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Richard Myles Turner, Munir Pirmohamed

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Cardiovascular Pharmacogenomics - Expectations and Practical Benefits

[1,2] Richard Myles Turner, [1,2] Munir Pirmohamed

[1]The Wolfson Centre for Personalised Medicine

Department of Molecular and Clinical Pharmacology

Institute of Translational Medicine

University of Liverpool

Block A: Waterhouse Building

1-5 Brownlow Street

Liverpool

L69 3GL, UK

[2] The Royal Liverpool University Hospital

Prescot Street

Liverpool

L7 8XP, UK

Corresponding author

Munir Pirmohamed

Correspondence address = [1]

munirp@liverpool.ac.uk

tel: +44 151 794 5549

fax: +44 151 794 5059

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Abstract

Cardiovascular disease is a leading cause of morbidity and mortality worldwide. Pharmacogenomics is the study of genetic determinants of inter-individual variation in drug response and aims to facilitate personalised medicine, through genotype-informed drug and dose selection, to maximise drug efficacy and/or minimise adverse drug reactions. Despite high expectations, no cardiovascular pharmacogenomic association is currently in widespread clinical practice; evidential, logistical, financial and knowledge implementation barriers exist. Nevertheless *VKORC1*, *CYP2C9* and *CYP4F2* variants have been associated with warfarin dose requirements, while *CYP2C19* variants are associated with perturbed antiplatelet response to clopidogrel. At present though, controversy exists over the clinical utility of these genetic associations. There is an increased risk of simvastatin-induced muscle toxicity in *SLCO1B1*5* carriers, *ADRB1* and *ADRA2C* polymorphisms are associated with differential response to bucindolol and rare congenital arrhythmia gene variants have been identified in drug-induced Torsades de Pointes. Practical benefits are still anticipated but much work remains.

1 Introduction

Cardiovascular disease is a leading cause of morbidity and mortality worldwide. Several strategies have been developed to ameliorate the burden from population-based health promotion initiatives through to patient-centred pharmacological and invasive interventions. There is notable inter-individual heterogeneity in response to cardiovascular drugs, affecting both efficacy and toxicity. This individual variability can be mediated through perturbation of drug pharmacokinetics, pharmacodynamics and/or differences in underlying disease processes. These mechanisms of variability are moulded by a trilogy of implicated factors: clinical (e.g. age, co-morbidities, body mass index (BMI), pregnancy), environmental (e.g. drug-drug and drug-food interactions) and genetic (human germline and gut microbiome). The contribution of each postulated factor likely varies with the cardiovascular drug and endpoint of interest.

Pharmacogenomics is the study of genetic determinants of inter-individual variation in response to a given drug and aims to facilitate the personalisation of pharmacological therapy, through genotype-informed drug and dose selection, to maximise drug efficacy and/or minimise adverse drug reaction (ADR) risk. Following the success of the Human Genome Project, the International HapMap Project and 1000 Genomes Project have sequentially and profoundly increased our understanding of genetic variation in the human genome. Concomitant extensive investigations have been undertaken for genetic biomarkers associated with disease susceptibility, disease prognosis and treatment response with candidate gene techniques, genome-wide association studies (GWAS) and now emerging next-generation sequencing technologies. It is interesting to note that pharmacogenomic candidate gene studies have been substantially more successful in identifying replicable common variants of appreciable effect size compared with candidate gene investigations into disease genetics. This is possibly due to a greater understanding of pharmacological pathways compared to disease processes.[1] Additionally, although large scale GWAS and subsequent meta-analyses[2–4] are now uncovering genetic associations for susceptibility to common cardiovascular conditions, the variant effect sizes are predominantly lower than for pharmacogenomic associations, particularly those related to

ADRs.[5–7] One reason for this may be evolutionary pressures selecting against common variants of large effect size that confer susceptibility to serious 'natural' pathology arising before or during reproductive age.[8]

Within pharmacogenomics, despite high expectations, translation of genetic associations into clinical practice has been slow. There are however some notable exceptions. The most widely cited is the widespread uptake of *HLA-B*57:01* genotyping prior to abacavir antiretroviral therapy, which was shown to significantly decrease the incidence of abacavir hypersensitivity syndrome (AHS).[9] The large effect size, high sensitivity and generalisability of *HLA-B*57:01* for immunologically confirmed AHS were instrumental in facilitating implementation. However, this is a safety phenotype while in cardiovascular disease, most pharmacogenomics associations have focused on efficacy end-points where the magnitude of effect is much smaller and thus no drug/gene association has made it into widespread clinical practice. Another notable exception is oncology where there is an expanding arsenal of licensed genotype-dependent therapeutics. This divergence is attributable in large part to the distinct biological basis of cancer, which results from acquisition of a critical combination of somatic genetic mutations. This has facilitated personalised medicine in oncology by two processes. First, disrupted cellular pathways resulting from critical mutations are rational pharmacodynamic targets for drug manipulation; the efficacy of such drugs is largely limited though to those tumours in which the targeted pathway is aberrant (sometimes irrespective of the phenotypic classification of the tumour and histology). Second, somatic tumour mutations or their phenotypes represent identifiable tumour biomarkers for patient selection. For example, tamoxifen is indicated for prevention of disease recurrence only in 'oestrogen receptor positive' breast cancer patients, and predates any of the genomic advances that occurred this century. More recently vemurafenib which inhibits V600E mutation-positive BRAF but not wild type BRAF has been approved for the treatment of BRAF V600E mutation-positive unresectable or metastatic melanoma. In contrast, the genetic contribution to common cardiovascular pathology is largely in the germline genome, and the variants that may affect drug response appear to be neither necessary nor sufficient, which has resulted in conflicting information in the literature and the calls for randomised controlled trials (RCTs) to show clinical

utility. Furthermore, pharmacogenomic studies in cardiology have largely focused on drugs that are already licensed (and many of them are off-patent, and therefore relatively cheap, unlike cancer drugs which tend to be more expensive) and in widespread clinical use. This undoubtedly represents an additional hurdle, because to change accepted clinical practice, and therefore physician behaviour, is difficult, and often requires a higher level of evidence.

This article will briefly explore the barriers in translating germline pharmacogenomics (hereafter termed pharmacogenomics) into clinical care before providing a broad overview of the cardiovascular drug-specific pharmacogenomics of warfarin, antiplatelets, statins, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors (ACEIs) and antiarrhythmics. Table 1 summarises contemporary cardiovascular pharmacogenomic associations and provides comparator select examples of genetic associations with common cardiovascular disorders.[2–7,10–21] In areas of controversy, we have sought to bring the existing literature together (using supplementary tables as appropriate) to provide an up-to-date reasoned interpretation.

2 Barriers to the implementation of pharmacogenomics

The barriers obstructing widespread implementation of pharmacogenomics can be categorised into evidential, logistical/financial and knowledge-based (Figure 1).[1] Research mediates variant discovery, replication, measurement of effect size in different ethnic groups and determination of clinical benefit. Although an adequately powered RCT versus current best practice might seem ideal to establish evidence of clinical utility, this approach will likely be unrealistic for every variant in the expanding list of pharmacogenomic associations. There is growing consensus that the level of required clinical evidence for a genetic (including pharmacogenomic) test should correlate with the potential impact of the test on a patient.[22] To illustrate, for a genetic test that leads to pre-emptive radical intervention (e.g. *BRCA1* and prophylactic mastectomy) it is imperative that a substantial evidence base exist before implementation. However, while it is important to have an evidence base for a pharmacogenomic test, the extent of that evidence should vary according to the drug response-gene association being studied, and may not necessarily always require a RCT. Furthermore, a badly

designed RCT is likely to create as much, if not more, confusion than an observational study. Thus, there is a need to evaluate all evidence in an intelligent manner. There is also a need for equity in criteria used for acceptability of genetic and non-genetic tests; for instance, we currently accept a change in dosing of some drugs when given to renally impaired patients based on data obtained through pharmacokinetic studies, but do not accept the same type of data when a drug is given to a patient with a genetic polymorphism in a metabolic pathway when the change in systemic exposure is equivalent.[1]

Logistical hurdles include the burden of test ordering on assiduous physicians, test turnaround times and follow up arrangements. One solution is pre-emptive genotyping platforms rather than spontaneous requesting of individual pharmacogenomic tests at the point of care.[23] The former can facilitate systematic incorporation of pharmacogenomic data into a patient's electronic medical record. This infrastructure can then be harnessed by updateable automated clinical decision support (CDS) algorithms, so consensus recommendations of clinically relevant pharmacogenomic information are presented to the clinician, but only at the relevant time (e.g. when a new drug is being prescribed), to maximise utility and potentially minimise test duplications, delays and lack of follow up.[22] These processes are being explored by programs such as the Electronic Medical records and Genomics (eMERGE) Network,[24] although the computational challenges are considerable. Financial concerns which exist include, for example, genotyping costs and procuring reimbursement.[1]

There are clinician and patient knowledge barriers regarding pharmacogenomics. Clinicians may be unfamiliar with the current variable evidence base, indications for and interpretation of pharmacogenomic tests. This knowledge gap could be addressed through institutional educational programs and/or impromptu self-directed learning accessed via electronic adjuncts brought to the clinician's attention as part of the CDS process during routine clinical practice. Patient unfamiliarity is conventionally addressed by the informed consent process. Given that the demand for genetic counsellors is predicted to soon outstrip supply, this role may additionally be outsourced to other professionals.[22] Although the implications of germline genetic variants for disease susceptibility can

make the informed consent process daunting for both sides of the doctor-patient relationship, obtaining consent for pharmacogenomics may be less intimidating, given that its clinical role is to optimise treatment for an already established disease.

As outlined, there are a broad range of implementation barriers to widespread adoption of pharmacogenomics; in the subsequent sections, we will focus on the contemporary evidential base of specific cardiovascular pharmacogenomic associations.

3 Warfarin

Warfarin is the most frequently prescribed oral anticoagulant worldwide,[25] and is indicated for patients with venous thromboembolism (VTE), atrial fibrillation (AF) and mechanical heart valves. Warfarin is a coumarin-derived racemic mixture that antagonises vitamin K epoxide reductase (Figure 2). The degree of anticoagulation is monitored by measuring the international normalised ratio (INR); the target INR range is 2.0 - 3.0 for most patients. There is a wide variation in therapeutic warfarin maintenance dose requirements ranging from 0.6mg/day to 15.5mg/day with patients spending only ~2/3 of the time in the therapeutic INR range.[26] Despite 60 years of clinical experience warfarin remains a leading cause of adverse drug events, as a result of haemorrhage or thromboembolism; the risks for both are INR-dependent.[1] Clinical factors together account for ~26% of inter-individual warfarin therapeutic dose variability.[27]

3.1 *VKORC1*

VKORC1 encodes the warfarin-sensitive target: vitamin K epoxide reductase complex subunit 1, which is the rate-limiting enzyme of the vitamin K cycle[28] (Figure 2). The noncoding single nucleotide polymorphism (SNP), rs9923231 (-1639G>A; G3673A), alters a *VKORC1* transcription binding site in the *VKORC1* promoter region and being an A carrier is associated with decreased enzyme expression[25] and lower warfarin dose requirements.[29] This association has been confirmed in several populations including African, Asian and Caucasian.[30] *VKORC1* rs9923231 alone explains ~20-25% of the variance in warfarin maintenance dose in Caucasian and Asian populations, but only

accounts for ~6% of dose variability in African Americans, attributable to their lower -1639A allele frequency (0.11 compared with 0.39 in Caucasians).[1,25] Several rare non-synonymous *VKORC1* polymorphisms have been identified that confer warfarin resistance leading to higher dose requirements.[25]

3.2 *CYP2C9*

CYP2C9 metabolises the more potent S-warfarin enantiomer and over 30 allelic variants are recognised. *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910) are the two most frequent minor *CYP2C9* alleles in Caucasians, with frequencies of 0.13 and 0.07 respectively.[25] In Asians, *CYP2C9*2* is very rare and the frequency of *CYP2C9*3* is 0.04[25]; in African Americans both *CYP2C9*2* and *CYP2C9*3* are absent or rare (allele frequencies 0 - 0.036 and 0.003 - 0.02 respectively).[31] *CYP2C9**2 and *CYP2C9**3 are each characterised by one non-synonymous SNP and encode proteins whose enzymatic activity is reduced by ~30-40% and ~80-90% respectively.[25] They are associated with prolonged warfarin half-life, increased time to reach therapeutic INR levels, reduced warfarin dose requirements,[25] and for patients with *CYP2C9*3*, an increased risk of bleeding has been confirmed by meta-analysis.[5] The reported hazard ratios for bleeding in **1/*3* and **3/*3* patients compared to **1/*1* wild type homozygotes were 2.05 (95% confidence interval (CI) 1.36 – 3.10) and 4.87 (95% CI 1.38 – 17.14) respectively, suggestive of a gene-dose trend.[5] Overall, *CYP2C9* genotype accounts for ~7-10% of warfarin dose variability.[1]

A recent GWAS in African American patients identified and replicated a clinically relevant association between a novel SNP (rs12777823) in the *CYP2C* gene cluster and stable warfarin dose requirements, following pre-specified conditioning for *VKORC1* rs9923231 and the composite genotype of *CYP2C9*2* and *CYP2C9**3.[32] rs12777823 was in low linkage disequilibrium with known *CYP2C9* alleles. The minor A allele frequency was 0.25 and was associated with decreased S-warfarin oral clearance in an adjunctive pharmacokinetic study of 60 African American patients. rs12777823 was associated with reduced warfarin dose requirements of ~7mg/week and ~9mg/week in individuals heterozygous and homozygous for the minor A allele respectively, and explained 5% of

the variability in warfarin dose.[32] rs12777823 is located within the *CYP2C* cluster, which includes *CYP2C9*, *CYP2C8*, *CYP2C18* and *CYP2C19*. rs12777823 has not been associated with warfarin dose variability in European and Japanese populations, despite being common, suggesting that it is in linkage disequilibrium with a causal variant in African Americans but not in other populations.[32]

3.3 *CYP4F2*

CYP4F2 metabolises reduced vitamin K to hydroxyvitamin K, depleting the vitamin K cycle of active vitamin K. The variant allele of rs2108622 (1297G>A, V433M) is associated with reduced steady state CYP4F2 hepatic concentrations, greater vitamin K availability[33] and has been identified as an independent predictor for higher warfarin dose requirements in Caucasian[29] and Asian[34] patients but not in African Americans,[32] after controlling for *VKORC1* and *CYP2C9*. However, it only explains an additional 1-2% of observed warfarin dose variability.[29,34]

3.4 Clinical utility

Over 20 warfarin dosing algorithms have been constructed that incorporate genetic determinants (mainly *CYP2C9*, *VKORC1 +/- CYP4F2*) with varying clinical and concomitant drug covariates into multivariable models for improving the prediction of individual patient warfarin dose requirements.[30] However, most were derived from small study populations. The International Warfarin Pharmacogenetics Consortium (IWPC) algorithm was derived from 4,043 patients from around the World and the IWPC pharmacogenetic algorithm significantly outperformed their clinical algorithm (i.e. no genetic covariates included) in the 46.2% of the population that required either ≤21mg or ≥49mg warfarin/week.[27] The IWPC pharmacogenetic algorithm is one of the high-performing validated algorithms recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for use by clinicians and is freely available at http://www.warfarindosing.org.[25]

Pharmacogenomic-based algorithms should benefit patient care through reducing the time to initial therapeutic and stable INRs, optimising the time spent within the therapeutic range and reducing adverse events.[26] Recently, the results of two RCTs which evaluated the clinical utility of genotype-

guided warfarin dosing have been reported.[35,36] The European trial showed that genotype-guided dosing (determined by an algorithm which took into account both genetic and clinical factors) was superior in achieving time within INR range of 2.0 - 3.0 over 3 months compared with current gold-standard clinical care.[35] However, the US trial showed that there was no difference in time within range over 1 month between dosing determined by an algorithm containing genetic and clinical factors, and by an algorithm containing clinical factors only.[36] Although both trials have tested whether genotype-guided dosing shows utility, the designs were different – of particular importance here may be the algorithmic strategy which comprised a loading dose algorithm in the EU study, but a maintenance dose algorithm in the US study. Despite the fact that the EU trial compared genotype-guided dosing to standard clinical care, it is important to note that the time within INR range in the EU trial was superior overall than that in the US trial. The US study population was also more heterogeneous compared to the EU population, as evidenced by the fact that the genetic algorithm actually performed worse than the clinical algorithm in African American patients, for reasons which are not completely clear. This also highlights the need for modified algorithms in sub-Saharan and African American patients, attributable to their lower frequency of *VKORC1* rs9923231, *CYP2C9*2* and *CYP2C9*3*.[31] The contradictory results seen in the two trials have added to the controversy of whether genotype-guided dosing is necessary for warfarin – there are other trials still currently under recruitment, and further data are expected from both these trials, which may help in determining future directions.

The novel oral anticoagulants (NOACs) rivaroxaban, dabigatran and apixaban have been approved for thromboembolism prevention in AF, having demonstrated either noninferiority or superiority to standard-of-care warfarin therapy with major bleeding rates not greater than with warfarin.[37] A fourth NOAC, edoxaban, is also in the late stages of development. These drugs are attractive to patients and clinicians alike as INR monitoring is not indicated and dose recommendations differ little between patients.[37] They also provide some choice to both patients and prescribers alike. However concerns remain regarding the lack of a procoagulant antidote and long term patient compliance (twice daily dosing is recommended for dabigatran and apixaban in AF). There are also no direct comparisons of

the new anticoagulant drugs, and these are unlikely to happen in the near future – thus prescribers are left with no definitive guidance as to which drug should be chosen in which patient groups. Research in the future needs to identify factors (clinical, genomic as well as other omics technologies) which will allow for stratification between these NOACs and warfarin (possibly using the same design as that used in the EU-PACT trial[35]).

4 Clopidogrel

Platelets are pivotal in the genesis of thrombotic cardiovascular pathologies. The oral prodrug clopidogrel is a second-generation thienopyridine whose active metabolite binds irreversibly to platelet membrane purinergic $P2Y_{12}$ receptors throughout the lifetime of a platelet (~10 days),[38] antagonising adenosine diphosphate (ADP)-mediated platelet aggregation. Its metabolism is complex: ~85% is rapidly hydrolysed to an inactive metabolite by hepatic carboxylesterase 1 (CES1),[39] and the remainder undergoes two sequential hepatic oxidation steps to form first the intermediate inactive metabolite (2-oxo-clopidogrel) and then the active metabolite (R-130964) involving CYP1A2, CYP3A4/5, CYP2B6, CYP2C9 and CYP2C19.[39]

There is substantial variability in platelet response to clopidogrel.[40] Clinical predictors of platelet resistance to clopidogrel, as evidenced by *ex vivo* high on-treatment platelet reactivity (HTPR), include older age (>65 years), high BMI, drug interactions that inhibit CYP enzymes (e.g. statins, proton pump inhibitors, erythromycin), diabetes mellitus, renal failure and reduced left ventricular function, although combined these factors only explain a small proportion of observed variability.[40] Cardiovascular endpoints still occur in ~12% of acute coronary syndrome (ACS) patients on clopidogrel.

4.1 *CYP2C19*

CYP2C19 largely mediates the conversion of inactive clopidogrel into its active metabolite.[39] *CYP2C19*1* is the wild-type allele but over 25 variants are recognised; the majority of identified reduced functioning alleles (e.g. **2-*8*) are rare with the exception of *CYP2C19*2* (rs4244285,

c.681G>A), and in Asian populations *CYP2C19*3* (rs4986893, c.636G>A).[38] *CYP2C19*2* is present with an allelic frequency of ~0.15 in both Africans and Caucasians and ≤0.35 in Asians; *CYP2C9*3* occurs in Asians with a frequency of ≤0.089.[38] *CYP2C19*2* and *CYP2C19*3* are loss-of-function alleles whose defining SNPs result in aberrant splicing and a premature stop codon respectively.[38] *CYP2C19*2* and *CYP2C19*3* are associated with reduced circulating active clopidogrel metabolite levels[41] and, for *CYP2C19*2*, meta-analysis has confirmed its association with per-allele significant increases in HTPR.[41] Although the *CYP2C19*2* allele has a stronger independent effect on *ex vivo* platelet function than any clinical risk factor, it still only accounts for up to ~12% of the observed variability in clopidogrel platelet response.[42]

*CYP2C19*17* (rs12248560, c.-806C>T) is another common polymorphism with estimated allelic frequencies of 0.16, 0.027 and 0.18 and in Africans, Asians and Caucasians respectively. *CYP2C19*17* leads to a gain-of-function as a result of enhanced transcription[38] and has been correlated with decreased prevalence of HTPR,[12] although the magnitude of effect is lower than compared to the loss-of-function alleles.[1]

To the authors' knowledge, 13 meta-analyses have been published to date since 2010 that evaluate the association between *CYP2C19* and clinical outcomes (Supplementary Table 1). A consistent and robust finding is that *CYP2C19* reduction-of-function alleles (predominantly *CYP2C19*2*) significantly increase the risk of stent thrombosis following percutaneous coronary intervention (PCI) compared to non-carriers. Further, a gene-dose trend is evident: the association of carrying one and two loss-of-function *CYP2C19* alleles (predominantly *CYP2C19*2*) with stent thrombosis were assessed separately in two meta-analyses, and the more conservative summary risk estimates were still 2.67 (95% CI 1.69 – 4.22) and 3.97 (95% CI 1.75 – 9.02) respectively.[11]

The impact of *CYP2C19* reduction-of-function alleles on major adverse cardiovascular events (MACE; e.g. cardiovascular death, nonfatal myocardial infarction or stroke) is more controversial. Interestingly though, differential risks of MACE with *CYP2C19* reduction-of-function alleles have

been suggested within patient subgroups taking clopidogrel. Firstly, when stratified by ethnicity the summary odds ratio (OR) for MACE appears to be marginally higher in Asian compared to Western patients with OR of 1.89 (95% CI 1.32 – 2.72) and 1.28 (95% CI 1.00 -1.64) respectively.[43] This may reflect the higher carriage of reduction-of-function *CYP2C19* alleles in Asian populations, or be a discrepancy as the included Asian studies had lower numeric event rates.[43] Secondly, there is a case for indication-specific clopidogrel pharmacogenomics.[1,38] This is because the overall risk reduction of MACE with clopidogrel is larger in conditions at high risk of MACE (e.g. PCI, especially after an ACS) compared to those at lower MACE risk (e.g. medically managed non-ST elevation myocardial infarction and AF). Therefore, the impact of *CYP2C19* genotype on MACE should be greater and more apparent for the high risk indications.[1] To support this hypothesis, the OR for MACE in *CYP2C19* reduction-of-function allele carriers compared to non-carriers is 1.57 (95% CI 1.13 – 2.16) in one meta-analysis, where 91.3% of included patients had undergone PCI,[11] compared to 1.18 (95% CI 1.09 – 1.28) in another meta-analysis which included a broader range of patient phenotypes.[41]

The balance of evidence is suggestive that *CYP2C19*17* confers a small but statistically significant reduced rate of MACE;[12,44] no meta-analysis has demonstrated a protective role of *CYP2C19*17* with stent thrombosis (Supplementary Table 1). Two meta-analyses reported a marginally significant increased risk of bleeding associated with *CYP2C19*17*.[12,44] However, this is not supported by a contemporary, albeit smaller (n = 6,659) meta-analysis that separated carriers of *CYP2C19* loss-of-function alleles from *CYP2C19*17* carriers and compared these patient groups directly (relative risk (RR) 1.05, 95% CI 0.72 – 1.53).[45] This meta-analysis did report though a non-significant trend for increased bleeding risk with *CYP2C19*17* in a subgroup analysis of studies that predominantly enrolled patients scheduled for PCI (RR 1.57, 95% CI 0.79 – 3.10), further hinting at indication-specific clopidogrel pharmacogenomics.[45]

4.2 Other genes affecting clopidogrel response

ABCB1 encodes ATP-binding cassette (ABC) subfamily B (MDR/TAP) member 1 (ABCB1), also referred to as P-glycoprotein: an efflux transporter that is extensively distributed. ABCB1 has broad

exogenous substrate specificity and has a role in eliminating substrates into the intestine, urine and bile. A commonly studied *ABCB1* variant is the synonymous SNP 3435C>T (rs1045642, Ile1145) SNP. TT homozygous patients have been shown to have reduced absorption of clopidogrel after a single oral loading dose compared to C/T and CC patients.[46] The most recently published meta-analysis to investigate the association between 3435C>T and clinical outcomes with clopidogrel therapy demonstrated no association with high platelet activity or stent thrombosis.[13] However significant results for increased risk of early (≤30 days) MACE (in carriers of the T allele compared to CC homozygotes) and decreased risk of bleeding (in TT compared to CC homozygous patients) were reported.[13]

As CES1 is the primary enzyme responsible for the biotransformation of clopidogrel, 2-oxo-clopidogrel and clopidogrel's active metabolite (R-130964) into inactive carboxylic acid compounds,[39] *CES1* variants may contribute to inter-individual variability in clopidogrel response. Recent *in vitro* research has demonstrated that the non-synonymous minor allele variant of G143E (rs71647871) from the *CES1A1* isoform of the *CES1* gene has completely impaired catalytic activity for metabolising clopidogrel, 2-oxo-clopidogrel and the established CES1-selective substrate, methylphenidate.[39] Furthermore, 143E has been associated with both significantly higher clopidogrel active metabolite levels (in healthy individuals) and significantly reduced ADP-stimulated platelet aggregation after but not prior to clopidogrel treatment (in healthy individuals and patients with coronary heart disease), compared to G143G homozygotes.[47] This same study though found no significant association between G143E and cardiovascular events after one year.[47] However this was a low powered comparison (only six of 350 patients carried 143E) and so, the effect of G143E on cardiovascular outcomes remains to be established.

Paraoxonase-1 (PON1) has a role in the hepatic conversion of 2-oxo-clopidogrel to the active thiol metabolite. Although an initial study suggested that *PON1* Q192R, a missense variant, determined clopidogrel efficacy, subsequent larger studies have failed to show any association between this variant and clopidogrel efficacy.[48]

4.3 Newer $P2Y_{12}$ receptor antagonists

Prasugrel is a novel thienopyridine prodrug that is converted in a single hepatic step to the active metabolite, principally by CYP3A4 and CYP2B6 and to a lesser extent by CYP2C9 and CYP2C19.[40] In the genetic substudy of the TRITON-TIMI 38 trial, cardiovascular outcomes with prasugrel were unaffected by *CYP* polymorphisms.[49] A recent study of 213 patients undergoing successful PCI for ACS and discharged on prasugrel has reported significantly higher and lower HTPR, assessed at one month post PCI, with *CYP2C19*2* and *CYP2C19*17* genotypes respectively.[50] They also observed a higher rate of bleeding complications in *CYP2C19*17* carriers.[50] Importantly, they assessed platelet response using platelet reactivity index vasodilator-stimulated phosphoprotein (PRI VASP), which is a highly specific test for $P2Y_{12}$ inhibition.[50] These finding contrast earlier results assessing ADP-induced platelet aggregation with light transmission aggregometry[49] and require independent replication.

Ticagrelor is a novel cyclopentyltriazolopyrimidine antiplatelet agent. It is a reversible, non-competitive inhibitor of $P2Y_{12}$ receptors that does not require bioactivation and is approved to reduce the rate of thrombotic cardiovascular events in patients with ACS in conjunction with low dose aspirin[40]. In the genetic substudy of the PLATO trial, the efficacy of ticagrelor was not influenced by *CYP2C19* or *ABCB1* genotypes.[51]

4.4 Clinical utility

There is substantial controversy surrounding the utility of *CYP2C19* genotyping and clopidogrel therapy. Collectively though, the evidence strongly affirms that *CYP2C19* reduction-of-function alleles (predominantly *CYP2C19*2*) predispose patients managed by PCI to stent thrombosis, and potentially but to a lesser degree MACE. Although the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) consensus guidelines do not support routine *CYP2C19* genotyping in clinical practice for clopidogrel therapy for any indication because of the lack of randomised data, they do permit consideration of *CYP2C19* genotyping for individual patients

believed to be at high risk for poor outcomes (e.g. patients undergoing PCI for extensive disease) prior to commencing clopidogrel.[42] RCTs are currently ongoing to assess the clinical utility of pre-prescription *CYP2C19* genotyping, as are studies using platelet function tests prior to clopidogrel use. The updated 2013 CPIC guidelines recommend that, for ACS patients managed with PCI in whom *CYP2C19* status is already known, alternate antiplatelet therapy (e.g. prasugrel or ticagrelor) be prescribed for carriers of two (strong recommendation) or one (moderate recommendation) reduction-of-function *CYP2C19* allele.[38]

5 Statins

Statins, the most commonly prescribed class of medication worldwide, are indicated for the primary and secondary prevention of cardiovascular disease. Their principal mechanism of action is the reduction in plasma low-density lipoprotein cholesterol through competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic *de novo* cholesterol synthesis. Genetic variation in the lipid lowering efficacy of statins has been extensively investigated and over 40 candidate genes have been described.[52] However, the statin pharmacogenomic association of greatest magnitude found to date is between *SLCO1B1* rs4149056 and muscle toxicity and is the focus here.

The clinical presentation of statin-induced muscle toxicity is heterogeneous and includes asymptomatic elevations in plasma creatine kinase (CK) level, myalgias with no detectable CK rise, severe myopathies with high plasma CK levels and rhabdomyolysis with its inherent risks of acute kidney injury, electrolyte disturbances and death. The frequency of statin-induced myopathy and rhabdomyolysis are estimated to be ~1/1,000 and ~1/100,000 respectively.[53] Several clinical factors are already known to increase the risk of statin-induced muscle toxicity including older age, female gender and low BMI.[53]

In 2008, a GWA case-control substudy of the SEARCH RCT was the first to demonstrate a strong association between *SLCO1B1* (which encodes the influx transporter OATP1B1) and myopathy.[6]

They compared 85 cases of severe myopathy with 90 controls, all of whom were prescribed 80mg simvastatin daily as part of the RCT. A single strong association between an intronic SNP variant, rs4363657, in *SLCO1B1* and severe myopathy was detected and regional genetic analysis revealed it to be in almost complete linkage disequilibrium with rs4149056 (521T>C, V174A; defines *SLCO1B1*5*), a non-synonymous SNP in exon 6. The frequency of the rs4149056 C allele was 0.15 and a gene-dose relationship was observed with ORs for myopathy of 4.5 (95% CI 2.6-7.7) per copy of the C allele, and 16.9 (95% CI 4.7-61.1) in participants homozygous for the C allele compared to the wild type TT genotype.[6] This association with myopathy has been validated through replication[6,54] and meta-analysis.[54] Further, the link with rs4149056 has been extended to milder, statin-induced adverse events including drug discontinuation for any side effect and myalgias.[55] The association though between rs4149056 and ADRs appears to be simvastatin-specific as there is a lack of supporting evidence for this association with pravastatin,[55] and rosuvastatin,[56] and less of an effect with atorvastatin.[54]

The rs4149056 variant perturbs OATP1B1 localisation to the hepatic cell membrane, reducing its transport capacity and has been associated with higher statin, including simvastatin acid, plasma concentrations that conceivably increase skeletal-muscle exposure.[55] However, the relationship between simvastatin plasma concentration and muscle toxicity is not straightforward and the precise mechanism(s) by which statin exposure provokes muscle toxicity remains ill-defined.

In 2012, the CPIC issued consensus guidelines for *SLCO1B1* rs4149056 and simvastatin-induced myopathy, including consideration of simvastatin dose reductions or use of an alternative statin for both carriers and homozygotes of rs4149056.[53] However, this is not widely practised and further predictors of myopathy risk need to be identified. To this end, two recent findings deserve a mention:

- Some patients on statins develop an immune-mediated necrotizing myopathy which continues despite discontinuation of the drug, and is characterised by anti-HMG CoA reductase antibodies. This sub-phenotype of myopathy has been associated with *HLA-DRB1*11:01* in

both Caucasians and African Americans, but not with the *SLCO1B1* rs4149056 polymorphism.[57]

- A recent study used gene expression profiles in lymphoblastoid cell lines exposed to statins and expression quantitative trait loci (eQTL) analysis to identify rs9806699, a cis-eQTL in the glycine amidinotransferase (*GATM*) gene which encodes for an enzyme involved in creatine synthesis, to protect against statin myopathy. The effect of the variant was shown in two patient cohorts, and *in vitro* studies suggest that this gene may provide a functional link between statin efficacy and toxicity.[14]

Finally, an alternative proposition recently suggested may be to develop dosing algorithms, as has been done for warfarin, in order to avoid excessive drug exposure.[58] The authors considered clinical (sex, age, BMI, ethnicity, dose, and time from last dose), genetic (SNPs in *SLCO1B1* and *ABCG2*) and metabolic (4β-hydroxycholesterol, a CYP3A activity marker, relevant for atorvastatin) variables to formulate dosing algorithms for rosuvastatin and atorvastatin. These algorithms predicted that for nearly 50% of patients taking the highest doses in routine clinical practice, systemic statin exposure exceeds the 90th centile.[58] However, whether this has clinical utility needs to be proven, since the relationship between systemic statin exposure and the risk of myopathy, is not as clear-cut as the relationship between INR level and the risk of haemorrhage/thromboembolism.

6 Beta-blockers

Beta-adrenoreceptor antagonists (β-blockers) are indicated for the management of hypertension, angina pectoris, following acute coronary syndromes, arrhythmias and heart failure (HF). We focus on the latter indication.

Sympathetic activity is increased in HF as an initial compensatory mechanism, which becomes deleterious in the long term. There is strong evidence of the overall prognostic benefit of β-blocker therapy in the patient population with heart failure, although clinically relevant proportions of HF

patients on β-blocker therapy derive no benefit or suffer ADRs necessitating dose reduction or drug withdrawal.

ADRB1 encodes the β_1 AR, the dominant cardiomyocyte β-AR subtype, and it has two common SNPs: Arg389Gly (rs1801253) and Ser49Gly (rs1801252). Arg389Gly is located within the intracellular eighth helix of the β_1 AR, which is involved in coupling to $G_{\alpha s}$.[59] Collectively, functional evidence demonstrates that the Arg389 receptor form confers greater noradrenaline (NA) affinity[60] and promotes greater agonist-mediated adenylyl cyclase stimulation and downstream signalling.[59] Ser49Gly is located in the amino terminus of β_1 ARs and, although its function is less well characterised, the Gly49 receptor form has been shown to exhibit enhanced agonist-promoted downregulation.[59] A recent meta-analysis reported that the Gly389 polymorphism increases susceptibility to HF in East Asians but has a protective trend against HF in Caucasians.[61] Furthermore, the authors found that in established HF patients on β-blocker therapy, Arg389Arg homozygotes are associated with significantly greater improvements in left ventricular ejection fraction (LVEF) compared with Gly389 carriers.[61]

Common polymorphisms in adrenergic system genes, especially *ADRB1*, *ADRB2*, *ADRA2C* and *GRK5*, have been extensively studied for pharmacogenomic associations with clinical endpoint outcomes in HF patients (Supplementary Table 2). The interesting pharmacogenomics of bucindolol merit highlighting. Bucindolol is a non-selective β-AR inhibitor with sympatholytic capability, weak α_1-AR antagonism,[62] and inverse agonist activity in failing hearts expressing Arg389Arg β_1-ARs.[16] The prognostic utility of bucindolol versus placebo in New York Heart Association (NYHA) functional class III and IV HF patients with LVEF ≤0.35 was assessed in the BEST RCT, which was terminated prematurely as bucindolol conferred no significant overall survival benefit.[15] Subsequent sub-analyses suggested that patients with NYHA class IV symptoms, African Americans and those with LVEF <0.2 in particular derived no benefit from bucindolol.[62] Using combinatorial genotyping in the 1,040 patient genetic substudy of BEST, a haplotype construct incorporating *ADRB1* Arg389Gly and *ADRA2C* Ins322-325Del polymorphisms has been published.[60] This construct helps

differentiate subgroups with enhanced (Arg389Arg homozygotes + any Ins22-325Del allele), intermediate (Gly389 carriers + Ins322-325Ins wild type homozygotes) and no (Gly389 carriers + Del322-325 carriers) bucindolol efficacy for six HF clinical endpoints (including mortality) compared with placebo.[60] α_{2C}-AR, encoded by *ADRA2C*, is located in the cardiac presynaptic junction and mediates negative feedback of NA release. The minor position 322-325 four amino acid deletion (Del322-325) results in a loss-of-function phenotype, and is associated with exaggerated sympatholytic response (as determined by an increased fall in circulating NA) to bucindolol.[60] It is postulated that the combination of carrying Del322-325 and the hypofunctional Gly389 β_1-AR neutralises bucindolol's efficacy because the exaggerated sympatholysis results in insufficient NA for the low NA-affinity Gly389 β_1-ARs to support the HF myocardium.[60]

Recently, this three-genotype construct has been further assessed for differentiating risks of ventricular arrhythmias (interaction test, $p = 0.028$)[18] and new-onset AF (interaction test, $p = 0.016$)[19] with bucindolol compared to placebo in the BEST genetic substudy cohort. Consistent with the all-cause mortality and cardiovascular mortality endpoints,[60] Arg389Arg HF patients experienced enhanced protection against onset of both types of arrhythmia.[18,19]

The Leu41 variant of the Gln41Leu SNP in *GRK5* (G protein-coupled receptor kinase 5) has been shown to consistently confer an inherent protective survival advantage compared to Gln41Gln wild type homozygotes in African American HF patients not on β-blocker therapy (Supplementary Table 2).[20] However, when comparing African American HF patients prescribed a β-blocker (predominantly metoprolol and carvedilol) versus those not prescribed a β-blocker *within* genotype, Gln41Gln homozygotes derived significant benefit from therapeutic β-blockade,[20] whereas Leu41 carriers did not.[20] The Leu41 variant is a gain-of-function polymorphism which enhances agonist-promoted desensitisation of β-ARs, conveying favourable 'genetic β-blockade'.[20] The minor Leu41 allele frequency is 0.231 in African Americans, but only 0.017 in Caucasians, which might help account for its associations being restricted to African Americans.

On review of the evidence, it is not unreasonable to conclude that preferential clinical outcome benefit from β-blocker therapy in HF patients is conferred by the *GRK5* Gln41Gln genotype, and for patients specifically prescribed bucindolol, the *ADRB1* Arg389Arg and to a lesser extent *ADRA2C* Ins322-325Ins (restricted to Gly389 carriers) genotypes. The association of *ADRB1* Arg389Gly with HF outcomes in patients taking other β-blockers is less clear. A pharmacogenomic role though for *ADRB1* Arg389Gly in HF is consistent with findings from hypertension studies, where Arg389 has been associated with higher blood pressure, a greater prevalence of hypertension[63] and, in the context of the Ser49Arg389 haplotype, an increased risk of cardiovascular endpoints in hypertensive patients, offset by atenolol.[64] Presently, the evidence supporting a role for *ADRB2* in β-blocker HF pharmacogenomics is insufficient.

At present, no recommendations can be made regarding the clinical utility of genotyping adrenergic system SNPs in HF (or hypertension) given the heterogeneity in results. Large prospective cohorts are required for validation of current results, preferably limited to a single β-blocker given their distinct pharmacological profiles. One such example is GENETIC-AF, a phase 2B/3 clinical RCT, which plans to begin patient enrolment early in 2014 to investigate prospectively the efficacy of bucindolol, compared to metoprolol CR/XL, for prevention of AF in *ADRB1* Arg389Arg homozygous HF patients with reduced LVEF.

7 Renin-angiotensin system inhibitors

ACEIs antagonise the renin-angiotensin-aldosterone system (RAAS) and are indicated in hypertension, acute coronary syndromes, chronic heart (left ventricular and congestive) failure and diabetic nephropathy. Angiotensin II receptor blockers (ARBs) provide a suitable alternative in those that do not tolerate ACEIs.

The 287-base pair Alu repeat element insertion/deletion polymorphism is located within intron 16 of the *ACE* gene (rs4646994, *ACE* I/D)[65], and the D allele is associated with increased plasma ACE activity. Its role in the pharmacogenomics of ACEI/ARB efficacy however remains controversial,

with a lot of contradictory data in the literature. Larger better designed studies are needed, not only to look at the effect of the *ACE I/D* but also to evaluate other SNPs throughout the whole genome in an unbiased fashion.

A rare serious ACEI ADR is angioedema, which has an estimated incidence from large epidemiological studies of 0.1-0.7%, and often manifests long after ACEI initiation. A recent meta-analysis of 12 studies including 1,136 cases, found no association between *ACE* I/D genotype and ACEI-induced cough (OR 1.12, 95% CI 0.88 – 1.43).[66] A recent GWAS of 664 subjects found no genome-wide significant SNP associations for ACEI-induced angioedema, suggesting that unlike other serious ADRs (e.g. statin-induced myopathy), common variants of large effect size are unlikely to be involved. They did replicate though two variants moderately associated with angioedema in the GWAS, rs500766 and rs2724635, in a second patient sample. These variants are in *PRKCQ* (protein kinase C θ) and *ETV6* (ETV6) respectively and both have roles in immune regulation.[67]

In summary, many studies have investigated the influence of genetic variants on indication-specific efficacy (ACEI/ARB) and ADRs (ACEI) associated with RAAS inhibition, but there are no clear candidates that could be implemented in clinical practice.

8 Antiarrhythmics

Drug-induced ventricular arrhythmias are rare, unpredictable but potentially fatal ADRs and are a leading cause of drug withdrawal after approval. The majority are related to medications that perturb cardiac repolarisation through blockade of I_{Kr}, prolonging the QT interval and predisposing to drug-induced Torsades de Pointes (DITdP)[68]. Most DITdP are related to antiarrhythmic medications, such as amiodarone, flecainide and sotalol. Multiple classes of non-cardiac drugs are also associated with QT prolongation, although only a proportion have been implicated in DITdP (e.g. erythromycin, chlorpromazine, domperidone). The incidence of DITdP has been estimated to be 1-3% for antiarrhythmic medications, although it is substantially less for non-cardiac drugs.[68] Several clinical

factors are known to increase the risk of DITdP including electrolyte imbalances (especially hypokalaemia), female gender and heart failure.

Drug-induced prolonged QT and Torsade de Pointes resemble congenital long QT syndrome (cLQTS), in which rare mutations (<1% minor allele frequency) in at least 13 genes which encode ion channels or proteins that modulate channel functions have been identified.[69] It has been estimated that ~10% of DITdP is predisposed to by rare mutations in the cLQTS disease genes, which constitutes cLQTS incomplete penetrance.[68] One example is the 22A>G variant in *KCNE2*, which causes the T8A substitution in potassium voltage-gated channel subfamily E member 2 (KCNE2). T8A has no pre-drug effect on QT interval but modulates QT interval sensitivity to sulfamethoxazole and has been associated with DITdP.[69,70] A recent candidate gene study utilised next-generation sequencing and reported that 23.1% of Caucasian subjects (6 of 26) with DITdP carried a variant within 22 congenital arrhythmia genes (which include the 13 cLQTS genes), compared to a background rate of 1.7% in 60 control subjects from the 1000 Genomes CEU data.[71]

More common ethnically restricted polymorphisms in genes that are infrequent causes of cLQTS, including *KCNE1* D85N (rs1805128) and *SCN5A* S1103Y (rs7626962), have been associated with DITdP.[68] *KCNE1* encodes potassium voltage-gated channel subfamily E member 1 (KCNE1) and rare mutations cause ~1% of cLQTS.[69] *KCNE1* D85N had an OR of 9.92 (95% CI 2.36 – 41.80) when comparing 176 Caucasian DITdP cases to 207 Caucasian drug-tolerant controls and occurred with a frequency of 1.8% in population controls.[7]

Nitric oxide synthase 1 adapter protein (NOS1AP) interacts with neuronal nitric oxide synthase (nNOS) and can accelerate cardiac repolarisation. Recently, variants in *NOS1AP* have been associated with DITdP and QT interval prolongation in Caucasians, which were most evident in the subgroup of patients prescribed amiodarone.[21] The most statistically significant *NOS1AP* SNP, rs10919035, occurred in 27.8% of amiodarone cases and 7.1% of healthy controls (OR 5.5, 95% CI 1.1-27.9). In

the replication study, rs10919035 showed a non-significant trend towards increasing amiodarone DITdP but meta-analysis of the two cohorts gave OR 2.81 (95% CI 1.62 – 4.89).[21]

DITdP is challenging to study given its rare and capricious phenotype; nevertheless rare and ethnically restricted variants have been identified. A further complexity is that case definitions and case validity have varied between studies. A collaborative Phenotype Standardization Project, championed by the international Serious Adverse Events Consortium (iSAEC), has recently set down consensus minimum definitions for classic, probable and possible DITdP to facilitate recruitment of well characterised cases into a large patient dataset for identification and validation of genetic variants.[72]

Meanwhile an institution-wide QT alert system has been implemented at Mayo Clinic that alerts physicians if a patient's electrocardiogram QTc is ≥500ms. For those with QTc ≥500ms, a retrospective multivariate model was built from established clinical predictors of QT prolongation (including drugs) that was a significant predictor of mortality.[73] Although the widespread clinical application of DITdP genetic variants cannot currently be recommended, in the long term, genetic predictors may be assimilated into risk models. Alternatively, as rare variants appear to contribute a large proportion of the genetic predisposition to DITdP, clinical risk models may be used to identify individuals in whom in-depth genotyping of candidate genes is warranted.

9 Conclusions and future perspective

Despite an increasing number of established drug response-gene associations, the majority of cardiovascular pharmacogenomic research remains in the discovery phase, as investigators struggle to identify and validate associations. This is partly a consequence of study methodological limitations including patient phenotype heterogeneity and inadequate sample sizes. Nevertheless, substantial progress has been made and clinical trials are ongoing with clopidogrel and planned for bucindolol. Furthermore, the creation of consortia facilitates the opportunity for standardising phenotype definitions, galvanising financial resources, conducting larger studies, assessing pharmacogenomic

associations in different ethnic groups, performing meta-analysis, determining evidence threshold requirements for genetic associations and developing consensus clinical guidelines.

There are of course many barriers to the implementation of pharmacogenomics. In order to overcome these, several pioneering 'early adopter' sites have initiated genomic medicines programs.[22] One example is the Vanderbilt University Medical Center, USA, where patients scheduled for coronary angiography are pre-emptively genotyped on a 184-variant platform and *CYP2C19* genotype-based recommendations are automatically provided electronically when physicians want to prescribe antiplatelet therapy.[23] Although such pre-emptive genotyping has been criticised as being premature given that there is no definitive supporting RCT evidence, Vanderbilt undertook appropriate and reasonable steps to ensure that equipoise was maintained. These included conducting patient focus groups, whose consensus decisions helped shape the design of the program, and seeking guidance from an ethics committee.[23] Taken together with the facts that (a) there is evidence that patients with *CYP2C19*2* undergoing PCI are at increased risk of stent thrombosis with clopidogrel; and (b) alternative antiplatelet agents are available, the pre-emptive genotyping strategy is likely to yield important real-world effectiveness data. Furthermore the clinical utilisation of this pharmacogenomic program has enabled Vanderbilt to develop solutions to the logistical, financial and knowledge implementation barriers and, along with other early adopter sites, has led to an outlined consensus implementation framework that will assist other institutions and the wider patient population if clinical pharmacogenomics becomes more widespread.[22] It is also important to note that several sites in the US are already undertaking genotyping before warfarin administration – given the negative result from the COAG trial[36] it will be interesting to see whether they continue with genotyping or revert to their original clinical practice of administering warfarin without any regard to genotype.

However, even if the Vanderbilt program shows some benefits, whether the broader cardiology community would accept such evidence is unclear. Hence there is probably still a need for RCTs to show that pre-prescription genotyping for *CYP2C19* has clinical utility in improving the efficacy of clopidogrel treatment. Such trials would be complementary to the implementation route, and provide a

stronger evidence base for the widespread implementation of pharmacogenomics testing prior to clopidogrel treatment. An example of one such trial, already in progress, is TAILOR-PCI (http://mayoresearch.mayo.edu/center-for-individualized-medicine/cardiovascular-disease-study.asp).

Looking into the future, there is clear need for better guidance on how biomarkers need to undergo qualification, what type of evidence is acceptable for such qualification, and more harmonization between regulatory agencies worldwide and also with organisations that write guidelines. This will provide clearer routes for reimbursement and implementation within clinical practice. It is important to re-iterate that RCTs are not necessarily always at the top of the of evidence tree, and all evidence needs to be evaluated in an integrative and intelligent manner. It is also important for researchers to be cautious in their recommendations at the discovery stage, and replication of any biomarkers in independent cohorts is essential.

Finally, this review has focused on genomic variation, although other –omics technologies (such as epigenomics, transcriptomics, proteomics, metabolomics and metagenomics) will undoubtedly also be important in determining variation in response to cardiovascular drugs. Two recent examples include the following: (a) microRNA, miR-133a, regulates VKORC1, a key determinant of individual variability in warfarin dose requirements[74]; and (b) microbiome studies have shown that gut bacteria, in particular *Eggerthella lenta,* lead to the inactivation of digoxin reducing its serum concentration, a key determinant of the action of digoxin.[75] Techniques that allow a systems approach integrating the different determinants of variability in drug response will ultimately be important in personalising drug therapy in cardiovascular disease.

In conclusion, despite the recent contradictory data from the warfarin trials,[35,36] it is our expectation that warfarin pharmacogenomics does offer real patient benefit in real-world clinical practice, although given the barriers to implementation, widespread adoption will still likely require several years. Over the coming decade, clarification of the clinical role of *CYP2C19* genotyping in antiplatelet therapy is anticipated and bucindolol may possibly become the first genetically-stratified

HF therapeutic. Slowly, painstakingly and arduously cardiovascular pharmacogenomics is edging towards mainstream clinical practice, although much work remains.

Conflict of Interest/Disclosure

The authors' have no conflicts of interest to declare.

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Table 1. Select examples of genetic variants associated with cardiovascular disease susceptibility and differential cardiovascular drug response

Clinical Outcome	Study Type	Study Size[i]	Locus/Gene	Variant(s)	Risk Estimate (95 % CI)	P-value[ii]
1.) Disease susceptibility associations						
Atrial fibrillation	MA[iii,2]	59,133	4q25 (*PITX2*)	rs6817105	RR 1.64 (1.55 – 1.73)	1.8×10^{-74}
			16q22 (*ZFHX3*)	rs2106261	RR 1.24 (1.17 – 1.30)	3.2×10^{-16}
			1q21 (*KCNN3 – PMVK*)	rs6666258	RR 1.18 (1.13 – 1.23)	2.0×10^{-14}
Coronary artery disease	MA[iv,3]	86,995	9p21	rs4977574	OR: 1.29 (1.23 – 1.36)	1.35×10^{-22}
			1p13 (*SORT1*)	rs599839	OR: 1.11 (1.08 – 1.15)	2.89×10^{-10}
			21q22 (*MRPS6*)	rs9982601	OR: 1.18 (1.12 – 1.24)	4.22×10^{-10}
Heart failure	MA[4]	20,926	15q22	rs10519210	HR 1.53 (1.05 – 2.24)	1.4×10^{-8}
	MA[4]	2,895	12q14	rs11172782	HR 1.46 (1.03 – 2.09)	6.7×10^{-8}
Hypertension	CG[10]	37,245	SBP GRS[v]		OR[vi]: 1.13 (1.12 – 1.15)	3×10^{-67}
			DBP GRS[v]		OR[vi]: 1.21 (1.18 – 1.24)	3×10^{-64}
2.) Pharmacogenomic associations						
Warfarin						
a) Haemorrhage	MA[5]	2,670	*CYP2C9*	*2, *3	*2 or *3 vs *1/*1: HR 1.64 (1.34 – 2.11)	<0.001
					*1/*3 vs *1/*1: HR 2.05 (1.36 – 3.10)	<0.001
					*3/*3 vs *1/*1: HR 4.87 (1.38 – 17.14)	0.01
b) Over-anticoagulation (INR>4)	MA[5]	4,717	*CYP2C9*	*2, *3	*2 vs *1: HR 1.52 (1.11 – 2.09)	0.01
					*3 vs *1: HR 2.37 (1.46 – 3.83)	<0.001
	MA[5]	3,691	*VKORC1*	-1639G>A	GA vs GG: HR 1.49 (1.15 – 1.92)	0.003
Clopidogrel						
a) Stent thrombosis	MA[11]	5,894	*CYP2C19*	*2, *3, *4 - *8	RFA present vs non-carriers: HR 2.81 (1.81 – 4.37)	<0.00001
					1 RFA vs non-carriers: HR 2.67 (1.69 – 4.22)	<0.0001
					2 RFA vs non-carriers: HR 3.97 (1.75 – 9.02)	0.001
b) MACE in patients at high risk of MACE (e.g. requiring PCI)	MA[11]	9,685	*CYP2C19*	*2, *3, *4 - *8	RFA present vs non-carriers: HR 1.57 (1.13 – 2.16)	0.006
					1 RFA vs non-carriers: HR 1.55 (1.11 – 2.27)	0.01
					2 RFA vs non-carriers: HR 1.76 (1.24 – 2.50)	0.002
c) MACE	MA[12]	9,428	*CYP2C19*	*17	HR 0.82 (0.72 – 0.94)	0.005
d) Early (≤30 days) MACE	MA[13]	4,195	*ABCB1*	3435C>T	OR 1.48 (1.06 – 2.06)	0.02
Simvastatin						
Myopathy	GWAS[6]	175	*SLCO1B1*	rs4149056, T>C	per copy of C allele: OR 4.5 (2.6 – 7.7)	<0.001
					CC vs TT: OR 16.9 (4.7 – 61.1)	<0.001
	CG[14]	4,421	*GATM*	rs1719247[vii]	OR 0.60 (0.45 – 0.81)	0.0007
Bucindolol						
a) Death	RCT[15]	2,708			B vs P overall[viii]: HR 0.90 (0.78 – 1.02)	
	CGS of RCT[16]	1,040	*ADRB1*	Arg389Gly	B vs P if Arg389Arg: HR 0.62 (0.40 – 0.96)	0.03
	CGS of RCT[17]	1,040	*ADRA2C*	Ins322-325Del	B vs P if Ins322-325Ins: HR 0.70 (0.51 – 0.96)	0.025
b) VT/VF	CGS of RCT[18]	1,040			B vs P overall[viii]: HR 0.42 (0.27 – 0.64)	

			ADRB1	Arg389Gly	B vs P if Arg389Arg: HR 0.26 (0.14 – 0.50)	0.00005
c) New onset atrial	RCT	2,392			B vs P overall[viii]: HR 0.59 (0.44 – 0.79)[19]	
fibrillation	CGS of RCT[19]	1,040	*ADRB1*	Arg389Gly	B vs P if Arg389Arg: HR 0.26 (0.12 – 0.57)	0.0003
Carvedilol/Metoprolol						
Death or HT	CG[20]	375	*GRK5*	Gln41Leu	βB vs no βB if Gln41Gln: HR 0.22 (0.12 – 0.40)	7.96×10^{-8}
					βB vs no βB if Leu41 carrier: HR 0.78 (0.35 – 1.7)[ix]	0.527
Mainly antiarrhythmic drugs						
DITdP	CG[7]	383	*KCNE1*	D85N, *G>A*	OR 9.92 (2.36 – 41.80)[7]	0.00177
	CG[21]	346	*NOS1AP*	rs10919035	OR 2.81 (1.62 – 4.89)[21]	0.00024

Abbreviations: AF = atrial fibrillation; B = bucindolol; βB = beta-blocker; CG = candidate gene study; CGS = candidate gene substudy; CI = confidence interval; DBP = diastolic blood pressure; DITdP = drug-induced Torsades de Pointes; GRS = genetic risk score; GWAS = genome-wide association study; HR = hazard ratio; HT = heart transplantation; MA = meta-analysis; MACE = major adverse cardiovascular events; OR = odds ratio; P = placebo; PCI = percutaneous coronary intervention; RCT = randomised controlled trial; RFA = reduction-of-function allele; SBP = systolic blood pressure; VT/VF = ventricular tachycardia/ventricular fibrillation

[i] = maximum study size - individual genotype analyses within a study include fewer participants; [ii] = p-values as reported in study; [iii] = only the three top genome-wide significant findings from the primary meta-analysis are included; [iv] = only the three top genome-wide significant findings from the study's primary meta-analysis, which are also previously published loci associated with coronary disease, are included; [v] = a genetic risk score developed from the weighted influence of 32 single nucleotide polymorphisms previously associated with SBP or DBP in GWAS; [vi] = OR for hypertension at baseline for a single unit increase in GRS; [vii] = rs1719247 is in linkage disequilibrium with rs9806699 ($r^2 = 0.76$); [viii] = overall risk estimate of drug versus placebo independent of genotype, provided when available to aid interpretation of corresponding statistically significant *within* genotype analyses; [ix] = provided to aid interpretation of the selective beneficial effect of β-blocker therapy shown for African American patients with Gln41Gl

Figure legends

Figure 1. Examples of evidential, logistical, financial and knowledge barriers to the successful implementation of pharmacogenomics in clinical practice to benefit patient care. Abbreviations: CDS = clinical decision support; EMR = electronic medical record

Figure 2. Warfarin is a racemic mixture of two enantiomers which inhibit vitamin K epoxide reductase complex subunit 1 (VKORC1). This antagonises the regeneration of reduced vitamin K, which is the essential cofactor for γ-glutamyl carboxylase (GGCX). This decreases post-translational activating γ-carboxylation of glutamate residues in clotting factors II, VII, IX and X. The more potent S-warfarin stereoisomer is inactivated by CYP2C9, whilst R-warfarin is metabolized to inactive alcohols by CYP1A1, CYP1A2 and CYP3A4. CYP4F2 depletes reduced vitamin K from the vitamin K cycle.[33]

Figure 1

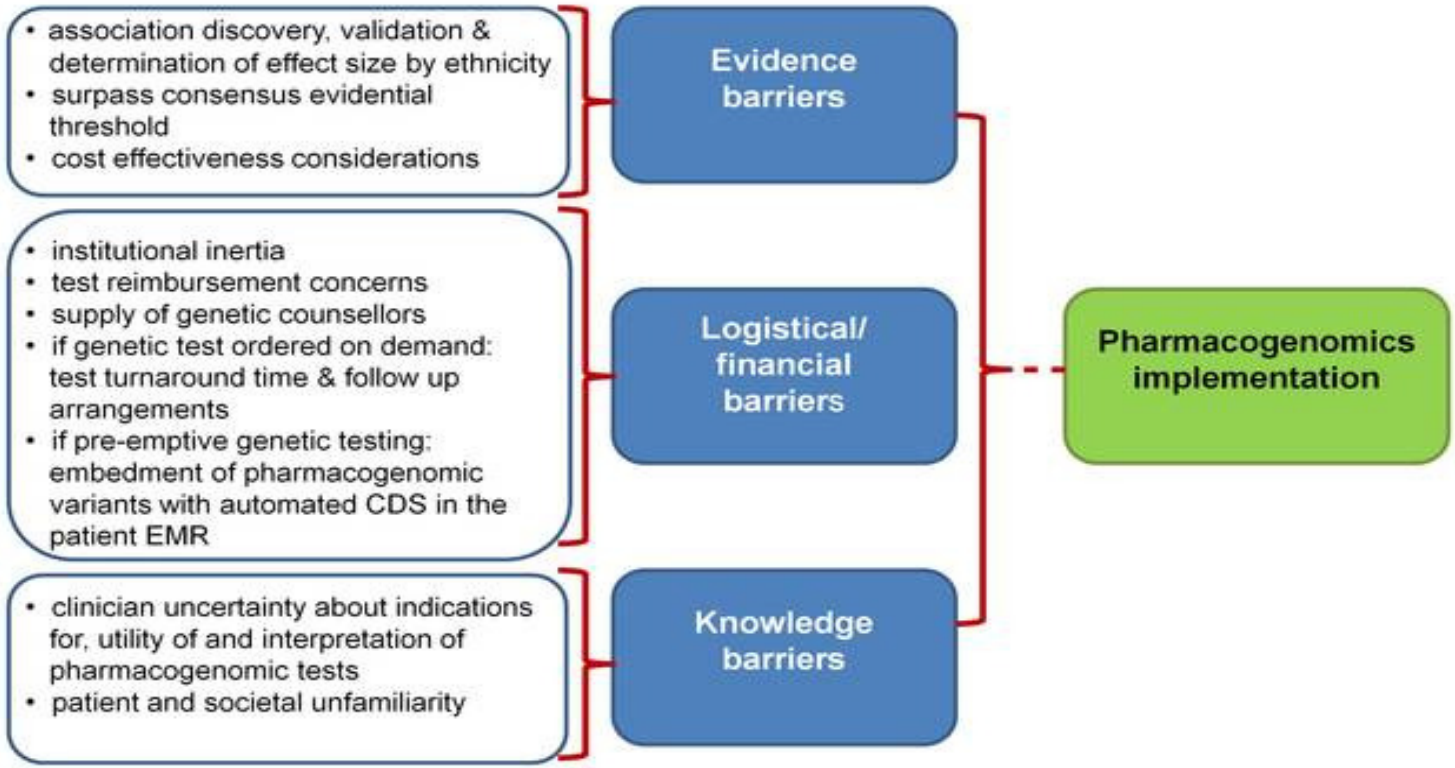

Figure 2