliary excretion of statins may influence the pharmacokinetics of statins and thereby its lipid lowering response. The TT genotype of the *SCLO1B1* T521C polymorphism has been associated with greater response to statins in lowering total cholesterol (113). A possible explanation for this finding is that the C allele is associated with impaired hepatic uptake of statins (114). The *ACCESS* study (86) and two small studies (115, 116) could not confirm the interaction between the *SCLO1B1* T521C polymorphism and the effect on lowering plasma lipid levels. The C3435T polymorphism of the *ABCB1* gene has been associated with modified response to statin therapy (117), which could not be replicated by Fiegenbaum et al. who found an association with the G2677T/A polymorphism of the *ABCB1* gene (118). Currently there is no evidence for an interaction between these polymorphisms in the *SCLO1B1* and *ABCB1* gene and statins on pharmacodynamic endpoints.

An excellent review published by Mangravite et al. gives a very comprehensive overview of all studies conducted in the pharmacogenetics of statins (119, 120).

Clinical implications

By addressing some of the most extensively investigated pharmacogenetic associated genes in cardiovascular drug therapy, it is clear that the evidence for clinical implications of pharmacogenetics currently is very scarce. Although each initial association of a polymorphism with modified drug response seemed very promising, the reality is that most associations cannot be replicated. Furthermore, most effects that were found are very small. Therefore implementation of these interactions in clinical practice is still far away. Only for coumarins, there is a real opportunity for pharmacogenetics by genotyping the *VKORC1* and *CYP2C9* gene to optimize anticoagulant therapy. Even though the effect of the existing variations in these genes are quite clear, clinical trials should provide evidence for the effectiveness of genotyping regarding prevention of adverse drug events and cost-effectiveness, before this procedure will be a part of every day anticoagulant therapy. These studies are currently underway and will possibly advocate for a global implementation of *VKORC1* and *CYP2C9* genotyping into the anticoagulant therapy guidelines.

Nevertheless, in August 2007 the Food and Drug Administration updated the warfarin prescribing information and highlighted the opportunity to use genetic tests to improve their initial estimate of warfarin dose. For other cardiovascular drugs, future research may well elucidate the role of genetics in the response to these drugs.

Future directions

Current approaches in pharmacogenetic research do not seem to lead to results that meet our expectations of individualized medicine. Therefore, new approaches are needed addressing issues and challenges such as the number of SNPs studied, study power, study design and application of new statistical methods in (pharmaco-)genetic analysis.

Most studies have only examined one polymorphism in a candidate gene associated with modified response to a certain drug. However, drug response is likely to result from complex interactions among various biologic pathways. Hence, future studies should consider a set of candidate genes and/or a genome wide scan (GWS) rather than addressing a single or small number of SNPs. In recent years, the costs for a GWS have considerably decreased and will be increasingly available.

Examining multiple SNPs will require sufficient sample size, that many studies to date lack.

Moreover, analysis of large numbers of SNPs to identify a combination of SNPs that influence drug efficacy, will be a huge challenge due to statistical problems. Not only the issue of multiple testing of many SNP should be addressed with new tools in statistical analysis, also possible important effects of gene-gene interactions should be considered (121). Although a definitive statistical method for characterising statistical patterns of epistasis is not known yet, conventional statistical methods only will not be the appropriate tool to decipher the complexity of pharmacogenetics.

Finally, to elucidate mechanisms that lie behind the genetic associations, other fields of research including proteomics and transcriptomics should be integrated in the field of pharmacogenomics.

Conclusion

In conclusion, although pharmacogenetic testing is already part of everyday clinical practice in some areas (chemotherapy,

psychiatry), for cardiovascular drugs currently only oral anticoagulant therapy seems to have a real opportunity to benefit from pharmacogenetic testing. In spite of the tremendous amount of publications in this field, there is no reason to advocate for genetic testing for any other cardiovascular drugs yet. Although future research will certainly benefit from emerging genetic technology as high throughput genome wide scans will be readily available, finding the genetic profile that will predict response to cardiovascular drugs will be a major challenge.

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