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Drug-Gene Interactions between Genetic Polymorphisms and Antihypertensive Therapy

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Abstract

Genetic factors may influence the response to antihypertensive medication. A number of studies have investigated genetic polymorphisms as determinants of cardiovascular response to antihypertensive drug therapy. In most candidate gene studies, no such drug-gene interactions were found. However, there is observational evidence that hypertensive patients with the 460W allele of the α-adducin gene have a lower risk of myocardial infarction and stroke when treated with diuretics compared with other antihypertensive therapies. With regard to blood pressure response, interactions were found between genetic polymorphisms for

endothelial nitric oxide synthase and diuretics, the α-adducin gene and diuretics, the α-subunit of G protein and β-adrenoceptor antagonists, and the *ACE* gene and angiotensin II type 1 (AT_1) receptor antagonists. Other studies found an interaction between ACE inhibitors and the *ACE* insertion/deletion (I/D) polymorphism, which resulted in differences in AT_1 receptor mRNA expression, left ventricular hypertrophy and arterial stiffness between different genetic variants. Also, drug-gene interactions between calcium channel antagonists and *ACE* I/D polymorphism regarding arterial stiffness have been reported. Unfortunately, the quality of these studies is quite variable. Given the methodological problems, the results from the candidate gene studies are still inconclusive and further research is necessary.

Hypertension is a major public health hazard because of its high prevalence, which is approximately 20% of the adult population in most developed countries,[1] and its high risk of cardiovascular diseases. Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients, and is responsible for a large proportion of cardiovascular disease in the population.[2-4] The average response to antihypertensive drugs is similar across different classes of antihypertensives. For example, in the Veterans Affairs study,[5] a randomised placebo-controlled clinical trial in which patients were randomly allocated to six different drugs or placebo, 31.7% of all hypertensive patients who were allocated to monotherapy with an antihypertensive drug failed to achieve a normal diastolic blood pressure. When the initial treatment failed, 85.9% of these patients were randomised to another antihypertensive which failed in 37.8% of these patients.[5] Although, the currently used 'trial and error' approach to antihypertensive drug therapy can be efficient in treating high blood pressure, it is not feasible with regards to long-term effects such as myocardial infarction (MI) and stroke. Important factors in interpreting the variability in outcome of drug therapy include the patient's general health, prognosis, disease severity, quality of drug prescribing and dispensing, compliance with prescribed pharmacotherapy and the genetic profile of the patient.[6,7]

Multiple susceptibility genes and the environment explain the phenotype of essential hypertension. From family, twin and adoption studies it has been estimated that 30–60% of the variation in blood pressure between individuals is caused by genetic factors.[8] However, there is a small proportion of familial forms of hypertension that have a single gene (Mendelian) inheritance pattern. These include:[9-11]

- apparent mineralocorticoid excess
- glucocorticoid remediable aldosteronism
- hypertensive forms of congenital adrenal hyperplasia
- Liddle's syndrome
- pseudohypoaldosteronism type II/Gordon's syndrome
- early-onset, autosomal dominant hypertension with severe exacerbation in pregnancy
- Bardet-Biedl syndrome types 2 and 4.

Pharmacogenetics focuses on the extent to which variability in genetic make-up is responsible for the observed differences in therapeutic response and adverse reactions between patients.[6,7] In other words, pharmacogenetics studies the interaction between drugs and genes, where interaction is defined as being present if the joint effect of a drug and genetic polymorphism is greater than the sum (additive scale) or product (multiplicative scale) of the individual effects of the drug and the genetic polymorphism. The purpose of pharmacogenetics is to understand the effects of genetic diversity on human response to drugs and other foreign substances and to use this information to avoid the occurrence of therapeutic failure and adverse drug reactions in

susceptible persons.[12] Drugs that are more specific for functional characteristics associated with an individual patient's polymorphism may contribute to a better response and reduced toxicity of pharmacotherapy.

Genetic polymorphisms may influence drug response in three ways.[13]

1. Through variation in pharmacokinetics that may pertain to absorption, but is mostly explained by altered metabolic clearance. Many pharmacokinetic drug-gene interactions are related to the cytochrome P450 (CYP) enzyme system.[14] The majority of these enzymes are located in the endoplasmic reticulum of the hepatocytes. This enzyme system can be modulated by genetic polymorphisms, causing some individuals to be poor (slow) metabolisers and others to be extensive (rapid) metabolisers. This is the case for many calcium channel antagonists that are metabolised by CYP3A4, many lipophilic β-adrenoceptor antagonists metabolised by CYP2D6, and losartan and irbesartan metabolised by CYP2C9.[15] However, it is unlikely that pharmacokinetic effects cause most of the antihypertensive drug-gene interactions.

2. Through altered pharmacodynamic drug-gene interactions. These involve, for example, gene products expressed as drug targets such as receptors and signal transduction molecules, which are relevant to the pharmacodynamics of drugs.

3. Through genes that are in the causal pathway of the disease and are able to modify the effects of drugs. It is also important to realise that most of the variation in blood pressure can be explained by a pharmacodynamic, rather than by pharmacokinetic, mechanism.[16] This is most apparent in studies in which pharmacokinetic and pharmacodynamic assessments are available in the same subjects, and in which inter-subject variability can be expressed as a coefficient of variation.

Four previous review articles presented pharmacogenetic concepts relevant to antihypertensive drug therapy. These articles included a brief overview of candidate gene studies with respect to blood pressure response and other cardiovascular responses to antihypertensive drug therapy.[17-20] Our review extends the previous overviews and discusses some of the reasons for inconsistent results regarding drug-gene interactions between genetic polymorphisms and antihypertensive drug therapy. Studies were identified in the Medline database from 1966 to October 2003 by combinations of keywords 'antihypertensive drug', 'genetics', 'polymorphism' and by checking the references of all identified papers. All studies that reported data on genetic polymorphisms and response to antihypertensive drug therapy were included. Response to antihypertensive drugs was not pre-specified and could include blood pressure response, change in left ventricular mass (LVM), risk of MI and stroke, and other cardiovascular effects.

1. Candidate Gene Studies and Antihypertensive Drugs

Thirty-six studies were found on the potential gene-drug interaction between genetic polymorphisms and antihypertensive drugs. Details of these studies are given in table I and table II. A summary of the findings of these studies is presented in table III.

1.1 Diuretics

One of the first polymorphisms examined for blood pressure response in patients treated with diuretics was the G460W (a Gly to a Trp substitution at residue 460) α-adducin polymorphism.[21,22] The human α-adducin 460W allele can be considered as a candidate gene for hypertension, because it may affect blood pressure by increasing renal tubular reabsorption of sodium through the activation of Na^+, K^+-ATPase (adenosine triphosphatase). Linkage and association studies, performed with markers mapping in the region (loci) of the α-adducin locus and with the G460W α-adducin polymorphism, respectively, yielded positive associations.[21] Compared with hypertensive patients who are homozygous for the 460G wild-type allele, hypertensive patients carrying at least one 460W allele have a less steep pressure natriuresis slope. This means that they need a higher arterial pressure to excrete the same amount of sodium after saline infusion.[55]

Table I. The influence of genetic polymorphisms on the effect of antihypertensive medicine in patients with essential hypertension

Sample	Duration of therapy	Gene (location)	Polymorphism	Allelic association[a]	References
Thiazide diuretic					
n = 143, Caucasian	8wk	*ADD1* (4p16.3)	G460W	460W allele associated with greater BP reduction	21,22
n = 1038, mixed	4y		G460W	460W allele associated with a lower risk of (combined) MI and stroke in comparison to other antihypertensive therapies. (observational study)	23
n = 87, Caucasian	2mo		G460W	460W allele associated with greater BP reduction	24
n = 585, mixed	4wk		G460W	No association (BP)	25
n = 87, Caucasian	2mo	*ACE* (17q23)	I/D	Insertion allele associated with a greater BP reduction	24
n = 376–585, mixed	4wk		I/D	No association (BP)	25,26
n = 387–585, mixed	4wk	*GNB3* (12p13)	825C/T	No association (BP)	25,27
n = 585, mixed	4wk	*ADRB1* (10q24–q26)	R389G	No association (BP)	25
n = 585, mixed	4wk	*ADRB2* (5q31–q32)	R16G	No association (BP)	25
n = 585, mixed	4wk	*LPL* (8p22)	S447Stop	No association (BP)	25
n = 585, mixed	4wk	*NOS2A* (17p11.2–q12)	E298D	E allele associated with greater DBP reduction	25
Selective β-adrenoceptor antagonist					
n = 63–91, Caucasian	4wk	*AGT* (1q42–q43)	M235T	No association (BP)	28
n = 84–86	1–3mo		M235T	No association (BP and LVM)	29,30
n = 84–86	1–3mo		T174M	No association (BP and LVM)	29,30
n = 63–91, Caucasian	4wk	*ACE* (17q23)	I/D	No association (BP)	28
n = 50, Caucasian	15d		I/D	No association with expression of AT_1R mRNA expression	31
n = 84–86, Caucasian	1–3mo		I/D	No association (BP and LVM)	29,30
n = 84–86, Caucasian	1–3mo	*AGTR1* (13q21–q25)	1166A/C	No association (BP and LVM)	29,30
n = 147, Caucasian	4wk	*ADRB1* (10q24–q26)	G389R	No association (BP and heart rate)	32
n = 40, mixed	>4wk		G389R	A allele associated with greater DBP reduction	33
n = 40, mixed	>4wk		S49G	S allele associated with trend towards DBP reduction	33
n = 84–86, Caucasian	3mo	*CYP11B2* (8q21–q22)	−344C/T	No association (BP and LVM)	30,34
n = 97, Caucasian	3mo	*ADRA2A* (10q24–q26)		SBP ↓ (278G/T) and DBP ↓ (1309G/A)	35 [b]
		ADRB2 (5q31–q32)		SBP ↓ (1342G/C) and DBP ↑ (1817G/A)	
		AGT (1q42-q43)		SBP ↑ (1015C/T)	
		EDNRB (13q22)		SBP ↓ and DBP ↓ (40G/A)	
		NOS3 (7q36)		SBP ↓ (498G/A) and DBP ↑ (2996A/G)	
		LIPC (15q21–q23)		DBP ↓ (110A/G)	
n = 90, Caucasian	48wk	*BDKRB2* (14q32.1–q32.2)	+9/−9	No association (LVM)	36

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Table I. Contd

Sample	Duration of therapy	Gene (location)	Polymorphism	Allelic association[a]	References
Selective and non-selective β-adrenoceptor antagonists					
n = 66, Caucasian	4wk	*GNAS* (20q13.2–q13.3)	FokI (+/−)	FokI + allele associated with greater BP reduction	37
ACE inhibitor					
n = 63–91, Caucasian	4wk	*AGT* (1q42–q43)	M235T	No association (BP)	28
n = 125, Caucasian	4wk		M235T	235T allele associated with greater BP reduction	38
n = 63–91, Caucasian	4wk	*ACE* (17q23)	I/D	No association (BP)	28
n = 125, Caucasian	4wk		I/D	No association (BP)	38
n = 104, Caucasian	6mo		I/D	Deletion allele associated with greater BP reduction	39
n = 50, Caucasian	15d		I/D	Deletion allele associated with reduced AT_1 receptor mRNA expression	31
n = 54, Japanese	>2y		I/D	Insertion allele associated with greater regression of LVH	40
n = 75, Japanese	6mo		I/D	Insertion allele associated with greater regression of LVH	41
n = 60, Japanese	1y		I/D	Deletion allele associated with positive effect on LVH and reduced diastolic filling	42
n = 57, Japanese	6wk		I/D	Insertion allele trend towards association with greater DBP reduction	43
n = 40, Caucasian	2mo	*AGTR1* (13q21–q25)	1166A/C	1166C allele associated with greater BP reduction and greater reduction arterial stiffness; no association heart rate	44
n = 125, Caucasian	4wk		1166A/C		
AT_1 receptor antagonist					
n = 84–86, Caucasian	1–3mo	*AGT* (1q42–q43)	M235T	No association (BP and LVM)	29,30
n = 84–86, Caucasian	1–3mo		T174M	174M allele associated with positive effect on LVM, no association BP	29,30
n = 84–86, Caucasian	1–3mo	*ACE* (17q23)	I/D	Insertion allele associated with greater DBP reduction, no association LVM	29,30
n = 84–86, Caucasian	1–3mo	*AGTR1* (3q21–q25)	1166A/C	1166A allele showed trend towards association with greater SBP reduction, no association LVM	29,30
n = 84, Caucasian	3mo	*CYP11B2* (8q21–q22)	−344C/T	−344T allele associated with greater SBP reduction, no association LVM	30,34
n = 84, Caucasian	12wk	*CYP2C9* (10q24)	*1 and *2	*1/*1 compared with *1/*2 associated with reduced DBP	45
n = 97, Caucasian	3mo	*APOA1* (11q23–q24) *CYP11B2* (8q21–q22)		SBP ↑ and DBP ↑ (1449A/G) SBP ↓ (267T/C)	35 [b]

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Table I. Contd

Sample	Duration of therapy	Gene (location)	Polymorphism	Allelic association[a]	References
		EDNRB (13q22)		SBP ↑ (40G/A)	
		NOS3 (7q36)		SBP ↑ (498G/A)	
		ACE (17q23)		DBP ↓ (12257A/G)	
		AGT (1q42–q43)		DBP ↑ (1198C/T)	
		LIPC (15q21–q23)		DBP ↓ (110A/G)	
n = 90, Caucasian	48wk	*BDKRB2* (14q32.1–q32.2)	+9/–9	No association (LVM)	36
Calcium channel antagonist					
n = 63–91, Caucasian	4wk	*AGT* (1q42–q43)	M235T	No association (BP)	28
n = 50, Caucasian	15d	*ACE* (17q23)	I/D	Insertion allele association with reduced expression of AT_1 receptor mRNA	31
n = 40, Caucasian	2mo	*AGTR1* (3q21–q25)	1166A/C	1166A allele associated with greater reduction arterial stiffness, no association BP and heart rate	46

a Comparisons are versus untreated or placebo, unless otherwise specified.

b 74 single nucleotide polymorphisms in 25 genes involved in BP regulation.

AT_1 = angiotensin II type 1; **BP** = blood pressure; **d** = days; **DBP** = diastolic blood pressure; **I/D** = insertion/deletion; **LVH** = left ventricular hypertrophy; **LVM** = left ventricular mass; **MI** = myocardial infarction; **mo** = months; **mRNA** = messenger RNA; **SBP** = systolic blood pressure; **wk** = weeks; **y** = years.

Moreover, they have lower plasma renin activity,[21] enhanced proximal tubular reabsorption,[56] and a more pronounced blood pressure decrease after acute sodium depletion or long-term diuretic treatment.[21] The 460W allele of the α-adducin gene is associated with a higher affinity for the Na^+, K^+-ATPase pump than the 460G allele.[57] This last finding is particularly relevant because the same functional protein alteration has been demonstrated in both the rat and human 'hypertensive' α-adducin variant, suggesting that the protein plays a crucial role in Na^+/K^+ metabolism.[57,58] In two trials, the 460W allele was associated with a greater blood pressure reduction in response to treatment with diuretics. In the heterozygous (G/W) hypertensive patients a mean blood pressure decrease of 14.7 ± 2.2mm Hg was found versus 6.8 ± 1.4mm Hg in the homozygous (G/G) hypertensive patients.[21,22] Recently, a second group of researchers also found an interaction between mean arterial pressure (diastolic blood pressure + [systolic blood pressure − diastolic blood pressure]/3) reduction and the G460W polymorphism. Homozygous (G/G) hypertensive patients had a reduction of 6mm Hg and patients with at least one 460W allele had a reduction of 12mm Hg in mean blood pressure.[24] In another study the 460W allele was associated with a lower risk (odds ratio [OR] 0.49; 95% CI 0.32, 0.77) of MI and stroke in diuretic users compared with users of other antihypertensive drug therapies.[23]

Recently, the *ACE* insertion/deletion (I/D) polymorphism was investigated for its role in blood pressure response to a diuretic (hydrochlorothiazide 25mg). In this study a significant association was found between *ACE* I/D polymorphism and response to hydrochlorothiazide. Hypertensive patients with the I/I genotype had a mean arterial pressure reduction of approximately 10mm Hg and those with the D/D genotype a reduction of 3.8mm Hg.[24]

A third polymorphism that may influence the effect of a diuretic is the 825C/T (cytosine into a thymine) polymorphism (exon 10) of the gene encoding for the β_3-subunit of the G-protein. The G-proteins mediate signal transduction across cell membranes.[59] The 825T allele of the β_3-subunit of the G-protein polymorphism has been related to an RNA splice variant that results in the deletion of nucleotides 498–620 of exon 9 and structural changes in the β-subunit.[60] Moreover, an enhanced signal was observed in lymphoblast lines from hy-

pertensive individuals carrying the 825T allele,[59] which suggests that this genetic variation may indeed affect signal transduction. In one trial, a positive association was found between the 825T allele and the effect of hydrochlorothiazide on blood pressure. Mean declines in systolic and diastolic blood pressures were 6 ± 2 and 5 ± 1mm Hg greater in TT than in CC homozygous patients, respectively.[27] However, with a larger sample size (585 vs 387 in the earlier study[27]) the investigators could not replicate these results.[25] Instead, they found an interaction between thiazides and the 298E allele of nitric oxide synthase gene on diastolic blood pressure. Hypertensive patients homozygous for the E allele had a diastolic blood pressure reduction of 8.6 ± 0.4mm Hg compared with 7.1 ± 0.6mm Hg for the other genotype groups. Additionally, their previously reported interaction with *ACE* gene[26] could also not be replicated with this larger sample size.[25]

Recently, an alternatively spliced transcript of the β_3-subunit of the G-protein referred to as $G\beta_3S2$ was identified. Transcripts of the $G\beta_3S2$ lack 129 base pair of coding sequence of the β_3-subunit of the G-protein. A close association between $G\beta_3S2$ expression and T-allele status of the 825C/T polymorphism of the β_3-subunit of the G-protein was found. The data suggest that $G\beta_3S2$ is a biologically active variant of the β-subunit of the G-protein, which may play a role in the manifestation of the complex phenotype associated with the 825C/T polymorphism.[61]

Table II. The influence of genetic polymorphisms on the effect of antihypertensive medicine in non-hypertensive patients with related diseases

Sample	Duration of therapy	Gene (location)	Polymorphism	Allelic association[a]	Reference
Selective β-adrenoceptor antagonist					
Nondiabetic nephropathy; n = 81, Caucasian	3–4y	*ACE* (17q23)	I/D	Deletion allele associated with reduced glomerular filtration	47
Selective and non-selective β-adrenoceptor antagonists					
Chronic heart failure; n = 328, Caucasian	2y	*ACE* (17q23)	I/D	Deletion allele associated with decreased chance of needing a heart transplantation	48
ACE inhibitor					
Proteinuric renal disease; n = 36, Japanese	12wk	*ACE* (17q23)	I/D	No association (proteinuria)	49
Diabetic nephropathy; n = 35, Caucasian	7y		I/D	Deletion allele associated with reduced glomerular filtration	44
Chronic heart failure; n = 34, Caucasian	6wk		I/D	Insertion allele associated with greater BP reduction (captopril), no association (lisinopril)	50
Post-PTCA; n = 126, Japanese	3–6mo		I/D	Insertion allele associated with reduced chance of restenosis	51
Nondiabetic nephropathy; n = 81, Caucasian	3–4y		I/D	Deletion allele associated with reduced glomerular filtration	47
Nondiabetic nephropathy; n = 88, Caucasian	4–12wk		I/D	Deletion allele associated with reduced proteinuria (when there is high salt excretion)	52
Post-coronary stents n = 345, Caucasian	6mo		I/D	Insertion allele associated with increased chance of restenosis	53
Cerebrovascular disease (stroke/TIA), n = 5688, mixed	4wk		I/D	No association (predicting cardiovascular risk or effect treatment)	54

a Comparisons are versus untreated or placebo, unless otherwise specified.

AT_1 = angiotensin II type 1; **BP** = blood pressure; **I/D** = insertion/deletion; **mo** = months; **PCTA** = percutaneous transluminal coronary angioplasty; **TIA** = transient ischaemic attack; **wk** = weeks; **y** = years.

Table III. Summary of the primary findings of candidate gene studies (comparisons are versus untreated or placebo, unless otherwise specified). The numbers of studies are listed based on allelic association with the outcome

Drug/ outcome	*ACE* A/N	*ADD1* A/N	*ADRA2A* A/N	*ADRB1* A/N	*ADRB2* A/N	*AGT* A/N	*AGTR1* A/N	*APOA1* A/N	*BDKRB2* A/N	*CYP2C9* A/N	*CYP11B2* A/N	*EDNRB* A/N	*NOS3* A/N	*GNAS* A/N	*GNB3* A/N	*LIPC* A/N	*LPL* A/N	*NOS2A* A/N
Thiazide diuretic																		
BP	1/1	3/1		1/0	0/1													
MI/St[a]		1/0																
β-Adrenoceptor antagonists																		
BP	0/2		1/0	2[a]/1	1/0	1[b]/3[c]	0/1	0/1		0/1	0/1	1/0	1/0	1/0				
LVH/LVM	0/1					0/2[c]	0/1		0/1									
AT_1R mRNA	0/1																	
HR				0/1														
ACE inhibitors																		
BP	1/3					1/1	1/1											
LVH/LVM	3[d]/0																	
AT_1R mRNA	1/0																	
AS							1/0											
HR							0/1											
AT_1R antagonists																		
BP	1[e]/0		0/1		0/1	0/2[c]	1[b]/0	1/0			2[f]/0	1[b]/0	1[b]/0			1[e]/0		
LVH/LVM	0/1					1/1	0/1		0/1		0/1							
Calcium channel antagonists																		
BP						0/1	0/1											
AT_1R mRNA	1/0																	
AS							1/0											
HR							0/1											

a The S49G and G389R polymorphisms of the β_1-adrenoceptor are counted as two studies.

b Only SBP.

c The M235T and T174M polymorphisms of the angiotensinogen gene are counted as two studies.

d Two studies found an association with insertion allele, one study with deletion allele.

e Only DBP.

f Compared with other antihypertensive therapies.

A/N = associated/not associated; **AS** = arterial stiffness; **AT_1R mRNA** = angiotensin II type 1 receptor messenger RNA expression; **BP** = blood pressure; **DBP** = diastolic blood pressure; **HR** = heart rate; **LVH** = left ventricular hypertrophy; **LVM** = left ventricular mass; **MI** = myocardial infarction; **SBP** = systolic blood pressure; **St** = stroke.

A polymorphism of the α-adducin gene may be used to identify patients with hypertension who are salt sensitive and respond relatively well to treatment with a diuretic. Nevertheless, two studies did not find an association between salt sensitivity and this gene in young men[62] or the general population.[63] The β_3-subunit of the G-protein was also proposed as a candidate gene, but Ciechanowicz et al.[64] could not find an association between polymorphism of the α-adducin gene and salt sensitivity of blood pressure. Furthermore, it has been suggested that polymorphisms of the angiotensin II type 1 receptor gene (*AGTR1*) and the γ-subunit of the epithelial Na^+-channel are also associated with salt sensitivity.[65,66] However, Giner et al.[67] could not find an association. Whether genetic polymorphisms that are associated with salt-sensitivity also modify the response to diuretics remains to be investigated.

1.2 β-Adrenoceptor Antagonists

In only two of 11 studies was a drug-gene interaction found between a genetic polymorphism and reduced blood pressure response to a β-adrenoceptor antagonist.[33,37] This reduced response was attributed to a FokI +/– polymorphism encoding for the α-subunit of the G-protein. Good responders (62.5% had a FokI + allele) had a mean arterial blood pressure decrease of >15mm Hg and poor responders (41.7% had a FokI + allele) had a decrease of <11mm Hg.[37] The α-subunit of each heterotrimeric G-protein contains the guanine nucleotide-binding site, which has intrinsic guanosine triphosphatase activity and confers the functional specificity on each G-protein that allows it to discriminate among multiple receptors and effectors. In the cardiovascular system, the α-subunit of the G-protein couples β_1- and β_2-adrenoceptors in order to stimulate cyclic adenosine monophosphate (cAMP) production. Johnson et al.[33] found an interaction between β_1-adrenergic receptor and metoprolol. Patients homozygous for arginine at codon 389 had a nearly 3-fold greater reduction in daytime diastolic blood pressure compared with those who carried the variant allele.

In patients with nondiabetic nephropathy, the presence of the *ACE* D allele was associated with a reduction of glomerular filtration.[47] Another drug-gene interaction with the *ACE* D allele was observed in patients with chronic heart failure. In this study,[48] treatment with a β-adrenoceptor antagonist was associated with a decreased need for heart transplantation.

The A (adenine) into a C (cytosine) transversion at nucleotide position 1166 is located in the 3′untranslated region of the *AGTR1* gene on chromosome 3q21-q25. Some studies have shown that it was associated with hypertension,[68,69] left ventricular hypertrophy (LVH),[70] coronary heart disease,[71] MI,[72] and progression of diabetic nephropathy.[73] The 1166A/C polymorphism of the *AGTR1* gene was, however, not related to variation in blood pressure response or degree of LVH during β-adrenoceptor antagonist therapy in small groups of hypertensive patients.[28-30]

Recently, Liljedahl et al.[35] tested a microarray-based minisequencing system on DNA samples of 97 hypertensive patients, of whom 49 were treated with atenolol. They genotyped 74 single nucleotide polymorphisms (SNPs) in 25 genes that were involved in blood pressure regulation. This group of researchers found a drug-gene interaction between atenolol and several genes which resulted in a change in blood pressure, including:

- 40G/A polymorphism of the endothelin receptor type B gene
- 278G/T and 1817G/A polymorphism of the adrenergic α_{1a}-receptor gene
- 1342G/C and 1309G/A polymorphism of the adrenergic β_2-receptor gene
- 498G/A and 2996A/G polymorphism of the endothelial nitric oxide synthase gene
- 110A/G polymorphism of the lipase hepatic gene.

However, this study[35] included only a small number of patients and was focused on the applicability of the minisequencing system rather than on finding drug-gene interactions. Because of the large number of SNPs tested without adjustment for multiple testing and the small number of patients, this

study could have resulted in a large number of false-positive and false-negative associations.

1.3 ACE Inhibitors

Most studies on interactions between ACE inhibitors and genetic polymorphisms have concentrated on the renin-angiotensin system (RAS). One of the steps of the RAS is the expression of angiotensinogen precursor in the liver. In response to lowered blood pressure it is cleaved by the enzyme renin. The resulting product, angiotensin I, is then cleaved by ACE to generate the physiologically active enzyme angiotensin II. This protein is involved in maintaining long-term blood pressure and in the pathogenesis of essential hypertension.

In one study, no drug-gene interaction was found between ACE inhibitors and the M235T (methionine into a threonine) polymorphism of the angiotensinogen gene.[28] However, in a larger study an interaction was found.[38] In this study, the reduction in systolic blood pressure was 20 ± 3mm Hg in patients with TT genotype compared with 22 ± 2mm Hg in patients with MT genotype and 13 ± 4mm Hg in patients with the MT and MM genotype. The reduction in diastolic blood pressure in patients carrying the TT allele was 11 ± 3mm Hg compared with 14 ± 2mm Hg in patients with MT genotype and 8 ± 2.5mm Hg in patients with the MM genotype.[38]

Most studies have concentrated on investigating the association between the I/D polymorphism (intron 16) of the *ACE* gene and the response to an ACE inhibitor. In one study,[39] a greater blood pressure reduction with ACE inhibitor therapy was observed in subjects with the D allele. In this study, the reduction in systolic blood pressure in patients with the DD genotype was 5.6 ± 3.1mm Hg compared with 3.1 ± 1.1mm Hg with the II genotype, and 3.6 ± 2.2mm Hg and with the ID genotype. The reduction in diastolic blood pressure in patients with the DD genotype was 8.9 ± 6.0mm Hg compared with 5.5 ± 3.4mm Hg with the II genotype 5.8 ± 4.0mm Hg with the ID genotype. In the other studies, the insertion allele was associated with a reduced regression of LVH in patients with hypertension,[40,43] greater reduction of glomerular filtration in diabetic and nondiabetic patients,[44,47] and less reduction of proteinuria in patients with nondiabetic nephropathy (primarily combined with a high excretion of salt).[74]

In two studies, the interaction between the 1166A/C polymorphism of the *AGTR1* gene and the response to ACE inhibitors was investigated. In a small study, no interaction was found.[38] In a larger study, the C allele was associated with greater reduction of arterial stiffness and blood pressure by ACE inhibition.[46] Patients with the AA genotype had a blood pressure reduction of approximately 6mm Hg and those with AC/CC genotype had a reduction of approximately 14mm Hg.[46]

1.4 Angiotensin II Type 1 Receptor Antagonists

Only one group has investigated the role of genetic polymorphisms and the response to an angiotensin II type 1 (AT_1) receptor antagonist (irbesartan).[29,30,34-36] The 174M allele of the angiotensinogen gene was associated with a positive effect of irbesartan on LVH. No interactions were found between the M235T polymorphism of the angiotensinogen gene or the –344C/T (cytosine to a thymine) polymorphism of the aldosterone synthase gene (*CYP11B2*) and irbesartan on reduction of LVH.[29,30,34] However, the –344T allele of the aldosterone synthase gene was associated with an increased reduction of systolic blood pressure after treatment with the AT_1 receptor antagonist. Patients with the TT genotype had a mean reduction in blood pressure of 21 ± 19mm Hg compared with 14 ± 18mm Hg in patients with TC genotype and 0 ± 17mm Hg in patients with CC genotype.[34] In contrast, the I allele of the *ACE* gene was associated with a reduction of diastolic blood pressure. Patients with the II genotype had a reduction of 18 ± 12mm Hg compared with 8 ± 11mm Hg in patients with ID genotype and 6 ± 9mm Hg in patients with DD genotype.[29] Aldosterone synthase is a key rate-limiting enzyme for the biosynthesis of aldosterone. The –344C/T polymorphism is associated with elevated plasma aldosterone concentration

through increased aldosterone synthesis.[75] These results suggest that the –344T allele is functionally associated with increased sodium reabsorption and thereby maintains blood pressure at a higher level due to volume expansion.

Liljedahl et al.[35] tested a microarray-based minisequencing system on DNA samples of 97 hypertensive patients of whom 48 were treated with irbesartan. They found that lowering of blood pressure by irbesartan was modified by several polymorphisms, including:

- 1449A/G polymorphism of the apolipoprotein A
- 267C/T polymorphism of the CYP, family 11, subfamily B, polypeptide 2 gene (*CYP11B2*)
- 40G/A polymorphism of the endothelin receptor type B gene
- 498G/A polymorphism of the endothelial nitric oxide synthase gene
- 1015C/T polymorphism of the angiotensinogen gene
- 12257G/A polymorphism of the *ACE* gene
- 110A/G polymorphism of the lipase hepatic gene.

1.5 Calcium Channel Antagonists

The influence of genetic polymorphisms on calcium channel antagonists has been examined in three studies. In one study, an interaction was observed between calcium channel antagonists and the *ACE* gene: patients with the I allele had a reduced expression of AT_1 receptor mRNA.[31] Another study found a drug-gene interaction with the A allele of the 1166A/C polymorphism of the *AGTR1* gene, which leads to greater reduction of arterial stiffness.[46] A drug-gene interaction between the M235T allele of the angiotensinogen gene and calcium channel antagonists could not be demonstrated regarding blood pressure response.[28,46]

2. Potential Reasons for Inconsistent Findings

Most of the studies exhibited inconsistent findings and did not yield conclusive results. In one[39] of four studies,[28,38,39,43] for example, an interaction was found between the *ACE* gene and ACE inhibitors regarding blood pressure response. The most promising result was the interaction between the G460W allele of the α-adducin gene and blood pressure reduction and risk of MI and stroke in response to diuretics. However, these results need to be replicated before definitive conclusions can be made.

2.1 Study Power

It is known that it may be problematic to demonstrate linkage or association consistently. For example, some groups were able to confirm an association between hypertension and the M235T polymorphism, while others could not.[76-78] A meta-analysis of 5500 subjects reported a significant (OR 1.2; $p < 0.0001$) but weak association between this polymorphism and hypertension.[79] This is most likely the same for studies investigating the role of genetic polymorphisms and the response to antihypertensive treatment. For this reason, pharmacogenetic studies require a large group of patients in order to have sufficient power to detect small genetic effects. This will reduce the likelihood of getting false-positive or false-negative results. For instance, Turner et al.[25] found no interaction between the *ACE* gene and β_3-subunit of the G-protein and the effect of hydrochlorothiazide on blood pressure response, in contrast to their previous reported finding of an association with a smaller sample size.[26,27] The sample size in the studies investigating antihypertensive drug-gene interactions in hypertensive patients ranged from 40 to 1048 persons. However, 71% of the studies had less than 100 patients, which is not sufficient for conclusive results when, besides a genetic factor, an interaction is also investigated. The number of patients needed to detect drug-gene interactions depends on the outcome studied (e.g. continuous versus categorical), the contrast between responders and nonresponders in different genotype groups (the amount of interaction), and the precision of the measurements.[80,81]

2.2 Genetic Diversity between Populations

Genetic diversity between populations can hinder replication of results. Gene variants that were select-

ed during evolution to conserve salt, for example, may play a larger role in hypertensive patients with ancestors from Africa.[18] In a study where the disease-causing allele is more prevalent, it might be easier to find an interaction. One example which was investigated for antihypertensive drug-gene interaction is the frequency of the I allele of the *ACE* gene which is different between Asian and Caucasian populations, i.e. 62% and 50%, respectively.[28,38,39,43]

2.3 Different Study Design

There are often different inclusion criteria for different study populations. If, for example, one study only included patients with severe hypertension, it is possible that these patients have a genetic profile which differs from moderate hypertensive patients examined in another study. This might be the case when the study of Stavroulakis et al.[39] (systolic blood pressure ≥140mm Hg and/or diastolic blood pressure ≥90mm Hg) is compared with Hingorani et al.[38] (systolic blood pressure >160mm Hg or diastolic blood pressure >90mm Hg). Furthermore, differences in treatment regimen were found between these studies. Both studies had a 4-week washout period, but in one study patients were given fosinopril 20mg once daily[39] (defined daily dose [ddd] equivalent = 1.33) while in the other study,[38] patients were given captopril 50 mg/day or enalapril 10 mg/day or lisinopril 10 mg/day or perindopril 4 mg/day (all ddd equivalent = 1). Another influence may be the variation in duration of therapy in different studies. The duration of therapy ranged from 15 days to 7 years in studies which focused on antihypertensive drug-gene interactions in hypertensive patients.

Different results may be explained by the use of different study designs, such as experimental (e.g. randomised clinical trial) and observational (e.g. cohort and case-control) studies. In observational study designs, for example, confounding may be a problem (e.g. population stratification).

Another potential explanation for different results relates to the definition of outcome. For instance, Scairrone et al.[24] used the reduction in mean blood pressure, while Turner et al.[25] used systolic and diastolic blood pressure separately.

2.4 Genetic Polymorphism and Disease-Causing Factors

Most of the examined polymorphisms are probably not the disease-causing factors.[49] An example is the M235T polymorphism of the angiotensinogen gene. There is now evidence that an A-for-G nucleotide substitution in the promoter region of the angiotensinogen gene 6 nucleotide upstream from the start site of transcription is the functional mutation.[82,83] The A substitution alters the binding of a nuclear protein, resulting in increased gene transcription compatible with increased angiotensinogen levels. Fortunately, it has been suggested that the –6G/A allele is nearly in complete linkage disequilibrium (LD) with the M235T polymorphism.[79,84] The same holds for the I/D polymorphism in the *ACE* gene. The I/D polymorphism predicts approximately half of the interindividual variability of ACE levels in serum[85,86] and tissue.[87] Thus, the probability that the *ACE* gene is not in linkage equilibrium with the functional polymorphism is considered very small.[86,88,89]

3. Considerations in the Design of Pharmacogenetic Studies

There are several ways to design studies to investigate interactions between antihypertensive drugs and genetic polymorphisms. All studies performed to date investigated (allelic) polymorphisms. This sort of study provides the most powerful approach to identify genes of small effect in complex traits,[90] because the markers that are used are either very close to the susceptibility locus or lie in the gene of interest itself. It is difficult to perform a linkage study, because a high number of patients would be needed and only relatives who use the same antihypertensive drugs can be included. In the future, genome-wide association studies using SNPs will become available, which will make it possible to use unrelated cases and controls to map regions of the genome, and eventually the whole genome.

It is possible to consider different endpoints when investigating hypertension. For example, studies could consider long-term outcomes such as MI and/or stroke, intermediate-term outcomes such as atherosclerosis and/or LVH, or short-term outcomes such as blood pressure. Another option is to investigate adverse effects of antihypertensive drugs or adherence to antihypertensive medication. Moreover, there are several potential inclusion criteria and there is no clear indication whether it is, for instance, best to choose mild or severe hypertension. It is important to consider the appropriate group of controls, because a strong difference in response can result in spurious drug-gene interactions.

The number of markers and the question which markers an investigator wants to test also need to be considered. There are biallelic (SNPs and deletion/insertion polymorphisms) and microsatellite markers (tandem repeats). Biallelic markers are relatively less polymorphic, but they are more abundant and accessible. The number of markers depends on whether there is significant LD in the candidate gene in the study population. LD occurs when two particular alleles at loci on the chromosome go together more often than may be expected from independent segregation in a population. LD can be determined with a small pilot sample. This can help to optimise marker selection and provide information for haplotype analysis.[91] Genotyping more markers gives more information and thus more power. It is, nonetheless, more expensive and time consuming, and the sample size has to be increased because more genotypes groups are identified. When more markers are tested, adjustment should be made for multiple testing. It is best to investigate only candidate genes, which can be linked to a biological system. For antihypertensive response, for instance, the genes in the renin-angiotensin-converting enzyme are prominent, and other regulatory mechanisms of pressure-natriuresis are important because of their role in blood pressure homeostasis.

A more crucial issue is whether checking for population stratification is needed. Population stratification refers to a form of confounding. The bias from population stratification is the distortion in the association between the genetic variant and the outcome that can occur when the variant is associated with an unknown risk factor which varies by ethnicity. Population stratification may also be important in drug response. For instance, African Americans may react differently to a specific antihypertensive drug class compared with Caucasians.[92-95] The impact of population stratification is, however, not yet clear. Some conditions must be met before a substantial bias occurs: (i) there must be substantial variation across ethnicities in the allele frequency of the relevant gene; (ii) there must be substantial variation in disease rates; (iii) allele frequencies must correlate with adjusted disease rates between ethnic groups; and (iv) adjustment for ethnicity must not reduce the relevant effect.[96] At present it is still unclear whether population stratification biases results in a substantial way.[91,97] To minimise the effect of population stratification, a solution could be typing additional markers unrelated to the outcome,[96] or match for ethnicity.[97,98]

4. Future Prospects

Although there are many difficulties to overcome, pharmacogenetics may yield successful strategies to optimise drug therapy. Several potential candidate genes are currently under investigation for their potential to modify response to antihypertensive drugs. Findings from previous studies require conformation in other studies to be able to make definitive conclusions about current positive drug-gene interactions. It is also important that research groups collaborate more in order to facilitate the conduct of a meta-analysis for conclusive results. With the development of efficient methods for analysing massive amounts of data, pharmacogenetic studies may eventually lead to the optimisation of antihypertensive drug therapy based on genetic profiles of patients.

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