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Published in:
Pharmacogenomics

DOI:
10.2217/pgs.15.120

Publication date:
2015

Document Version
Peer reviewed version

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Total Words: ~~4,917~~ 5444 References: 10~~195~~ Tables: 1 Figures: 0

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Disclosures: none

Key words: Heart failure, pharmacogenetics, pharmacogenomics, candidate genes, genome wide association study

Abbreviations:

A2AR Alpha 2 Adrenergic Receptors

ACE Angiotensin Converting Enzyme

A-HeFT African American Heart Failure Trial

Ala Alanine

Arg Arginine

Asp Aspartic acid

B1AR Beta 1 Adrenergic Receptors

B2AR Beta 2 Adrenergic Receptors

BEST Beta blocker Evaluation of Survival Trial

BNP Brain natriuretic peptide

cAMP Cyclic adenosine monophosphate

CHF Chronic Heart Failure

CYP2C19 Cytochrome P450 2C19

D Deletion

Del Deletion

FDA Food and Drug Administration

GUCA1B Guanylate Cyclase Activator 1B

Gln Glutamine

Glu Glutamic acid

Gly Glycine

GRACE Genetic Risk Assessment of Cardiac Events

GRAHF Genetic Risk Assessment in Heart Failure

GRK G protein Receptor Kinase

GWAS Genome Wide Association Study

H/IDN Hydralazine and Isosorbide Dinitrate

I Insertion

Ile Isoleucine

Leu Leucine

LVEF Left Ventricular Ejection Fraction

MERIT HF Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure

MRPS10 Mitochondrial Ribosome coding Protein S10

NGS Next Generation Sequencing

NOS Nitric Oxide Synthase

NYHA New York Heart Association

RAAS Renin-angiotensin-aldosterone

RRAGD Ras Related GTP binding D

RCT Randomised controlled trials

Ser Serine

SNP Single Nucleotide Polymorphism

SOLVD Studies Of Left Ventricular Dysfunction

STEMI ST Elevation Myocardial Infarction

T Thymine

Thr Threonine

TPMT Thiopurine Methyl Transferase

V-HeFT Vasodilator Heart Failure Trial

Abstract

Heart failure is a common disease with high levels of morbidity and mortality. Current treatment comprises of beta-blockers, ACE inhibitors, aldosterone antagonists and diuretics. Variation in clinical response seen in patients begs the question of whether there is a pharmacogenetic component yet to be identified.

To date, the genes most studied involve the beta-1, beta-2, alpha-2 adrenergic receptors, and the renin-angiotensin-aldosterone pathway, mainly focusing on single nucleotide polymorphisms (SNPs). However results have been inconsistent. Genome wide association studies (GWAS) and next generation sequencing (NGS) are seen as alternative approaches to discovering genetic variations influencing drug response.

~~It it hoped that~~Hopefully future ~~genetics~~ research will lay the foundations for genotype-led drug management in ~~patients with heart failure~~these patients with the ultimate aim of improving their ~~response and~~ clinical outcome.

Executive Summary

- Heart failure is a common cause of hospitalisation and its incidence is set to rise.
- Mainstay of treatment comprise of beta blockers, ACE inhibitors, aldosterone antagonists, and diuretics.
- Single nucleotide polymorphisms (SNPs) potentially influencing drug response include the Arg 389 Gly variant and the Ser 49 Gly variant in the beta 1 adrenergic receptor, the Arg 16 Gly, Gln 27 Glu and Thr 164 Ile polymorphisms within the beta 2 adrenergic receptor, an insertion at the

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287th base pair in the angiotensin converting enzyme and the Gly 264 Ala mutation in the sodium-chloride co-transporter.

- Next generation sequencing (NGS) and systems biology approach might provide better alternatives of understanding the underlying disease process of heart failure.

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Introduction

Heart failure (HF) is one the most common cause of hospitalisation and represents the end stage of a variety of heart conditions; it is associated with significant morbidity and mortality (1-2). Prognosis is poor, with 1-year mortality estimates ranging between 25-45%. Furthermore its incidence is rising and with an ageing population, this disease is likely to increase in importance in its impact of the healthcare burden (3-5). The pathophysiology of HF is centred on increased activity in the adrenergic and renin-angiotensin-aldosterone systems (RAAS), which leads to vasoconstriction and fluid restriction with further deleterious effect on cardiac function. Beta-blockers, ACE inhibitors/ angiotensin II receptor blockers and aldosterone antagonists reduce activity in these pathways and have shown prognostic benefit, thus are the foundation of HF therapy (6-12).

HF mortality remains high in spite of numerous validated randomised controlled trials (RCT) to guide HF management but meta-analysis of these RCTs relating to B-blocker therapy has shown varying treatment response, correlated with cohort geographical location (5,13). To account for this varying treatment response, firstly it is worth appreciating that the underlying HF aetiology has been shown to affect drug response in HF and beyond this, ethnicity, gender, body mass index, renal function are likely implicated (14-16). But genetic polymorphisms manipulating drug response may also account for these findings (17-21).

In the past decade there has been considerable progress in cardiovascular pharmacogenetics, with genetic determinants defined for both warfarin and clopidogrel leading to alteration of product label for these drugs, suggesting the use of genetic information to guide therapy (22-23). There is a growing body of evidence that variation in proteins within the sympathetic axis and RAAS influence drug response thus increasingly pharmacogenetics of HF research is being sought as a way to optimise HF treatment and advance new drug development in this area (24-

31). Although drug response variation in HF is likely multi-factorial, pharmacogenetic variation may partially account for therapeutic failure contributing to the remaining high mortality in HF. Identifying novel gene variants affecting treatment response may reveal unrecognised pathways and new potential therapeutic targets. Few studies to date have attempted to assess the extent to which variation in drug-response was exclusively due to genetic factors and therefore expounding the likely clinical benefit of using pharmacogenetics to guide HF therapy. One of the prerequisites to bridging this gap is to consider likely trial designs and criteria that will lead to a consensus upon using pharmacogenetics-based variants to guide therapy in clinical practice.

We will review the current key body of evidence on pharmacogenetics candidate genes for HF and the challenges and framework required to advance the goal of pharmacogenetics based HF therapy.

Pharmacogenetics and heart failure: candidate genes

Antagonism of Renin- angiotensin-aldosterone system (RAAS)

Enhanced RAAS activity augments afterload and cardiac workload with subsequent adverse ventricular remodelling leading to worsening cardiac failure making RAAS inhibition a fundamental of HF therapy. ACE inhibitors, angiotensin 2 receptor blockers and aldosterone antagonists have been shown to reduce mortality and promote reversal of cardiac remodelling (8,12,32). The "I/D" single nucleotide polymorphism (SNP) variant in the ACE gene (referring to presence or absence of an insertion at the 287th base pair), with DD genotype are over three times as likely to have raised plasma aldosterone in a dose dependent manner, despite long term treatment with ACE inhibitors (31). A prospective study into the 'I/D' variant in 479 HF subjects with systolic dysfunction found the D variant was associated with worse transplant free survival at 1 year (89% survival in II, 80% ID, 74% DD). Furthermore it was also showed that the negative effect of the D allele was most evident in subjects on low dose ACE inhibitors or not taking beta-blockers and was negated by high dose ACE inhibitors (21). This is one of the few studies to date to have attempted to assess the extent to which variation in drug-response was exclusively due to an SNP. Despite this compelling evidence for interaction between ACE I/D SNP and ACE inhibitor dose on survival with the authors postulating a combined use of ACE

inhibitors and angiotensin II receptor blockers may benefit DD homozygotes, it has not translated to genotyping at this locus to influence prescribing.

On top of this, the negative effect of the D allele was greater in those not taking beta-blockers and other studies have shown that sympathetic stimulation increased sympathetic response in subjects with the Arg389 allele and relatively increased plasma renin. These findings suggest neuro-hormonal interactions are important and considering genotypes across these two systems may allow more effective tailoring of HF therapy (33). Notwithstanding the good evidence for a drug-gene interaction that is more consistent than any other loci in this setting, ACE I/D genotyping still does not guide ACE inhibitor prescription. The 'bench to bedside' link remains elusive, clinicians remain either unaware or unconvinced of how genetic variation could influence ACE inhibitor response. Framework for establishing such a link is detailed later. From a pragmatic perspective, patients whose genotype infers benefit from high-dose inhibitors can only do so if their renal function permits. This illustrates that pharmacogenetics can only be useful in guiding heart failure therapeutics as part of a wider picture.

The adrenergic system

Reduced cardiac output in HF promotes increased sympathetic activity via the alpha-mediated vasoconstriction leading to an increase in total peripheral resistance and activation of beta adrenergic receptors increasing chronotropy and inotropy. This subsequently results in increased wall tension, myocyte necrosis, oxygen demand and is dysrhythmogenic. The understanding of this pathophysiology and RCTs on B-blockers (metoprolol, bisoprolol and carvedilol) that have shown beneficial effect on survival and disease progression have formed the cornerstone of HF treatment (6-7, 9-10, 34). Most of the research on pharmacogenetics in heart failure focuses on response/outcomes with B-blocker therapy. The key SNPs relating to this system will be discussed followed by the clinical impacts of these SNPs at the end.

Beta 1 adrenergic receptor (B1AR)

B1AR is a major pathologic β-adrenergic subtype in heart failure. Gene variants in B1AR may influence beta-blocker response as there is a varying degree of response seen among the different subsets of heart failure patients despite receiving the same class of drug. The polymorphisms Arg 389 Gly and Ser 49 Gly are the most studied,

with allele frequencies for both SNPs estimated around 50-70% and 15-20% respectively. Although other SNPs have been found in relation to B1AR, the allele frequencies are <1% and thus not likely to be of public health significance (35).

The potential role of the Arg 389 Gly polymorphism in reducing mortality was seen in a genetic substudy of the B-blocker evaluation of Survival Trial (BEST) (28). The trial was prematurely terminated at 2 years due to a lack of mortality benefit overall, however non-blacks who were more likely to carry the Arg 389 Gly polymorphism had a significantly improved mortality (28). The genetic substudy demonstrated that those who were Arg 389 homozygotes had a 34% mortality benefit with bucindolol and this was supported with ex-vivo cell data (29). This apparent mortality benefit of bucindolol in Arg 389 homozygotes was initially attributed to the added property of bucindolol in reducing noradrenaline levels in the body but the benefit was still demonstrated in a subset of patients who had stable noradrenaline levels, lending support to the hypothesis of enhanced beta-blockade effect as a benefit mechanism (28). Unfortunately the lack of consistency of this effect across large clinical trial data and the relatively small difference in allele frequencies between the racial groups suggest alternative explanations for racial differences in beta-blocker response rather than purely due to SNP Arg 389 (29).

At a molecular level, the SNP Arg 389 Gly is associated with greater agonist-promoted G protein coupling, making it the more active form of the receptor, this was elucidated by looking at dobutamine response in 10 healthy Arg 389 homozygotes and 8 Gly 389 homozygotes (35). Arg 389 homozygosity is associated with enhanced chronotropy, contractility and bisoprolol more effectively reduced sympathetic response. Furthermore Arg 389 homozygotes showed increased plasma renin activity suggesting neuro-hormonal cross-talk is also important (33), thus in this instance structural changes here apparently have the expected effect.

The Ser 49 Gly variant was investigated using left ventricular myocytes from HF patients, since cAMP is the second messenger in beta 1 stimulated signal transduction, this was used as a marker for agonist response in cells with Gly 49 versus Ser 49. This study found basal and agonist stimulated cAMP levels were greater in cells with the Gly 49 variant, which showed greater desensitisation and receptor down-regulation with saturating catecholamine levels, this may account for improved survival in HF patients carrying Gly 49 (36-37). Despite this, there is inconsistent evidence that this translates to differences in drug response.

Downstream, G-protein receptor kinases (GRKs) phosphorylate B1ARs and B2ARs, reducing agonist-promoted receptor function promoting desensitization. The main cardiac GRKs are GRK2 and GRK5, screening identified 4 relevant genetic variants in the GRK 5 gene, but none in GRK2 (20). The Gln 41 Leu SNP is the most studied variant in the GRK 5 gene, this SNP is represented in the GRK5 regulatory domain. The Leu 41 variant is uncommon in Caucasians but is carried by up to 40% of African Americans, HF appears to develop independently of carrying the Leu 41 variant but it may convey survival benefit in established HF (38). Interestingly the Leu 41 variant is more prevalent in Takotsubo cardiomyopathy sufferers suggesting associated susceptibility to sudden adrenergic rushes (39).

Beta 2 adrenergic receptors

Beta 2 adrenergic receptor (B2AR) gene variation may also be important, three potentially functionally significant SNPs identified include: Arg 16 Gly, Gln 27 Glu and Thr164 Ile. The Arg 16 Gly and Gln 27 Glu polymorphisms, represented in the extracellular amino terminus, are in linkage disequilibrium. Animal studies of the Gly 16 variant was associated with a reduction in agonist promoted down-regulation, while the Glu 27 variant was correlated with complete resistance to down-regulation (40). The Thr 164 Ile polymorphism is represented within the proposed ligand-binding pocket, it exists purely in the heterozygous state and is in linkage disequilibrium with Gly 16 and Glu 27 (41-42). Cells expressing Ile 164 *in vitro* displayed lower binding affinity for adrenaline, suggesting attenuated *in vivo* response (41). Ile 164 may be associated with a reduced response to B2AR mediated vasodilatation, potentially accounting for why carriers had reduced HF survival rates. Higher phenylephrine doses were required to induce 50% dilatation in the dorsal hand vein for Thr 164 carriers whilst higher doses of isoproterenol were required in Ile 164 carriers (43). Subsequent work showed B2AR mediated vasodilatation depended on complex inter-play between the above 3 SNPs (44).

Alpha adrenergic receptors (A2ARs).

A2ARs influence blood pressure via central and peripheral mechanisms, and studies suggesting variation in response to alpha adrenergic agonists may have a genetic component have focused on deletion of three glutamic acid residues at base pairs

301-303 in the A2AR. A2AR del 301-303 variant may effect desensitisation, facilitating attenuation of chronically increased vascular tone with increased sympathetic activity in HF but other studies have not been able to demonstrate that del 301-303 predicts variability in A2AR mediated vasoconstriction (45-46).

Another example of polymorphisms affecting the alpha adrenergic receptors is seen in the ADRA2c gene which encodes the α_{2C}AR receptor. Regulation of noradrenaline levels via a negative feedback mechanism is controlled by the α_{2C}AR receptor and a multiple-nucleotide polymorphism α_{2c}322-325 deletion (Del) here has been associated with increased heart failure in black patients (29).

Clinical Significance of Adrenergic system Genetic Variants on Therapeutic Response and Outcome

Do these genetic variants modulate therapeutic benefit in HF patients? Initial studies showed Arg 389 Gly and Ser 49 Gly in the B1AR gene and Glu 27 Gln in the B2AR gene influenced beta-blocker response in HF patients, also showing greater improvement in echocardiographic parameters with beta-blockers than Gly 389, Ser 49 and Gln 27 carriers (27, 47-48). Homozygous carriers of Gln 27 in HF sufferers respond less well to carvedilol than Glu 27 carriers (18-19). Subsequently it was postulated that B2ARs expressing Gln 27 were sensitive *in vitro* but 'pre-desensitised' *in vivo* by chronic endogenous agonist exposure. B2ARs expressing the Gln 27 variant may have reduced desensitization *in vitro*, potentially explaining in vitro desensitization resistance with Gln 27 (40,49-50).

On the contrary, other studies failed to show a drug-gene interaction with the above variants. A sub-study from the MERIT HF trial (9) showed the Arg 389 variant did not predict outcome with either placebo or metoprolol (51), although notably the 2 treatment arms were not compared by genotype. Thus whether Gly 389 carriers benefitted less from metoprolol than Arg 389 homozygotes is unknown. De Groote *et al* also found no difference in beta-blocker response correlated with any of the above genetic variants for the B1AR and B2AR gene in assessing change of heart rate and ejection fraction among HF patients (52).

These discrepancies may due to the above studies only involving a relatively small sample size, which may not yield statistically meaningful results given the rarity of

some of the variants studied (see Table 1). Also combining adrenergic receptor genetic variants may be more fruitful than individual polymorphisms, one study addressed this by dividing heart failure patients into 2 groups: one including those who were Arg 389 homozygous with at least one copy of the Gly 27 variant and the second included all other genotypes. The first group had shorter survival with carvedilol than the second group, although there was no difference with metoprolol (53).

Genetic variation in GRK may influence beta-blocker response. One study demonstrated in African American HF sufferers not on beta-blockers, Leu 41 conveyed a survival advantage mimicked by beta blockade (20). This suggests that , genotyping here could avoid needless side effects and cost from patients taking a drug unlikely to be beneficial. However, few other studies have looked at drug-gene interaction here.

Given these inconsistent results and insufficient studies, it is unsurprising that usage of genotyping to guide beta-blocker prescription has not materialised. Despite the above research, relatively few clinicians are aware of the genetic variations influencing beta-blocker response. Therefore the case for the clinical value of pharmacogenetics in HF is fragile since no consensus exists on whether these variants consistently predict drug response.

Nitric oxide

Nitric oxide regulates myocardial remodelling, vascular reactivity and thrombosis (54). The hydralazine/isosorbide dinitrate (H/IDN) combination releases nitric oxide, reducing vascular tone. Interest in genetic variation in nitric oxide synthase (NOS) was initiated from V-HeFT study, which showed African American patients had better survival rates on H/IDN whilst Caucasian did better on ACE inhibitors (55). This prompted the AHeFT study: a double-blind trial of 1050 African Americans with heart failure NYHA class III-IV randomized to H/IDN or placebo, mortality was significantly higher in the placebo group (54).

SNPs may account for racial difference in drug response. One study investigated genetic variation in NOS 3 as the Glu 298 variant is more common in black patients. There was a significant improvement in quality of life in patients taking H/IDN with the

Glu 298 variant and a trend towards LV reverse remodelling and no trend was evident with other genotypes (56). H/IDN was licensed for use in black patients with HF but controversy regarding racially-guided drug prescribing ensued. H/IDN was infrequently prescribed and the manufacturers discontinued production (57). This was arguably racially-guided therapy rather than personalised medicine, although it is cheaper and more cost-effective to direct therapy based on race rather than genotype, by overlooking the social context of prescribing and not pursuing biological accuracy the end result was counter productive for personalised medicine.

Renal sodium transporter and response to loop diuretics

Although diuretic prescription is common in HF patients yet significantly less is known regarding pharmacogenetics involved here. Reasons why two clinically similar patients require widely differing loop diuretic doses evade us (59). One study looked at genetic variation in renal sodium transporters, measuring excretion of sodium, potassium, chloride and volume of urine in 97 healthy subjects taking bumetanide, torasemide and furosemide to identify differences related to genotype. The SLC12A3 Ala 264 polymorphism in the sodium-chloride co-transporter gene was associated with increased excretion of potassium and chloride ions with all loop diuretics. Similar trends towards stronger excretion were also seen with sodium, calcium and volume of urine excreted (60), but the significance of how this applies to HF patients is unknown so there is currently no prospect of genotyping here guiding HF therapeutics.

Novel positional candidates: *discovered through GWAS*

Genome wide association studies (GWAS) provide a mechanism for discovering relevant genetic loci and have identified drug-gene interactions in treatment of other cardiovascular diseases (61-62). To date virtually no published GWAS has sought relevant genes in HF drug response, GWAS have been used to identify loci implicated in the development of HF and outcome in patients with HF (63-65). Candidate gene studies are intrinsically limited, investigators can only hope to find an association between the specific genes examined and the outcome measured since any of the 2.5 million SNPs coded for may be significant. This method may yield new pathological pathways, potentially pointing towards new treatment targets. To date,

there are limited GWAS looking for genetic variants associated with heart failure that have been published (66-67). Norton and colleagues carried out exome sequencing on a large family with DCM, discovering a mutation in the heat-shock protein BAG3 was correlated with heart failure (64,66). Smith and colleagues on the other hand, identified 2 loci that were associated with the development of heart failure among those of African and European descent (63 – 65). Another study carried out a population-based GWAS comparing patients with HF with controls and discovered new susceptibility loci in HSPB7 and FRMD4B (67).

The only GWAS investigating HF pharmacogenomics was performed in a canine model of heart failure, where the investigators aim to discover SNPs predictive of beta-blocker response. Three SNPs reached genome wide significance, one was located within the MRPS10 gene, which encodes a small subunit of mitochondrial ribosomes, and variation here could logically influence myocardial energetics (68). This was a tentative first step towards using GWAS to identify variants influencing drug response in HF. Using GWAS to identify novel loci in HF pharmacogenomics has broad potential but this is largely unchartered territory and careful analysis will be required upon emergence of new SNPs. This may be partly due to the known pitfalls with GWAS, including the high false discovery rate and the need to replicate any findings in a second, large clinical study (69). Furthermore, although GWAS may identify a specific locus, it does not identify the specific variant or combination of variants within the locus that exert biological effects (25).

As we might expect, SNP analysis is time consuming and has some limitations. There is a frequent finding of false positives and and it is unable to reliably analyse rare variants, and gene-gene interactions. We do not yet understand the exact biological function of most SNPs that are discovered and one SNP alone does not always translate into a clinically relevant pharmacogenetic loci as there is often a lot of other genetic interactions involved.

A potential alternative approach is the utilisation of next generation sequencing (NGS). This involves millions of small fragments of DNA which can all be sequenced at the same time. Whole exomes can be analysed and this will provide a large amount of data. NGS has the advantage of looking at large amounts of genetic data in a shorter perios of time, and will be able to identify complex haplotypes, gene-gene interactions and rare variants in order to identify a novel pharmacogenetic loci. A potential problem however is the large amount of data that needs to be handled at any one time.

~~An potential alternative approach which has shown promise is the utilisation of next generation sequencing (NGS).~~

Non-genetic factors influencing drug tailoring in heart failure

As suggested earlier ethnic variation in heart failure therapeutic response is unlikely to be exclusively genetic, Bloche wrote a comprehensive analysis of the VHeFT and AHeFT studies and highlighted the importance of psychosocial, environmental, cultural and economic factors that may influence ethnic difference in response to heart failure therapy, further noting that research has neglected these areas (70). One study found social isolation was associated with greater vascular resistance in response to perceived stressors and impaired repair mechanisms, such as wound healing (71).

No studies to date have attempted to estimate the proportion of drug-response variability that may be due to genetic variation versus environmental factors in clinically similar patients. Also differences in pharmacokinetics and pharmacodynamics should be considered, as Roden *et al* points out, any physiological variable impacting the interaction with a drug or its receptor can modulate drug action, e.g. catecholamine levels affect drug-adrenergic receptor interaction (23).

Another area gaining momentum is tailoring medication in response to biomarker levels as there is considerable evidence for the relationship between brain natriuretic peptide (BNP) levels and worse prognosis in those with HF (72-73). A recent meta-analysis looked at six trials in which patients with HF were randomized to titration of their medical therapy according to circulating BNP levels or a parallel control group (total n=1 627). All six studies showed mortality benefit in the BNP-guided therapy group compared to controls and no studies reported increased incidence of adverse effects in this group (74). Therefore eventually it may be possible to use a combination of pharmacogenetics and biomarker levels to guide HF therapy, which could further optimise the therapeutic benefit (75). Investigation into the proteomics of HF may also reveal variation that can be used to guide HF therapy hand-in-hand with biomarkers and pharmacogenomics, which would facilitate bridging the gap of genotype and phenotype. Disparity between genotype and phenotype may also account for the inconsistent results with current SNPs, further appreciation of this relationship would be a significant step forward. Although, research into proteomic variation in HF is largely at the animal model stage, there are preliminary data that show that proteomics may offer promise in determining outcome in HF (76 – 77)

Future directions and consensus building

Pharmacogenetics already guides therapy for oncological use of biological agents and assays determining TPMT activity guide azathioprine prescription (78-79). It should also be possible to implement pharmacogenetics into practice with HF therapies. A major obstacle to clinically implementing HF pharmacogenetic discoveries has been the inconsistent results; hence stronger evidence for specific drug-gene interactions is needed. GWAS discovery has been used in over 250 human traits, which includes disease susceptibility, physical attributes and serum biomarkers (80). Global collaboration is needed to gain suitably powered study samples. The genomics of drug response in HF requires both retrospective analysis of randomized-control trials where DNA has been collected and observational studies where genomic information is linked to medical records in a large population to provide robust estimates of the genetic architecture of the drug response traits. One such large observational study has already been initiated (81).

Any genetic algorithm proposed for predicting drug response must be shown to be efficacious in randomised, prospective studies. Flynn proposes confirmatory trial designs for pharmacogenetic studies including targeted and stratification designs (82). In the targeted design, patients are genotyped at screening and those with the genotype being studied are included and randomised to one of the treatment groups, restricting results to one genotype. Varying drug effects across different genotypes may be investigated using the stratification design, where patients are allocated to groups based on genotype then randomized to treatment. Notably both designs require considerable prior knowledge of the genetic marker. Even though targeted and stratification studies provide methods for translating pharmacogenetic knowledge into prescribing guidance, they do not address the interplay of how different genetic combinations influence drug-response. Although a version of the stratification design may be useful, this would be increasingly difficult as more complex genetic and therapeutic criteria determine which group a patient is allocated to, as number of subjects per group will fall, reducing statistical power.

Several genetically guided HF drug-trials are being devised. In 2010, ARCA Biopharma (Bloomfield, CO) and the FDA agreed on a safety and efficacy trial of bucindolol versus metoprolol CR/XL in 3200 patients homozygous for B1AR Arg38 (83). Also a study evaluating the impact of genetic variations on candesartan response in approximately 300 HF patients already on an ACE inhibitor has been completed, results are awaited (84).

Studying patients with HF to determine whether a specific genetic variant is protective or deleterious in HF patients taking certain drugs is fraught with difficulties, given the multi-factorial causation of variation of drug response. Individuals with HF recruited into these studies are likely to have less severe disease or subject the results to survivor bias (85). Reduction of survivor bias may be possible by storing genotypic information in an electronic medical record and making this data available at the time of drug prescription. Roden *et al's* pre-emptive approach would allow 'automated delivery of point-of-care decision support' (23) and appears to be a pragmatic proposal as part of establishing the bench to bedside link.

The challenges to population-wide implementation of pharmacogenetic discoveries were highlighted with CYP2C19 and clopidogrel and CYP2C9 / VKORC1 and warfarin (86-87). Although, the US FDA added pharmacogenomics labels to clopidogrel and warfarin, existing challenges include the required threshold of evidence to translate it into practice and the demonstration of an increased value above the current functional testing practices (88-89). Additionally, newer agents may be used instead of warfarin and clopidogrel, potentially without the same requirement for genetic stratification (90-92), but have been associated with increased bleeding. In this regard, a recent study has shown the benefits of point of care testing of CYP2C19 in the choice between anti-platelet agents clopidogrel and prasugrel (93).

Although functional assays may help guide prescription of specific drugs (94) there are no functional tests available to guide and monitor efficacy for HF therapies at present. Low-cost genome sequencing of patients at disease diagnosis may be more beneficial. Storing whole genome information from the time of diagnosis would allow systematic annotation of the patient's medical record with appropriate pharmacogenomic information. If this information was accessible at the point of prescription it could be used to guide the use of multiple drugs across different diseases. Physicians would not have to know about, commission or await results of a specific test before making prescribing decisions. But the challenges of reducing the cost of genome sequencing and developing the infrastructure to support this decision making process remain to be fully addressed.

In 2005 the US FDA approved the use of isosorbide dinitrate and hydralazine combination for HF treatment in African-Americans and this approach of race-targeted drug development is not without its flaws. Race is a difficult concept in the 21st century as its definition is becoming more homogenous as the world becomes more globalised. There are ethical questions surrounding this approach and accepting that race alone influences the course of a disease ignores the other economics and socio-cultural factors at play.

Currently, despite many studies looking for variants influencing drug therapy in HF, this has not been applied clinically. There is little prospect of this changing in the immediate future given the inconsistent results to date. But the approach outlined here may be the initial step towards improving the process of deriving evidence for pharmacogenetics based HF therapy. It is important to note that HF is a heterogeneous trait and multiple factors beyond genetic variability influence drug response in HF, as described above. Comorbidities and HF aetiology are particularly pertinent when considering how to tailor HF therapy to the individual. Respiratory and renal comorbidities are particularly important in HF as they may limit the dose of beta-blockers and ACE inhibitors that may be used, regardless of whether the patient's genotype suggests higher doses would be beneficial (95-99).

Finally, the recent systems biology approach that takes into account the integration of genes and molecules, as well as interpreting the relationship between molecular units and the corresponding mechanical counterparts will hopefully be able to better increase our understanding of heart failure and help us develop effective therapy (100). Through the construction of mathematical models and simulation, systems biology allows us to integrate the information from fields such as genomics and proteomics with clinical-epidemiological data and provide better understanding of the disease process in HF (101).

Conclusion

Mounting evidence suggests drug-gene interactions influence treatment with all agents used in heart failure therapeutics. The GWAS approach may help identify

more loci, with potential for new drug development. Expansion upon current evidence using the above methods, results of which may ultimately be the foundation for tailoring of HF therapy to an individual's genome and clinical profile helping to minimize side effects including improvement in cost-effectiveness. Disappointingly, there are no short term prospects of widely employing genetically guided HF therapy. Given current trends, HF cases are likely to continue to increase over the coming years, making the enhancement of HF therapeutics' more important than ever.

Table 1: Summary of most studied genetic variants in the pharmacogenetics of heart failure, including the minor allele frequencies values and rs ID if available, taken from Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID:

Receptor/ transporter	Genetic Variant	Minor allele frequency; rsID	Pharmacogenetic association
A2AR	Del 301-303	n/a	Deletion associated with resistance to desensitization (38)
ACE	Insertion/ deletion (I/D)	n/a	Deletion associated with raised serum ACE (20)
B1AR	Arg 389 Gly	0.304; rs1801253	Arg form gain-of-function allele Associated with enhanced response to bucindolol(27)
B1AR	Ser 49 Gly	0.173; rs1801252	Gly 49 associated with enhanced desensitisation and agonist-promoted down-regulation (28)
B2AR	Thr 164 Ile	0.005; rs1800888	Ile 164 loss of function allele, attenuated response to sympathetic stimulation (35)
B2AR	Gln 27 Glu	0.234; rs1042714	Gln 27 associated with reduced response to carvedilol (13, 14)
B2AR	Arg 16 Gly	0.474; rs1042713	Arg 16 loss of function variant; shorter duration stimulation (41). Reduced response to beta blockade (89).
GRK5 (acts on B1AR and B2AR)	Gln 41 Leu	0.087; rs17098707	Leu 41 gain of function allele Associated with improved survival in patients not on beta-blockers (15)
NOS 3	Asp 298 Glu	0.196; rs1799983	Glu 298 associated with good response to H/ IDN (53)
Renal sodium transporter	SLC12A3 Ala 264	n/a	Associated with enhanced excretion potassium and chloride (50)

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