Genomic prediction of individual drug response

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Abstract

A novel medical approach, personalized medicine, seeks to use genetic information to "personalize" and improve diagnosis, prevention and therapy. The personalized management of cardiovascular disease involves a large spectrum of potential applications, from diagnostics of monogenic disorders, to prevention and management strategies based on modifier genes, to *pharmacogenomics*. Several lines of evidence suggest that common polymorphic variants of modifier genes can influence the response to drug response in cardiovascular disease. Using pharmacogenomics approaches to affect management of heart failure, arrhythmias, dyslipidemia and hypertension, warfarin anticoagulation and antiplatelet therapy appears very promising. In heart failure, common genetic variants of beta-adrenergic receptors, alpha-adrenergic receptors and endothelin receptors among others significantly alter the response to heart failure therapy. This knowledge could be used to personalize and optimize cardiovascular therapy based on the patient's genetic profile.

While the advances in technologies will continue to transition personalized medicine from the research to the clinical setting, physicians and in particular cardiologists need to reshape clinical diagnostics paradigms, learn how to use new genomic information to change management decisions, and provide the patients with appropriate education and management recommendations.

Keywords: pharmacogenomics; genetic variation; cardiovascular genetics; drug response; heart failure.

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Introduction

The International Human Genome Project was completed in 2003 after 13 years of extensive work by a network of laboratories and an approximately $3 billion investment [1]. The sequence of the 3 billion base pairs of the human genome became publicly available: this and the extraordinary change in technology have changed the way we perceive medicine.

Today, with the novel sequencing technologies (next-generation sequencing), the cost of sequencing a human genome is less than $5,000 and can be completed in about a week. Furthermore, it is also possible to sequence only the coding portion of the genome, the *exome*, for approximately $1,000. It is expected that the combination of improved technology and improved computational methods to handle the huge amount of data generated by the new sequencing platforms will rapidly push the cost of genome sequencing below the $1,000/genome as projected by the National Human Genome Research Institute (The Road

to the $1000 Genome — A Roundup of Sequencing Technology Developments, http://www.genome.gov). Meanwhile, taking advantage of the next-generation sequencing technologies, the "1000 Genomes Project" is currently sequencing the genomes of a 2,500 people of various ethnic origin, to provide a comprehensive resource on human genetic "common" variation [2].

The advances in genomics and high throughput technologies will soon have a profound impact in the management of cardiovascular medicine [3, 4]. *Genomics*, the science studying the genes of a genome and how they interact with each other, is at the foundation of *personalized medicine*, a form of medicine that uses the patient's genomic information to improve diagnosis, prevention and therapy. Out of 3 billion base pair in the human genome, there are likely over 10 million common genetic variations that occur every 100-300 base pair of genomic sequence, and there are at least 100 new polymorphic variations per person [5]. Common variations of the nucleotide sequence are called genetic *polymorphisms*. Many of these polymorphisms are frequent in the general population and have no clinical effect. However, some genetic variation in genes functionally important account for variations in susceptibility to diseases and different response to drugs (*pharmacogenomics*) observed in population studies and even within families. Genes that modify the individual response to disease or therapy are called *modifier genes*.

Pharmacogenomics

More than 100,000 deaths each year are due to adverse drug reactions, and pharmacogenomics may contribute in reducing this number by better tailoring the therapy on the genetic profile of each individual patient [6]. Pharmacogenomics is defined as the study of genes that influence the response to drugs, and has the purpose of maximizing the benefits and minimizing the side effects of therapies based on the individual's genetic profile.

Genes associated with different drug response have been identified using two approaches. The first one is the *candidate gene* approach based on the identification of "candidate variants" in pharmacokinetic pathways. The second and most recent approach is based on genome-wide association studies (*GWAS*). The Catalog of Genome-Wide Associations studies of the National Human Genome Research Institute (www.genome.gov/gwastudies) currently lists 1305 published GWAS at $P \leq 5 \times 10^{-8}$ for 210 traits (accessed 2/2012). In GWAS, the approach is based on the genome-wide screening of hundreds of thousands of selected polymorphic variations (SNPs) rather than candidate genes. Once an association is discovered between a polymorphism and the disease, the modifier gene in proximity to the SNP is identified. This novel approach, developed thanks to the technological advances in high throughput sequencing methods and bioinformatics approaches, is based on the screening of large populations of patients and controls, and has already been successfully utilized in cardiovascular medicine in complex common disorders such as hypertension and coronary artery disease. GWAS studies are demanding in terms of cost, technology and size of the study population, but they are important since it is expected that they will provide innovative personalized genomic information about risk of disease and generate novel therapeutic targets.

Pharmacogenomics of heart failure: a paradigm

Heart failure (HF) is one of the most serious and expensive conditions in health care worldwide due to its high prevalence (1–1.5% of adult population) and high morbidity (frequent hospitalizations). In the United States, HF affects approximately 4 million people and causes about 200,000 deaths per year; and it has a generally rapid course with a mean survival of only 1.7 years for males and 3.2 for females after diagnosis [7]. In Europe, data are substantially similar and together it suggests that in spite of the improvement of HF therapy, disease progression has not changed and HF still remains one of the most important health problems in the world.

HF is a syndrome characterized by primary pathophysiological processes, which interact with a wide number of complex secondary interrelated pathophysiological mechanisms: rare mutations in single Mendelian genes, common genetic variations (polymorphisms) in *modifier* genes, which can modify the natural history of the disease, such as genes of the renin-angiotensin-aldosterone (RAAS) system and adrenergic system, genetic polymorphisms that can modify the response to therapy (*pharmacogenomics*), gene-gene interactions, such as β_1 and α_2 adrenergic

receptors, and environmental factors, such as ischemic heart disease, viral infections, hypertension, infiltrative diseases, toxins, diabetes [8].

Several studies have provided evidence of the existence of modifier genes in HF that can modify the severity and progression of the disease [9–15]. A more comprehensive answer toward the question of identification of modifier genes in HF is expected to come from GWAS, which are currently ongoing, including the Framingham study. Two recent papers have reported a large metanalysis of the risk of heart failure and mortality in the CHARGE Consortium [16, 17]. These studies, which included over 20,000 subjects of various ethnicities, found 2 loci (*USP3* and *LRIG3)* associated with risk of developing HF and one locus (*CMTM7)* associated with the risk of HF mortality. To have clinical impact, these studies should be replicated in independent prospective studies, and the functional significance of the modifier genes identified elucidated.

In HF, pharmacogenomics has already shown a promising role. Indeed, in spite of the improvement in the natural history of HF thanks to the therapeutic advancement in the last 20 years and the development of practice guidelines, large trials such as BEST (Beta Blocker Evaluation Survival) and AHeFT (African American Heart Failure Trial) have suggested that some patients have a different response to treatment (*responders* versus *nonresponders*) due to underlying genetic differences [18]. The most important genetic variations associated with a different pharmacological response are listed in Table 1.

In the BEST trial, the anticipated effect of bucindolol, a β-blocker/sympatholytic agent, on patients with HF in class 3 and 4 was disappointing and did not reach statistical significance. However, when the investigators analyzed the response to treatment based on the β_1 adrenergic receptor genotype, they found a strong association with the Arg389Gly polymorphism. Arg389Arg homozygous carriers responded significantly better than Gly389 carriers to the treatment with a 38% reduction in mortality. The Arg389Arg carriers' response was even better than the response previously reported for carvedilol [11]. The different behavior of the two allelic variants is explained by the fact that the Arg389 variant is more responsive to the agonist stimulation (isoproterenol) than the Gly389 allele [11], a behavior confirmed by other studies involving different β-blockers including metoprolol and carvedilol [18].

More recently, the BEST investigators reported the results of a substudy on the pharmacogenetic effect of the α_{2C}-adrenergic receptor, whose role is to inhibit norepinephrine release in the prejunctional adrenergic nerve terminals. The polymorphism α_{2C} Del322-325 had previously been associated with a worse prognosis in HF, with evidence for a synergistic effect with the β_1 Arg389 allele in Black patients [13, 19]. In the current study, Bristow et al. showed that the norepinephrine-lowering and clinical therapeutic responses to bucindolol were strongly influenced by the α_{2C} receptor genotype: α_{2C} Del322-325 carriers had an excessive sympatholytic effect and had no evidence of any therapeutic benefit from bucindolol, whereas wild-type α_{2C} carriers had a 30% reduction in mortality [20].

Other polymorphisms, such as the β_1 extracellular adrenergic receptor-Ser49Gly and polymorphisms of β_2 adrenergic receptor, can modify HF (Table 1) [18]. In the RAAS system, patients with the ACE *DD* genotype had a worse prognosis but at the same time were the best responders to β blocker therapy compared to the other genotypes (*II* and *ID*) [21]. It is interesting to note that the evidence of a genetically driven response to therapy in HF dates back to the AHeFT study. In this trial, the investigators found that African-American patients had a much better response to the therapy with hydralazine and isosorbide dinitrate compared to Caucasian patients. Indeed, this is the first FDA approved therapy for HF based on racial differences and therefore on genetic background [22]. Preliminary data of the GRAHF substudy (Genetic Risk Assessment of Heart Failure in AHeFT) suggests that at least one of the genetic causes lies in the -344C/T polymorphism located in the promoter of the aldosterone synthase gene, and that this is associated with a worse prognosis but with a better response to the hydralazine/isosorbide dinitrate therapy in carriers [23]. The same polymorphisms had previously been associated with higher enzymatic activity, hypertension and myocardial remodeling [18].

The studies on β_1 – α_{2C} receptors indicate the existence of complex gene-gene interactions in the genetic determinants of HF. The gene-gene interaction and the functional effect in the case of the adrenergic receptors are of particular interest. The β_1 Arg389 receptor is more responsive to the adrenergic stimulation:

DISEASE	GENE	POLYMORPHISM	FUNCTION	THERAPEUTIC IMPLICATIONS
Heart Failure				
RAAS	ACE	D/I	ACE D: higher ACE activity and A II levels	ACE inhibitors Beta-blockers
	Aldosterone synthase	Promoter -344 T/C	-344 C: increased transcriptional activity and aldosterone production	ACE inhibitors Aldosterone receptor antagonists
β-adrenergic receptors	β1-adrenergic receptors	Arg389Gly	Arg: increased adrenergic signal	Beta-blockers ACE inhibitors
		Gly49Ser	Gly: enhanced down regulation	Beta-blockers
	β2-adrenergic receptors	Gly16Arg Gln27Gly	Receptor down regulation	Beta-blockers
α-adrenergic signaling	α-2C receptor	α-2C Deletion	Deletion: decreased uptake of norepinephrine	Beta-blockers
	G protein β3 subunit	C825T	C825T: increased α-adrenergic signaling, lower plasma renin	ACE inhibitors
Nitric oxide	*NOS3*	Asp298Glu	Asp: associated with lower NOS3 activity	ACE inhibitors
Endothelin system	*EDN1*	IVS-4 G/A Lys198Asn	Unknown	Beta-blockers
Atherosclerosis/lipid disorder				
LDL uptake	*LDLR*	L5	LDL receptor	HMG-CoA reductase (statins)
HMG-CoA reductase	*HMGCR*	H7	Cholesterol biosynthesis: attenuate sensitivity to statins	HMG-CoA reductase (statins)
Cytochrome P450	*CYP3A4*	*4	Increases metabolism of statins, increases effect	atorvastatin, simvastatin, lovastatin
		*1G	Decreases effect	
Myotoxicity	*SLCO1B1*	Val174Ala	Increased risk	simvastatin
Arrhythmia				
QT interval	*SCN5A, KCNH2, KCNQ1, KCNJ2, KCNE1*		Ion channel function	QT-prolonging antiarrhythmic drugs, antibiotics, antipsychotics
	NOS1AP		NO synthase pathway	QT prolonging agents
Anticoagulant therapy				
Cytochrome P450	*CYP2C9*	*2 and *3	Clearance of warfarin, risk of bleeding	Warfarin
Vitamin K oxidase	*VKORC1*	1173C/T	Reduced metabolism of vit. K, higher warfarin dose requirement	Warfarin
Antiplatelet Agents				
Cytochrome P450	*CYP2C19*	*2	Decreased conversion of active metabolite, loss-of-function	Clopidogrel

Table 1 *Pharmacogenomics of cardiovascular disease.* ACE *angiotensin-converting enzyme,* RAAS *renin angiotensin aldosterone system,* NOS3 *Endothelial nitric oxide synthase,* EDN1 *endothelin 1,* A II *angiotensin II,* D *deletion,* I *insertion. From McNamara et al., 2008 [18], and Roden et al., 2011 [4].*

patients homozygous for the Arg389 allele carrying also the α_{2C} Del322-325 receptor characterized by decreased uptake of norepinephrine seem to have an enhanced adrenergic response, worst prognosis but the greatest improvement in ejection fraction with β blocker therapy [13, 19].

Finally, we have studied the association of polymorphisms of the endothelin system with HF in the BEST cohort [15]. Two genetic variations (IVS-4 G/A and Lys198Asn) on a common haplotype in the endothelin-1 gene were associated with differential response to bucindolol in terms of a combined endpoint of HF hospitalization and all cause death (Fig. 1). The effect of the endothelin-1 haplotype was only evident in the treatment group, supporting a pharmacogenetic interaction between bucindolol and the haplotype. Ultimately, these types of data could be used to tailor beta-blocker therapy for individuals based on their underlying endothelin-1 haplotype [15].

Pharmacogenomics of lipid disorders

Another important area of research is the pharmacogenomics of HMG-CoA reductase inhibitors (statins). Both *in vitro* and *in vivo* (human) studies have shown

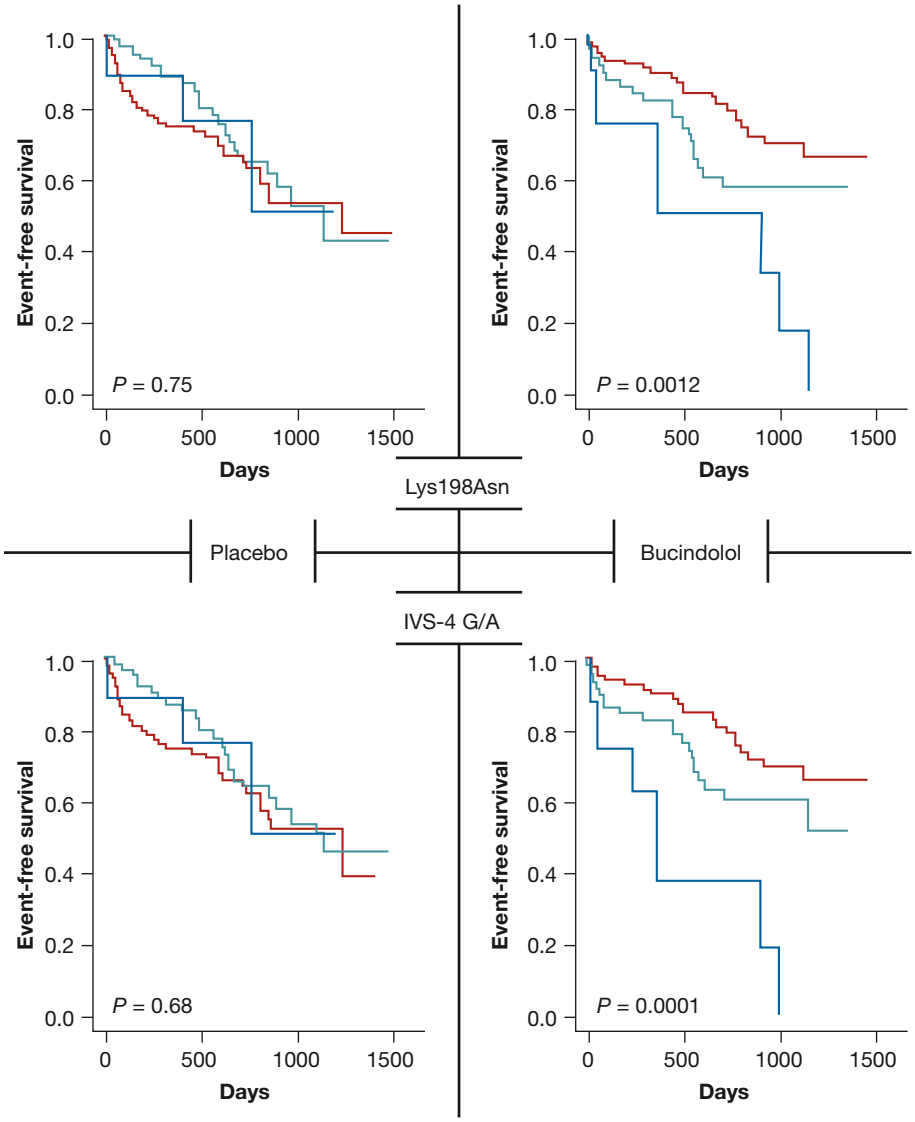

Fig. 1 *Time to the combined event of first heart failure hospitalization or death for endothelin-1 polymorphisms Lys198Asn (top) and IVS-4 G/A by genotype. Common homozygotes (G/G-Lys198; and IVS-4 G/G, respectively), heterozygotes (G/T-Lys198Asn; IVS-4 G/A), and rare homozygotes (T/T-Asn198; IVS-4 A/A) are depicted by the red, turquoise and dark blue lines respectively. The data are separated by treatment group, with placebo treated and bucindolol treated subjects on the left and right, respectively. Reproduced with permission from Taylor et al. [15]*

that the HMGCR H7 polymorphism and the LDLR L5 polymorphism are associated with a lower efficacy of the statin therapy in a number of ethnically different populations [4]. Similarly, various polymorphisms of the *CYP3A4* have been associated with either lower or higher LDL level with statins (Table 1). Also statin-related myotoxicity has been associated with genetic variations in modifier genes, CYP3A5 and SLCO1B1, initially identified as candidate genes and then, in the case of SLCO1B1, replicated also in GWA studies [4]. Current studies are evaluating the clinical impact of genetic variations in pharmacogenomics of lipid therapy and risk of cardiovascular events.

Pharmacogenomics of arrhythmias

Several drugs can induce arrhythmias by causing a prolongation of the QT interval (drug-induced long QT syndrome): these include antiarrhythmic drugs (sotalol, dofetilide, quinidine), anti-psychotics, antibiotics, and methadone. Subclinical LQT syndrome appears to be the cause of a large proportion of these cases and seems to be associated with genetic variations of LQT genes [4]. Other modifier genes have been found in association with LQT by GWAS approach, such as NOS1AP, implicated in the nitric oxide synthase pathway, although the modifier mechanism is unknown. The identification of patients at risk of potentially life-threatening arrhythmia induced by drugs has significant clinical impact.

Pharmacogenomics of warfarin anticoagulation

A major field of interest has been the study of pharmacogenomics of warfarin therapy. The genes that appear to play the most important role are CYP2C9 and VKORK1. CYP2C9*2 and CYP2C9*3 are associated with lower warfarin dose requirement and increased risk of bleeding. Despite of the several studies on warfarin pharmacogenomics, the clinical utility of a pharmacogenomics approach over anticoagulation control is not established [24]. Large ongoing randomized trials are currently addressing this question.

Pharmacogenomics of antiplatelet therapy

Another field of interest in cardiovascular pharmacogenomics is the dose-response of antiplatelet agents. Clopidogrel shows a wide range of dose-responses, and this variability has been associated with the loss-of-function CYP2C19*2 polymorphism, which causes a lower conversion of clopidogrel (prodrug) into hits active metabolite [4]. Carries have an increased risk of cardiovascular events and in-stent restenosis. The Food and Drug Administration has recently approved a warning alerting about these pharmacogenetic findings and the opportunity of alternative therapies in CYP2C19*2 carriers.

Conclusions

A new era in which personalized medicine will enter in our clinical practice and the $1,000 genome will be available is just around the corner [25]. Tailoring therapy based on pharmacogenomics tests may save lives and improve patients' care. Advances in technologies continue to transition from the research to the clinical setting, reshaping clinical diagnostic paradigms and challenging the healthcare team to consider how new genomic information may be leveraged to influence management decisions and to approach the promise of personalizing medical care. The problem is then for the physician and in particular the cardiologist, to understand and manage this new genomic information and provide the patient with appropriate education and management recommendations. Finally, the possibility to study large cohorts of patients with cardiovascular disease will provide fundamental data for the profiling of risk factors and the optimization of the therapy. •

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