Abstract

Heart failure represents the composite endpoint of various cardiovascular disorders. Advanced pharmacotherapy resulted in significant improvement of overall survival, however with highly variable outcome, possibly due to genetic modification of drug disposition and action. This review highlights the role of genetic polymorphisms in systems responsible for disposition of drugs, used in heart failure patients (e.g. the polymorphic drug metabolizing enzymes such as cytochrome P450 enzymes, as well as polymorphic ATP-membrane transporters like P-glycoprotein (P-gp)). In addition, genetic variants in physiological systems, being target of drug action, particularly beta-adrenergic receptors, the renin–angiotensin–aldosterone system (RAAS)- and endothelin system, and the endothelial nitric oxide (NO) synthase are reviewed. The current situation in pharmacogenomics of heart failure with respect to drug disposition and action is characterized by multiple studies investigating single components of a complex system. Therefore, overall conclusions regarding treatment and/or outcome of heart failure patients based on individual genetic traits require large prospective trials allowing for simultaneous assessment of multiple genetic variants in different systems. Using advanced screening technologies, such trials can be carried out in the near future.

Keywords: Heart failure; Gene polymorphism; Pharmacokinetics; Adrenergic antagonists; Renin–angiotensin system

1. Introduction

Congestive heart failure represents the final stage of a continuous disease process. It originates from prevalent cardiovascular diseases, e.g. dilated cardiomyopathy or ischemic cardiomyopathy based on coronary artery disease. Although pharmacotherapy has provided significant improvement of overall survival, the prognosis of advanced heart failure is still rather poor. To further improve therapy, a detailed understanding of molecular events resulting in structural and functional changes of the heart would be pivotal. In particular, characterization of a possible genetic contribution to the overall disease process would allow to predict the course of the disease and to help selection of the appropriate therapeutic approach. Progress in identifying such disease modulating factors has been limited (at least compared to monogenic diseases, e.g. familial dilated cardiomyopathy), which is readily understandable based on the heterogeneous nature of the disease process. A number of excellent recent reviews addressed the interaction of genetics and disease progress with particular focus on susceptibility and/or modifier genes [1,2].

Numerous studies have resulted in guidelines for pharmacological treatment of heart failure [3]. ACE-inhibitors [4] and/or AT1-blockers [5], anti-adrenergic drugs [6], diuretics (no prospective mortality trial available), digitalis [7], and lately aldosterone antagonists [8] comprise standard treatment regimens, while others such as endothelin antagonists are still rather experimental [9]. Such standardized pharmacotherapy, however, leads to variable clinical outcome. One factor possibly contributing to this variability is the modification of drug disposition and action by genetic traits. This review highlights the influence of genetic polymorphisms
(monogenic traits leading to at least two phenotypes with a frequency of more than one percent) both in systems responsible for disposition of drugs used in heart failure patients and physiological systems determining effects of these drugs.

2. Pharmacogenomics of drug disposition

Highly variable plasma concentrations and hence effects following administration of standard doses have been described for many cardiovascular drugs. This phenomenon can be attributed to variable expression of drug metabolizing enzymes (in particular cytochrome P450) and, less well explored, to variable expression of drug transporters. Some drugs used in treatment of heart failure patients are metabolized by polymorphic enzymes (e.g. beta-adrenergic blockers and AT1-antagonists) or subject to polymorphic transport (e.g. digitalis).

2.1. Polymorphic metabolism

The large prospective trials such as MERIT-HF [10], and CIBIS [11] gave clear evidence that treatment with selective beta1-adrenoreceptor blockers like metoprolol and bisoprolol improves survival of patients with mild to moderate heart failure. Lately, the COPERNICUS-Study [12] demonstrated beneficial effects of carvedilol also for NYHA IV patients. In contrast to beta1-selective blockers, carvedilol inhibits beta1- and beta2, but also alpha1-receptors. Metoprolol and carvedilol, but not bisoprolol, are metabolized by cytochrome P4502D6. This enzyme has been studied in detail and 7–10% of the Caucasian population exhibit no CYP2D6 activity due to well characterized hereditary polymorphisms, which has a major impact on CYP2D6 dependent clearance of metoprolol and carvedilol [13,14]. For example, CYP2D6 poor metabolizers provide a six-fold elevated metabolic ratio of metoprolol [15]. From a survey on adverse events after metoprolol treatment, the same group concluded that side effects may be due to CYP2D6 genotype, since the frequency of poor metabolizers was elevated in the group of patients, who reported pronounced adverse symptoms [16]. The number of poor metabolizer patients studied in this trial, however, was rather limited. In contrast, an unexpected low drug effect may arise from the phenomenon of gene duplications of CYP2D6 leading to high protein expression. The frequency of the CYP2D6 duplications is given with 1–3% in Middle Europeans but up to 29% in Ethiopia [17].

A recent study from our group addressed the question of the impact of CYP2D6 phenotype on disposition of carvedilol during chronic treatment. The data indicate a pronounced increase of steady state plasma concentration in poor metabolizers (bioavailability 38% vs. 25%; P<0.01 [18]). As carvedilol has additional alpha-blocking properties, an unexpected high concentration following administration may lead to pronounced orthostatic problems based on vasodilation. There is, however, no data available whether the clinical outcome of beta-blocker therapy in heart failure is influenced by genetic polymorphisms of CYP2D6.

Aside selected beta-blockers, angiotensin-II type-1 (AT1) receptor antagonists such as losartan and irbesartan are subject to polymorphic metabolism [19–21]. The prodrug losartan is activated in the liver by the drug metabolizing enzyme cytochrome P450 2C9 (CYP2C9) to the active metabolite EXP3174, and Sekino et al. [22] demonstrated in healthy Japanese subjects that CYP2C9 wild type carriers have lower systolic blood pressure after losartan therapy than poor metabolizers. Irbesartan, in contrast, is inactivated by CYP2C9. The two major hereditary polymorphisms of this enzyme lead to amino acid replacements causing diminished enzyme activity. Consequently, hypertensive patients being low active CYP2C9 carriers showed a stronger effect on reduction of the diastolic and—less significant, of the systolic blood pressure—following treatment with irbesartan [19]. The impact of the variable pharmacokinetics of AT1-antagonists to the outcome on heart failure, however, has not yet been verified, since prospective studies considering this aspect are lacking.

2.2. Polymorphic transport

Digitalis glycosides have been used in the treatment of heart failure since more than 200 years. In contrast to the beneficial effects of beta-blockers and ACE-inhibitors on long-term survival in patients with heart failure, the digitalis trial did not detect differences in mortality [7]. Nevertheless, digitalis glycosides are still frequently prescribed by physicians particularly in Middle Europe. Digoxin undergoes only minor metabolism, but is a high affinity substrate of the P-glycoprotein (P-gp), a member of (ATP-binding cassette) superfamily of membrane transporters, which is encoded by the MDR-1 gene. P-gp is responsible for the apical transport of various other lipophilic drugs like the above-mentioned digoxin, but also for beta-blockers such as celiprolol, pafenolol, carvedilol and talinolol, cholesterol synthesis inhibitors like lovastatine, atorvastatine, and simvastatine, and many other compounds. Since P-gp mediates the transport of these important drugs via membranes of the intestine or the endothelial cells of brain capillaries [23,24], it may serve as functional barrier against drug entry [25] or contributes to excretion (expression at the canalicular site of hepatocytes or tubular cells of the kidneys). The important role of P-glycoprotein as duodenal apical transporter for digoxin was shown with co-administration of the P-gp inhibitor verapamil to healthy volunteers [26] leading to markedly increased bioavailability of digoxin. On the other hand, the digoxin uptake is decreased in subjects who received the antituberculous agent rifampicin prior to therapy. Rifampicin is an effective inducer of P-gp as well as of MRP2 [27,28] due to interaction with the pregnan X receptor (PXR) [29].
P-gp expression in various organs has a broad interindividual variability [30]. The attempt to investigate the association of P-gp expression and activity in relation of polymorphisms in the MDR1 gene, coding for P-gp revealed a significant correlation of a silent polymorphism in exon 26 (C3435T) with intestinal P-gp expression levels and oral bioavailability of digoxin [31]. The C3435T SNP had an allelic frequency of 53.9% in a sample of 461 German Caucasians [32], but varies significantly between different ethnic groups (0.17–0.27% in African Blacks, 0.41–0.47% in Orientals) [33–35].

The finding was supported by a study on volunteers, receiving 0.25 mg/day digoxin. Subjects being homozygous 3435TT had a 20% reduced bioavailability within the first 4 h and the differences to CC-carriers were more pronounced considering haplotypes with G2677T [36]. Similar results were obtained in a French study on digoxin [37]. However, the functional impact of C3435T is not consistent. In two Japanese studies on digoxin kinetics, the area under the curve in the first 4 h was significantly higher in the CC group than in subjects homozygous for TT [38,39]. The latter study had also investigated the effects of MDR1 polymorphisms on duodenal mRNA expression, demonstrating elevated expression in case of TT carriers, which could explain the lower digoxin levels in 3435CC-carriers. Investigations in a German sample with 55 volunteers using the beta-blocker talinolol as probe drug revealed that the exon 26 SNP is of minor importance [40].

Recent data from our group identified expression of P-gp in endothelial cells of ventricular tissue taken from human heart suggesting a role in modifying intracardiac drug concentrations [41]. In fact, patients with dilated cardiomyopathy had a significantly reduced expression of P-gp in heart. It is tempting to speculate that these patients have a higher susceptibility towards therapeutic and toxic effects of P-gp substrates. Subsequent analysis of the association of MDR1 genotype and P-gp expression, however, revealed no major impact of genetic polymorphisms on cardiac P-gp expression [42].

In summary, the functional importance of MDR1 polymorphisms remains to be elucidated and there is still no functional explanation for the association of C3435T with P-gp expression, described in some studies. Therefore, it may be hypothesized that C3435T represents a genetic marker for variants not yet identified in regulatory regions of the MDR1 gene.

3. Pharmacogenomics of drug action

Aside from inherited variations in drug metabolizing enzymes, genetic polymorphisms in drug effector systems have been explored in detail. Thereby, the same plasma concentration can lead to different effects based on genetic variability in receptors (adrenergic system) or enzymes (RAAS).

3.1. Adrenergic receptors

The increased adrenergic drive of the failing heart results in desensitizing of this pathway in patients with heart failure [43]. Cardiospecific over-expression of both β1- or β2 receptors in animal models resulted in cardiac dilatation and heart failure [44]. The question arises whether genetic polymorphisms in the adrenoceptor system affect drug response. For the β1-adrenoceptor gene, a total of 18 polymorphisms have been described, 7 of which lead to amino acid changes in the coding region [45]. Of particular interest is the Arg389Gly polymorphism which was shown to affect signal transduction in vitro. The Gly389 variant had a lower adenylate cyclase activity following isoproterenol stimulation [46]. Although exercise response was shown to be more pronounced in Arg389 patients [47], other careful investigations in healthy volunteers did not support a prominent functional role of this polymorphism in humans. Nevertheless, the idea that a diminished signal transduction of the Gly389 variant could have a protective role in heart failure has been recently supported [48]. The authors describe cardiac targeted expression of the Arg389Gly polymorphism in mice. Depending on age of the animals, Arg389 mice had a decreased cardiac contractility, expressed fetal and hypertrophy genes and presented finally with fibrosis and heart failure. Consequently, response to β-receptor blockade was more pronounced in Arg389 mice. Accordingly, the authors report improved ventricular function in heart failure patients homozygous for Arg389. A recent paper addressing the issue in a subset of 600 patients from the MERIT-HF study, however, did not detect any genotype dependent event free survival as a function of the Arg389-Gly polymorphism [49]. Therefore, the noteworthy experimental data by Mialet-Perez et al. [48] remain to be confirmed in larger clinical trials.

Interestingly, a previous paper had suggested a synergism of the above Arg389Gly polymorphism with an α2C-adrenoceptor variant which is characterized by a deletion of four amino acids at codons 322–325 [50]. Black patients being homozygous for both, Arg389 and the α2C-deletion, had a 10-fold increased risk to develop heart failure. The effects of the α2C-deletion are readily explained by a reduction of synaptic autoinhibitory feedback which in turn leads to increased pre-synaptic release of norepinephrine. The combination of enhanced receptor activation together with a more pronounced signal transduction could explain the deleterious effects observed.

For the β2-adrenoceptor, 13 genetic variants have been described. Two polymorphisms in the coding region, namely Arg16Gly and Gln27Glu have been associated with altered downregulation following chronic agonist exposure. Functional exercise capacity was higher in patients with reduced downregulation (16Gly/27Glu), whereas oxygen consumption was higher in patients prone to downregulation (16Gly/27Gln) [51]. A third polymorphism (Thr164Ile) had in vitro characteristics of a de-
creased binding resulting in reduced signal transduction for the Ile164 variant. In fact, one study in patients with heart failure indicated a negative influence of the 164Ile polymorphism on clinical outcome.

The association of genetic polymorphisms in adrenoceptors with susceptibility and/or treatment response in patients with heart failure shows promising results of both the mechanistic and the clinical side. At present, however, it is difficult to assess the relevance for drug therapy, and therefore, controlled clinical trials are needed.

3.2. The renin–angiotensin–aldosterone system

Based on the above guidelines, angiotensin converting enzyme inhibitors and angiotensin-receptor-1-antagonist belong to drugs of first choice in the treatment of hypertension and heart failure. It is firmly established that the renin–angiotensin–aldosterone system (RAAS) plays a major part in the regulation of blood pressure, electrolyte homeostasis and gene regulation [52]. There is an ongoing debate, however, whether genetic modulations of the RAAS-system which affect function of this system contribute themselves to the development of cardiovascular diseases or modify the outcome. Moreover, activity of this system may be altered by the progression the disease and the extent of changes can be influenced by genetics.

The renin gene appears to be relatively conserved. However, in a family with hyperproreninaemia, a premature stop codon was found in exon 10 at codon 387 [53]. Later, two single nucleotide polymorphisms (SNPs) were identified in the promoter region accounting for an elevation of 54% of in vitro transcription activity [54]. Very recently, SSCP analysis of the renin gene revealed further intronic polymorphisms and a missense mutation in exon 9. The frequency of a G1051A polymorphism differed significantly between 212 patients with hypertension and 209 normotensive controls [55]. Data in patients having reached the endpoint of congestive heart failure, however, are lacking to our knowledge.

In contrast to polymorphisms in the renin gene, a hereditary variant in the gene of the renin substrate, namely angiotensinogen, seems to play a moderate role in the etiology of cardiovascular diseases [56,57]. In the CARDIGENE study, investigating 433 patients with idiopathic cardiomyopathy and 401 gender- and age-matched controls, there was lack of evidence of an association of any out of 10 polymorphisms in candidate genes, including T174M and M23T [58]. In a recently published study, 158 Czech patients with chronic heart failure (NYHA II–IV) were genotyped for the polymorphism G-6A in the 5'-UTR and M235T and compared with 200 controls. There was a slightly increased frequency of the 235T-variant and the authors concluded from a combined analysis that subjects being homozygote for –6G and carrying at least one copy of 235T would have a significantly increased risk of heart failure [59].

Within the gene of the angiotensinogen converting enzyme (dipeptidyl carboxypeptidase, kininase II), a large number of genetic variants have been identified. The most prominent one is a 287-bp insertion/deletion polymorphism in intron 16 of the gene. In 1990, it could be shown that it modulates ACE activity [60] and a first genotyping study revealed a significant elevated proportion of subjects being homozygous for the deletion among CAD patients. Since the deletion variant is associated with increased ACE plasma concentrations, it was believed that the ACE insertion/deletion (I/D) polymorphisms acts as a potential risk factor for CAD [61]. Due to this pronounced difference (and possibly also due to the simple mode of detection of the ACE deletion), a large number of subsequent studies was published within a short time. In a large prospective study, the initial observation could not be confirmed [62]. Moreover, there was no statistically significant difference of the I/D allelic frequencies between 1319 myocardial infarction patients and 2381 population-based controls from the MONICA study [63] and a meta-analysis could show that with increasing sample sizes, the odds ratios for the overall risk of myocardial infarction in dependence of the ACE genotype approach a value of 1 [64].

Individual ACE activity differs widely, but the I/D-polymorphism contributes only 9 U/l on average, leading to a broad overlap of ACE activity in respective genotypes [65]. Therefore, the question arises whether the ACE genotype has in vivo functional consequences. As reviewed by Schunckert [65], the ACE DD genotype has no impact on the conversion rate of exogenous angiotensin I to angiotensin II, when the concentrations of angiotensin I are low, but has a markedly effect on conversion rates and blood pressure, when higher angiotensin I doses have been used during the investigation.

However, there are interesting observations on the impact of the ACE genotype, which may be not directed via effects of angiotensin II on the angiotensin receptor AT1. After the initial observation that the left ventricular mass increased differently after physical exercise dependent of the ACE genotype [66], in a further attempt, 1200 military recruits preselected for their ACE genotype underwent a 10-week physical training. Half of the subjects were treated with a subhypotensive dose of 25 mg/day of the AT1-inhibitor losartan, while the others received placebo. Strikingly, in the ACE DD-group, there was a significant nearly three times higher increment of left ventricular mass compared to II group, in both the losartan as well the placebo group [67]. Similar differences attributable to this genotype have been confirmed in several subsequent trials [68–70], however, others showed no significant effects [71–73]. The exact mechanisms which may in turn explain the conflicting data remains unclear.

The association of heart failure and ACE genotypes remains controversial. As reviewed by van Berlon et al. [74], initial studies with smaller sample sizes showed associations whereas larger studies with well-defined
matched control groups revealed only marginal or no association. However, some studies show that the ACE DD-genotype appears to have an influence on the progression of heart failure. For example, in a survey of 193 patients, having a similar ACE genotype distribution as controls, the 5-year mortality was 49% in the II/ID group but increased to 72% in the DD-group \( (P=0.001) \) [75].

There are also numerous studies investigating whether the ACE genotype influences the response to the treatment with ACE inhibitors. The majority of drug-related studies was performed in hypertensive patients. However, as reviewed by Koopmans et al. [76], there is still no clear picture on the impact of one of the genetic variants investigated. Similarly, a second review by Niu et al. [77] came to the same conclusion in patients with heart failure.

### 3.3. AT1 receptors

The angiotensin-II-receptor 1 gene displays also certain polymorphisms, however, the majority of studies gave no evidence for associations to cardiovascular diseases [78,79]. Moreover, there was a lack of association between the AT1-R polymorphisms and idiopathic dilated cardiomyopathy as demonstrated in 433 patients and 401 controls [58].

Since the AT1-receptor is also an important pharmacological target for drugs such as losartan, candesartan, etc., the question arises whether the polymorphism in the AT1-receptor may influence the drug effects. Although the number of studies published so far is rather limited, there is some evidence that this genetic trait indeed affects the clinical outcome. Diez et al. [80] observed among hypertensive patients treated with 100 mg losartan that the AT1-R 1166AA carriers exhibited lower serum concentrations of procollagen type I, a marker of extracellular collagen synthesis, compared with carriers being AC or CC. Moreover, the myocardial stiffness was significantly reduced as determined by Doppler echocardiography.

From the substantial number of trials dealing with polymorphisms of the RAAS-system, it can be concluded that the risk of cardiac diseases and the treatment outcome is only marginally influenced by a single hereditary variant. However, in some cases, the consideration of combinations of selected polymorphisms revealed highly interesting results [81]. Regarding heart failure, prospective studies on the clinical outcome considering the various genetic variants are necessary to judge the impact of the of polymorphic RAAS system.

### 3.4. The endothelin system

Endothelin is a potent physiological counterplayer of NO, an oligopeptide that mediates contraction of vessel smooth muscles and stimulates cell proliferation via endothelin receptors [82]. Hereditary variants in the genes of the endothelin system could therefore disturb the sensitive balance between dilatating NO and vasoconstricting endothelin [83]. Variants in ET(A) and ET(B)-receptors seem to have only minor impact on CAD [84], but for the development of hypertension [85] and dilated cardiomyopathy [86]. Certain variants had been identified in the endothelin gene. One of these variants, a frequent adenine insertion located in the 5'-UTR, was reportedly associated with hypertension [87]. We could show in in-vitro assays using umbilical vein endothelial cells that mRNA and protein expression was significantly elevated in the insertion variant compared to the wild type, implicating functional relevance also for cardiovascular diseases or heart failure [88].

Similar to noradrenalin, enhanced concentrations of big-endothelin predict a reduced survival in patients with heart failure [89]. Consequently, attempts have been made to use endothelin receptor antagonists as a therapeutic option. The current results, however, do not support the use of these drugs in heart failure.

### 3.5. Endothelial NO synthase

Nitrogene monoxide (NO) is one of the most potent relaxing compounds release from the endothelium after stimulation by certain factors such as bradykinin, thrombin, or acetylcholine [90]. The beneficial effects of NO resolve also from inhibition of platelet aggregation, adhesion of platelets and leukocytes to the surface of the endothelium, inhibition and adhesion, as well as from inhibition smooth muscle proliferation [91]. Due to this unique properties, genetic polymorphisms that decrease the activity of the endothelial NO synthase could promote the development of atherosclerosis and progression of coronary artery disease. A functional significant Glu298Asp-exchange was suggested as a risk factor for coronary artery disease [92] and a VNTR polymorphism in intron 13, firstly described by Nadaud et al. [93], was strongly associated with an elevated risk of CAD [94]. Possibly, C/A-rich motifs act as splicing enhancer [95], since it could be demonstrated that a 65-kDa heterogenous nucleic ribonucleoprotein (hnRNP) L binds to the CA-motifs dependent on the length of the repeats. Although there is a large amount of data showing the favorable effects of elevated eNOS expression in heart failure [91,96,97], no studies are available on the role of genetic polymorphisms in this system for both susceptibility and treatment outcome.

### 4. Potential impact of genetic polymorphisms on the outcome of heart failure patients

In this review, we describe the genetic variability in drug disposition and action with respect to compounds used in heart failure. Numerous polymorphisms are reported in drug metabolizing enzymes, transporters and effector systems. Studies addressing the combined impact of all these factors on outcome of heart failure patients have not been carried out so far. This is in part due to
recent limitations in technology for simultaneous assessment of multiple polymorphisms. The development of high-throughput techniques such as MALDI-TOF [98] and pyrosequencing [99] or design of high-density SNP-chips enables the parallel investigation of more than 10,000 SNPs [100]. These techniques are currently applied in multi-center epidemiological studies [101] and may be useful in outcome assessment of heart failure patients. Regarding clinical relevance of the currently available data on pharmacogenomics in heart failure best evidence is given for the impact of drug metabolizing enzymes. Reduced enzyme activity leads to decreased clearance, higher plasma concentrations and more side effects [16]. Polymorphisms in export pumps like p-glycoprotein do not seem to be suitable for the exact prediction of an individual’s bioavailability of, e.g. digoxin, however, there are first studies showing that polymorphic uptake-transporters like OATP-C may influence significantly the pharmacokinetics of statins [102]. Although the RAAS system is highly polymorphic, the influence on the clinical outcome of most genetic variants is low or controversial in relation to the development or treatment of heart failure. Polymorphisms in the endothelial NO synthase and to a less extent within the endothelin system were clearly shown to extent within the endothelin system were clearly shown to influence on pharmacogenomics in heart failure best evidence is given for the impact of drug metabolizing enzymes. Reduced enzyme activity leads to decreased clearance, higher plasma concentrations and more side effects [16]. Polymorphisms in export pumps like p-glycoprotein do not seem to be suitable for the exact prediction of an individual’s bioavailability of, e.g. digoxin, however, there are first studies showing that polymorphic uptake-transporters like OATP-C may influence significantly the pharmacokinetics of statins [102]. Although the RAAS system is highly polymorphic, the influence on the clinical outcome of most genetic variants is low or controversial in relation to the development or treatment of heart failure. Polymorphisms in the endothelial NO synthase and to a less extent within the endothelin system were clearly shown to be associated to the risk of coronary artery disease, however, there is currently also no evidence in heart failure patients. In summary, the current situation in pharmacogenomics of heart failure is characterized by multiple studies investigating single components of a complex system. Overall conclusions on the potential of individualized therapy in heart failure based on pharmacogenomic approaches, however, require large prospective trials.

References


[73] Fatini C, Guazzelli R, Manetti P, et al. RAS genes influence exer-


