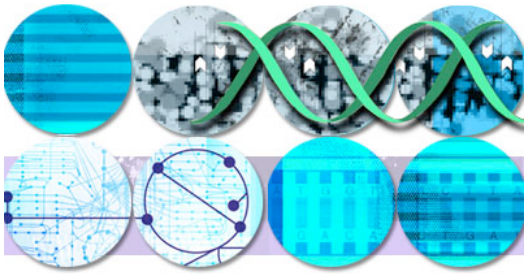
Final Assignment:

Cardiovascular Pharmacogenomics in Adults and Children with Heart Failure: The Future of Precision Cardiovascular Medicine



BIOMEDIN 258:
Genomics, Bioinformatics, and Medicine

Professor:
Doug Brutlag, PhD

Student:
Anthony C. Chang, MD, MBA, MPH
AChang007@aol.com

It is a capital mistake to theorize before one has data.

- Sherlock Holmes, *A Study in Scarlett* (by Arthur Conan Doyle)

Introduction

Cardiovascular drugs are usually prescribed at standard doses despite highly variable individual responses to these medications, ranging from no or subtherapeutic efficacy to a serious adverse drug reaction (ADR) ([1]). In addition, the conventional "mg/kg" therapeutic strategy is particularly inadequate and challenging in pediatric cardiac patients with their wide range of body sizes and differing degrees of organ maturation ([2])([3]). The combined undertreatment of medical conditions and ADR from medications result in economic costs that are estimated to be well over $100 billion per year ([4]). With the advent of **next-generation sequencing**, also termed high-throughput sequencing, this transformative escalation of sequencing capacity has rendered genetic analysis both rapid and inexpensive and launched numerous areas of genetic investigations on over 3 million **single nucleotide polymorphsims**, or SNPs, in human genome ([5])([6]).

Pharmacogenomics and Pharmacogenetics

Genetic factors are now known to be involved in the wide variation of individual patients' responses to medications with over 2,000 genes involved in response to medications ([7])(see Figure 1). **Phamacogenomics** is the "study of the role of inherited and acquired genetic variation in drug response" of all genes collectively with use of high-throughput data ([8]) whereas **pharmacogenetics**, the narrower term, refers to the study of genetic influences on one individual's response to drugs of a specific gene or group of genes ([9])(see Figure 2). Of the two related

terms, pharmacogenomics has become the preferred term as it is now clear that many genes interact with each other in gene-drug interactions.

Nebert et al elegantly classified pharmacogenomics into two main groups of genes that can have influence over variation in drug responses: differences in genetic variants associated with **pharmacokinetics** (such as drug transporters) and variations in phenotypic expressions of **pharmacodynamics** (such as drug targets like ion channels or receptors) ([10]).

In addition, the three current methods of pharmacogenomic analysis are: 1) **genetic association by candidate gene polymorphism**- a relatively oversimplified strategy to relate individual gene variants (SNPs, which usually occur in ≥ 1% of the population as opposed to mutations, which affect < 1% of the population) to drug response; 2) **haplotype mapping**- a haplotype refers to the inheritance of a cluster of SNPs inherited together so these genes are studied for variations in drug response; and 3) **systemic genome-wide association studies (GWAS)**- involves a comprehensive investigation of all the possible candidate genes in the genome that result in a drug response (see Table 1). While most of the pharmacogenomic studies to date have focused on candidate gene variants that encode proteins that are involved with absorption, distribution, metabolism, and excretion of these medications, GWAS are now being utilized to facilitate finding multi-gene pharmacogenomic associations.

New pharmacogenetic discoveries about how cardiac medications such as warfarin, clopidogrel, and statins are influenced by genetic variants

are modifying dose strategies and leading the FDA to modifying drug labels ([11]). For example, genetic variations in the well-studied *CYP2C9* and *VKORC1* genes account for up to 63% of variability in warfarin dosing in adults that result in reduced dose requirement ([12]). In addition, pharmacogenetic information is also applied to identification of nonresponders to clopidogrel and toxicity of statin medications as well as labeling of medications ([13])([14]).

More recently, a few pharmacogenomic investigations have also focused on adult patients with heart failure ([15])([16])([17])([18])([19])([20]). Examples of genes that have influence over drug response include diuretics, digoxin, angiotensin-converting enzyme, β1-adrenergic receptor, and even endothelial nitric oxide synthase (eNOS)([21]).

There are growing numbers of pediatric pharmacogenomic investigations on attention deficit disorder and asthma medications as well as chemotherapy agents, but there remains a paucity of investigations in cardiovascular pharmacogenomics in children with the exception of the drug warfarin ([22])([23]). In the pharmacogenomic study of warfarin in children by Nowak-Gottl, age was the more important factor determining dose rather than the *VKORC1* and *CYP2C9* genotype variants that were influential in adults on warfarin. In addition, while the enzyme cytochrome P450 *CYP3A7* is highly expressed early in life, another enzyme *CYP3A4* is more active later in life ([24]). Pharmacogenomic studies in children can be challenging due to relatively smaller study populations and difficulties with replication studies. In children, age-related changes in body sizes and pharmacokinetic profiles add additional challenges to study genetic effects on medications.

Pharmacogenomics in Adults with Heart Failure

Both angiotensin-converting enzyme inhibitors (ACEis) and β-adrenergic receptor blockers (β-blockers) have been the mainstay of therapy for heart failure in adults. Both clinical efficacy and adverse effects of these medications, however, vary significantly from patient to patient. The first report of a pharmacogenetic effect of a heart failure medication on clinical efficacy was in 1998 with a report on an ACEi ([25]). Since then, pharmacogenetic associations for heart failure medications have been found to be race, dose, sex, and drug-specific and are known to be highly complex.

β-Adrenergic Receptor Blocker Therapy. While some β-blockers are selective β_1-blockers (eg, atenolol and metoprolol), others act all all three adrenergic receptors (eg, carvedilol and propranolol). Since 2000, there have been over 30 reports on β-blocker pharmacogenomics; most of these studies tested genetic variants related to the sympathetic adrenergic system, specifically the **β-adrenergic receptor**.

The gene for **β_1-adrenergic receptor *(ADRB1)***, consisting of 1,714 base pairs, has two variants: Ser49Gly (rs1801252) and Ser389Gly (rs1801253), both with amino acid substitutions of glycine for serine but at positions 49 and 389, respectively ("rs number" is the reference single nucleotide polymorphism, or SNP identification number assigned by the National Center for Biotechnology Information, or NCBI). These two variants result in improved response to β-blocker therapy; there appears to be a dose relationship to the genetic influence with medications, however, so that the genetic effects are attenuated at

higher doses ([26]). A haplotype combining Gly16/Gln27 (Gly16Arg (rs1042713) and Gln27Glu (rs1042714)) in the gene for the **β_2-adrenergic receptor *(ADRB2)*** also was discovered to have increased morbidity and mortality risks ([27])(see Figure 3).

Other promising candidate genes for pharmacogenetic effects of β-blocker therapy for heart failure include 1) the **□-2C adrenergic receptor *(ADRA2C)*** insertion/deletion of amino acids 322-325 (rs61767072) that result in improved response to metoprolol in deletion carriers (when combined with ADRB1 Arg/Arg genotpe) ([28]) and 2) **G-Protein-Coupled Receptor Kinase 5 *(GRK5)*** Gln41Leu (rs17098707) with reduced mortality in Leu41 carriers treated with propranolol in African Americans ([29]). The variants for the β-blocker metabolizing enzyme ***CYP2D6*** did not demonstrate any association with therapeutic outcomes. These data suggest that pharmacogenomic knowledge in heart failure patients can be useful for both clinical efficacy as well as deleterious effects of β-blockers.

Angiotensin-converting Enzyme Inhibitor (ACEi) Therapy. Even though the first report of pharmacogenomic effects of heart failure medications was on ACEis, the pharmacogenomic literature for ACEis is not as robust as that of β-blockers and has not demonstrated any clear evidence for genetic variation in ACEi response.

The genes of the renin-angiotensin-aldosterone system (RAAS) have been the focus of the pharmacogenetic studies on ACEis in adults. The gene for **angiotensin I-converting enzyme (*ACE*)** has a variant that is an insertion/deletion (I/D) polymorphism in intron 16 that involves the 287 bp Alu repeating sequence (rs4646994) ([30]); this variant has been discovered to have increased plasma and tissue levels of ACE but

clinical implications of this finding is controversial ([31]). Other candidate genes reported in the literature on ACEi include: 1) **angiotensinogen gene (*AGT*)** and its variant Met235Thr (rs699) ([32]); 2) gene for **angiotensin II type-1 receptor (*AGTR1*)** and its variant 573C>T (rs5182) ([33]); and 3) **bradykinin type-1 receptor gene (*BDKRB1*)** variant A>G (rs12050217) ([34]).

The role of the RAAS gene variants (*ACE I/D*, *AGT* (Thr174Met), *AGTR1* (1166A>C), and others) in **angiotensin receptor blocker** therapy remains undefined ([35]).

Pharmacogenetic variability is thought to be an underlying reason for some of these observed clinical differences amongst individual patients to heart failure medications, but pharmacogenetic associations have often failed to be replicated in cohort populations in order for these associations to be validated (see Figure 4). The reasons for these failed studies also include 1) lack of statistical power due to low event rates; 2) low sample sizes/ short follow-up times; and 3) publication and sampling biases. In addition, as most patients are now on dual or triple medical therapy as well as assist devices, pharmacogenomic implications of multiple medical and surgical regimens have become at best difficult if not impossible to determine.

Pharmacogenomics in Children with Heart Failure

There are presently no studies on pharmacogenomic effects of β-blocker or ACEi in children with dilated cardiomyopathy and heart failure, and the aforementioned adult β-blocker pharmacogenomic data on *ADRB1* Ser49Gly, *ADRB2*, and *ADRA2C* polymorphisms have only limited application to children. Two areas of investigation of interest, however, are summarized below in the area of pharmacogenomics in children with heart failure.

Congenital Heart Disease and Heart Failure. The sole pediatric pharmacogenomic study of heart failure medications in children was a study of 154 single ventricle infants who were enrolled in a randomized, double-blind, placebo-controlled trial by the Pediatric Heart Network to determine the somatic growth effects with or without enalapril ([36]). These infants were genotyped for polymorphisms in five genes of the RAAS system: *AGT*, *ACE*, *AGTR1,* aldosterone synthase (*CYP11B2*), and chymase (*CMA1*). The upregulation of the RAAS genotype, in this study, are associated with unfavorable remodeling (persistent elevation in ventricular mass and volume) as well as growth impairment after volume reduction surgery (see Figure 5).

The findings, therefore, suggest that there may be clinical implications for genetic risk stratification and pharmacotherapy in this cohort of single ventricle infants to minimize maladaptive ventricular remodeling. This investigation also highlights the significance of the compound effects of multiple single nucleotide polymorphisms (SNPs) in a biochemical pathway rather than separate analyses of SNPs in a single gene. A major limitation of this study,

however, is that the pharmacogenomic association between enalapril and the outcomes was not replicated in a separate cohort, a necessary condition for the pharmacogenomic association to be valid ([37])(see Table 2).

Anthracycline-induced Cardiomyopathy (ACT). Anthracyclines used in children with cancer has lead to progressive left ventricular dysfunction in 6-16% of children with higher risk on protocols with cumulative doses of > 300 mg/m^2 ([38]). The mechanism of cardiotoxicity seems to be related to reactive oxygen species, formed during the reduction of anthracyclines, and these oxygen radicals then irreversibly damaging DNA in cardiomyocytes as well as inducing apoptosis ([39]). In addition to higher cumulative doses of anthracyclines, clinical risk factors include concomitant cardiac radiation, shorter infusion time, younger age, longer time since treatment, and female sex ([40])([41]).

An important application of pharmacogenetics is to see which genetic factors are involved in ACT in children as ACT has been observed even at relatively low doses of anthracyclines. Interestingly, three genes that encode **NAD(P)H oxidase** (*NCF4*, *RAC2*, and *CYBA*) as well as two genes that encode **anthracycline transporters** (*ABCC1* and *ABCC2*) are found to have significant association with cardiotoxicity by SNP analysis ([42]).

Increased risk for late cardiotoxicity was also observed in C/C homozygotes for the **catalase gene** *CAT* c.66+78C>T variant ([43]). In addition, the anthracycline metabolizing **carbonyl reductase gene** *CBR3* and its variant Val244Met was associated with increased risk for late cardiotoxicity, even at doses < 250 mg/m^2 ([44]). Very recently,

Visscher et al discovered the several gene variants that can be predictive of ACT in children and were subsequently confirmed with replication cohorts: rs17863783 in *UGT1A6* and also rs7853758 and rs885004 in *SLC28A3* genes as well as several **adenosine triphosphate-binding cassette transporters** (*ABCB1*, *ABCB4*, and *ABCC1*) [45]. The addition of some of these genetic variants to clinical risk factors in a prediction model improved the identification of high- and low-risk pediatric patients: 75% of the children in the high-risk group were accurately predicted to develop ACT and 96% of children were accurately predicted not to develop ACT in the low-risk group [46](see Figure 6).

Precision Medicine in Children with Heart Failure

Personalized medicine refers to the "coupling established clinical-pathological indexes with state-of-the-art molecular profiling to create diagnostic, prognostic, and therapeutic strategies precisely tailored to each patient's requirements" [47]. For some, **precision medicine** is preferable to personalized medicine as precision medicine calls for disease treatment that integrates genomic knowledge and other molecular research with input from patients' medical records as well as social and environmental data from the population as a whole (whereas personalized medicine has a connotation of individual anecdotes).
As with warfarin, studies will need to compare pharmacogenomics-guided vs conventional heart failure therapy in both adults and children to see if there is a reduced incidence of morbidity and mortality and a favorable cost-effectiveness profile [48][49]. Higher doses of certain medications as we have observed in anthracyclines are not genetically relevant but lower doses of these same mediations can be affected by genetic variables.

This new brand of precision medicine will require strategic management of massive volumes of pharmacogenomic data in the form of bioinformatics as well as attention to socioeconomic and ethical considerations. Future pharmacogenomic data will need to incorporate variants in multiple genes, on-chip investigations of cell-drug interactions ([50]), and novel molecular biomarkers like ST2 and microRNA ([51]) as well as clinical information to maximize efficacy while minimizing risk to children with heart failure. The convergence of genomic information, electronic records, and computer capablity will demand a higher level of artificial intelligence in biomedical sciences in order to interpret the data and to improve pharmacogenomic knowledge and research ([52]).

Conclusion

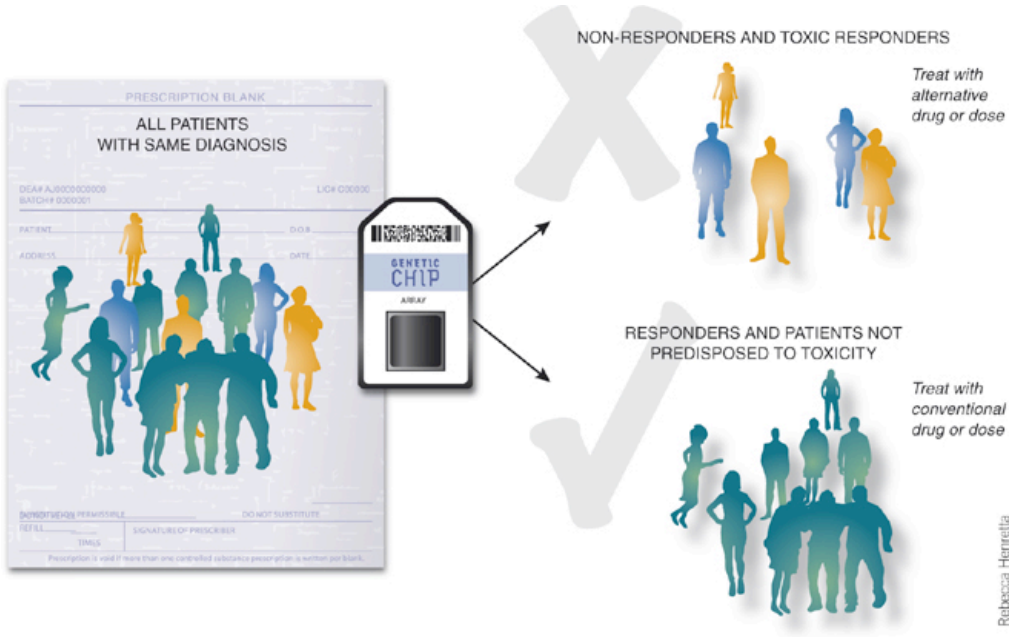
The advent of modern pharmacogenomics as a result of rapid-throughput DNA sequencing and GWA studies has changed the therapeutic dosing strategies with cardiovascular medications in adults such as warfarin, clopidogrel, and statins. Although there is preliminary evidence for few genetic variants that may influence medications presently used for heart failure, even β-blockers have yet to show clear benefit of modifying therapy due to pharmacogenomic association.

There is presently a paucity of literature on the pharmacogenomic aspects of cardiac medications for heart failure in children as these investigations in this population are even more difficult than adults. Adult cardiovascular pharmacogenomic data often do not have direct aplicability to children. In addition, heart failure outcomes are not always objective and therefore determination of influence of genetic variables is rendered more daunting. Number of pediatric patients with heart failure are often small so large clinical trials with different medications are difficult to organize.

There needs to be a clarion call for physicians to be educated in the revolutionary advances in pharmacogenomics and its impact ([53]). Each child with heart failure will have medications specifically tailored to the genetic, molecular, and clinical information and precisely formulated to maximize efficacy and minimize harm. A new multidisciplinary team including pediatric cardiologists, pharmacogenomic expertise from geneticists/genetic counselors as well as pharmacists, bioinformaticists/ data scientists, and even experts in proteomics, metabolomics, and transcriptomics will all be needed to

formulate ideal precision medicine for each child with heart failure and to to set forth transformative strategies for acute and chronic cardiac medications as well as socioeconomic guidelines, policy recommendations, and advanced training ([54])([55]).

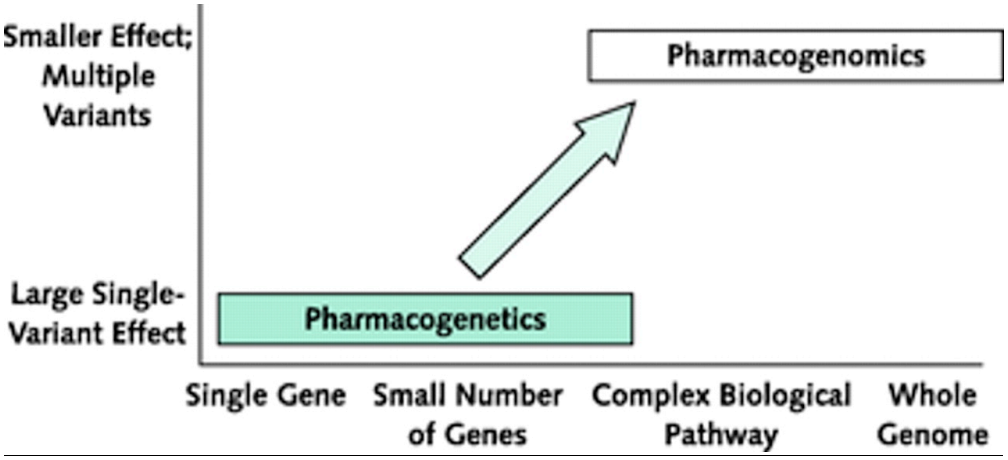
Figure 1. Pharmacogenomics Approach.



Pharmacogenomic approach in the future will involve individualizing precision therapy based on the genetic profile of each patient so that only **responders** and patients not predisposed to toxicity are given the conventional drug or dose. The **non-responders** and toxic responders will be treated with alternative drug or dose in order to maximize benefit and minimize risk.

(Piquette-Miller M et al. The Art and Science of Personalized Medicine. *Clin Pharmacol and Therapeutics* 2007; 81: 311-315.)

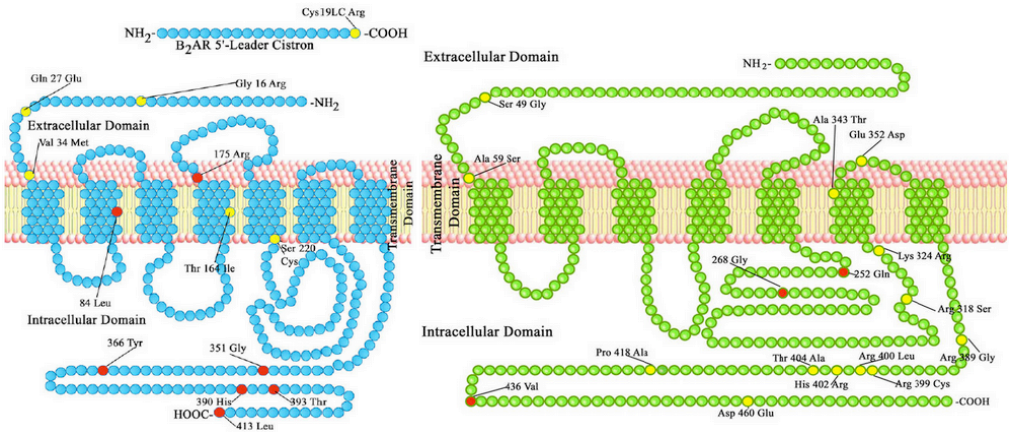
Figure 2. Pharmacogenomics vs Pharmacogenetics.



Pharmacogenomics refers to genomic and complex biological pathway effects on variability to medications with multiple variants whereas **pharmacogenetics** involves smaller number of genes or a single gene.

(Roden DM et al. Pharmacogenomics: Challenges and Opportunities. *Ann Intern Med* 2006; 145: 749-757.)

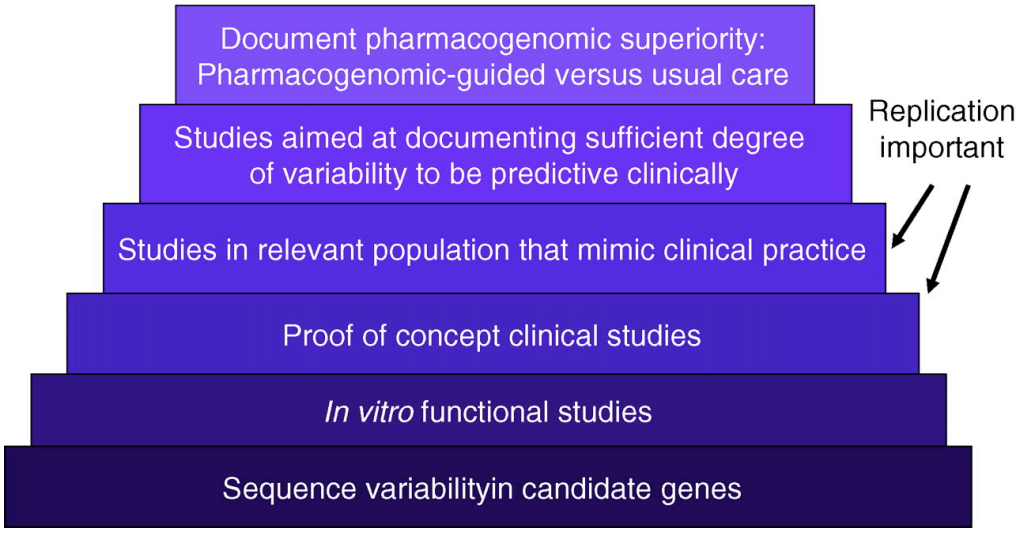
Figure 3. Location of *ADRB1* and *ADRB2* Polymorphisms.



The locations of ***ADRB1*** and ***ADRB2*** polymorphisms genes are shown with the red filled circles indicating sites of missense polymorphisms and yellow filled circles indicating silent polymorphisms.

(Mestroni L. Pharmacogenomics, Personalized Medicine, and Heart Failure. *Discovery Medicine* 2011.)

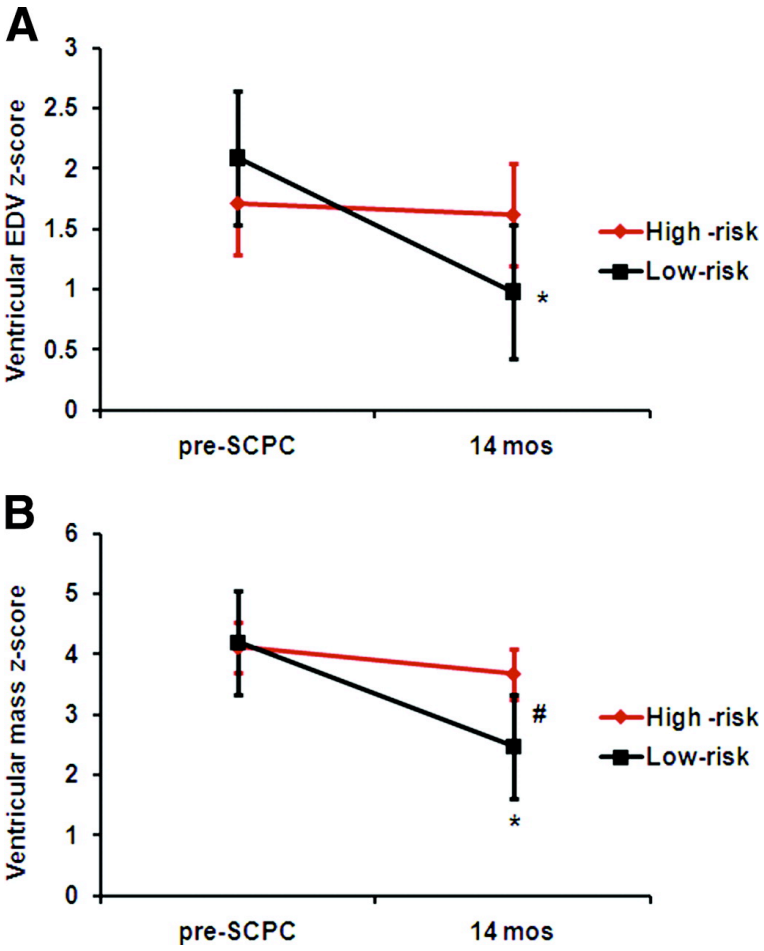
Figure 4. Moving Pharmacogenomics to Clinical Practice.



The six-step sequence from pharmacogenomic findings to eventual change in clinical practice when a study demonstrates pharmacogenomic-guided care to be superior. Note that **replication studies** will be essential in the intermediate steps to bring molecular data to clinical practice.

(Johnson JA et al. Cardiovascular Pharmacogenomics. *Exp Physiol* 2005; 90(3): 283-289.)

Figure 5. Ventricular EDV and Mass z-Scores.

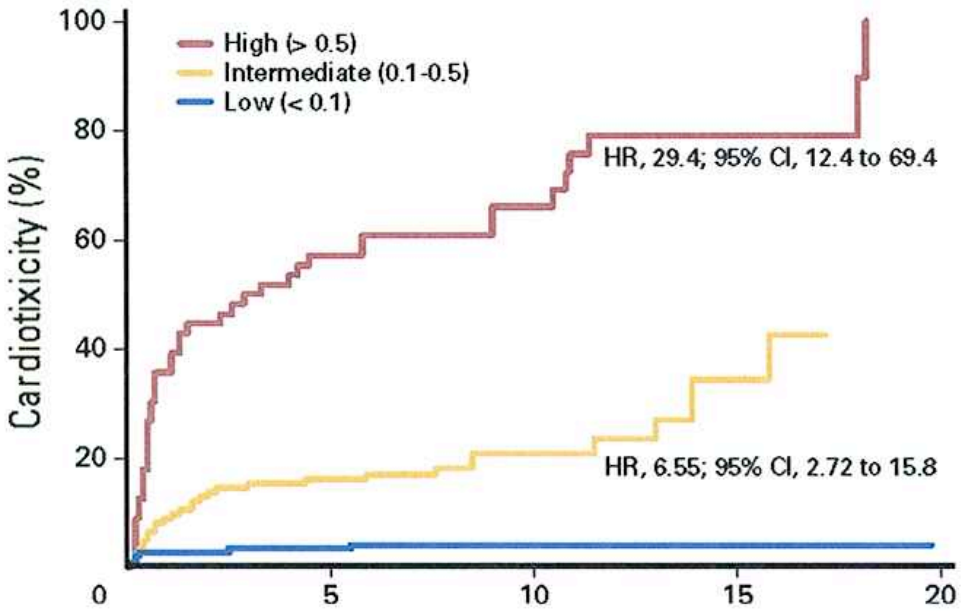


In A, the ventricular EDV z-scores and in B, the ventricular mass z-scores before and after the superior cavopulmonary connection surgery are plotted. The **high-risk patients** (in red) did not have a change in ventricular EDV nor mass, indicating an unfavorable remodelling process.

EDV, end-diastolic volume; SCPC, superior cavopulmonary connection.

(Mital S et al. Renin-Angiotensin-Aldosterone Genotype Influences Ventricular Remodeling in Infants with Single Ventricle. *Circ* 2011; 123: 2353-2362.)

Figure 6. Kaplan-Meier Curves of Anthracycline-Induced Cardiotoxicity.



The Kaplan-Meier curves of anthracycline-induced cardiotoxicity in low (blue), intermediate (yellow), and high-risk (red) groups based on multimarker regression model. The incidence of children who develop anthracyline-induced cardiotoxicity continues to increase in both **intermediate** and **high-risk** groups after the first few years.

HR, hazard ratio vs low-risk group.

(Visscher H et al. Pharmacogenomic Prediction of Anthracycline-Induced Cardiotoxicity in Children. *J Clin Oncol* 2011; 30: 1422-1428.)

Table 1. Methods of Pharmacogenetic Analysis.

Method	Description	Benefits	Shortcomings	Ref.
Genetic Association by Candidate Gene Polymorphism	Relate individual gene variants (SNPs) to drug response	Definitive association of variants with drug response	Slow, oversimplification, potenital miss of new variants	1,2
Haplotype Mapping	Study a group of associated gene variants, or in linkage disequilibrium	A group of associated genes such as those of a common pathways can be studied together	Potential miss of new variants	3,4
Systemic Genome-wide Analysis	Study large sets of candidate genes by genome-wide analysis	All the possible candidate genes in the genome that may contribute to drug response can be investigated	Too complex, costly	5

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The three methods of pharmacogenetics analysis are listed and described: 1) genetic association by candidate gene polymorphism; 2) haplotype mapping; and 3) systemic genome-wide analysis (GWAS).

(Mestroni L. Pharmacogenomics, Personalized Medicine, and Heart Failure. *Discovery Medicine* 2011.)

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