Published in final edited form as:

Heart Fail Clin. 2010 January ; 6(1): 1–10. doi:10.1016/j.hfc.2009.08.013.

Pharmacogenetics in Heart Failure: How it will Shape the Future

Eman Hamad, M.D. and Arthur M. Feldman, MD, PhD

Magee Professor and Chairman, Department of Medicine, Jefferson Medical College, Editor-in-Chief, Clinical and Translational Science, 1025 Walnut Street, Philadelphia, PA 19107, 215-955-6946, arthur.feldman@jefferson.edu

Pharmacogenomics is a growing field of research that focuses on how an individual's genetic background influences their response to therapy with a drug or with a device. Recent studies suggest that an individual's genetic background combined with environmental factors can predict the occurrence of disease, an individual's response to pharmacological interventions, drug toxicity and/or prognosis. Increasing evidence from clinical trials in patients with heart failure (HF) due to systolic dysfunction suggest that genetic variations can predict the occurrence of heart failure, influence the effects of standard therapies and influence outcomes of HF patients. This Chapter will review the underlying principles of pharmacogenomics, discuss some of the complex variables that influence the investigational approach to pharmacogenomics, demonstrate how variations in genes encoding a variety of different proteins can influence the effects of pharmacologic agents and describe the potential impact of pharmacogenomics on the treatment of patients with heart failure.

Pharmacogenomics – a historic perspective

That different individuals respond differently to the same “drug” or substance has been known since the earliest days of the practice of medicine. For example, in 510 B.C. Pythagoras noted that ingestion of fava beans resulted in a potentially fatal reaction in some, but not all, individuals.[1] In the 1950's, alterations in glutathione metabolism were detected in the erythrocytes of patients with hemolytic anemia that were induced by ingestion of a variety of agents including fava beans and “Favasim” was attributed to Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency [2] [3]. Another major observation of genetic variation in drug response dating from the 1950s involved the muscle relaxant suxamethonium chloride – a drug metabolized by N-acetyltransferase [4]. One in 3500 Caucasians was found to have a less efficient variant of the enzyme (butyrylcholinesterase) that metabolizes suxamethonium chloride. In patients with the variant the drug's effect is prolonged because of slow metabolism resulting in delayed recovery from surgical paralysis. Patients with “normal” N-acetyltransferase activity were referred to as “fast acetylators” while those with the decreased enzyme activity were referred to as “slow acetylators.” Because many drugs undergo acetylation, this variant also influenced how people responded to a family of drugs including isoniazid (antituberculosis) and procainamide (antiarrhythmic) [5].

Attempts to understand such variations, led to family, twin and racial distribution studies that focused on plasma concentrations and the factors known at the time to influence drug levels including absorption, distribution, metabolism and excretion. [6] These studies demonstrated

Correspondence to: Arthur M. Feldman.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

that the G6PD enzymatic defect in erythrocyte metabolism was inherited and that there was a higher incidence in African Americans [7]. Subsequent genetic studies identified that the G6PD gene is linked to the X chromosome and over 160 different mutations have been identified in various populations. These mutations most often alter the stability of the protein by disturbing protein folding and patients with the mutation are more susceptible to neonatal jaundice and hemolytic anemia.[8] The finding that genetic variability could alter an individual's response to a pharmacologic therapy led to the evolution of the field of "Pharmacogenetics."

The development of pharmacogenetic research was paralleled by rapid advancement in genomic science that lead to the Human Genome project (HGP)[9,10] which identified approximately 25,000 genes of the human genome. This lead to the evolution of "pharmacogenomics;" the study of drug response variation to multiple genes and how genetic and environmental factors act together to determine drug responses in patients. For purposes of this discussion we will use pharmacogenetics and pharmacogenomics interchangeably.

It is now increasingly being recognized that gene mutations can cause disruption of the normal function of a drug's metabolizing enzyme, transporter or receptor, leading to variability in efficacy and toxicity. Due to the individual genetic variability the ideal drug that treats all patients with out any toxicity does not exist. With many drugs there is a compromise between efficacy and toxicity that must be evaluated by the treating physician. A meta analysis of 39 prospective studies from US hospitals suggest that 6.7% of in patients have serious adverse drug reactions and 32% have fatal reactions, causing about 100,000 deaths per year in the USA [11,12]. By the same token, many currently approve medical regimens in cardiovascular disease and other areas are not effective in at least 20% of patients – but enhance survival in others. These observations may be due to a single genetic variation but may also be multigenic or multifactorial in etiology.

Currently, standard of care often does not take into consideration any of the factors that might influence an individual's response to therapy. Clinical trials carefully assess the phenotype of the patients that are enrolled; ie, their sex, age, medical regimen and severity of disease, but rarely assess their genotype. The absence of "pharmacogenetics" in industry-sponsored clinical trials has been largely due to the concern that by identifying a small number of patients that will respond to a pharmacologic agent or medical device, the sponsor limits the number of individuals that can be treated and thus limits the growth of the market. Indeed, many of today's blockbuster drugs would have far smaller markets if there use were predicated on the patient's genotype. Corporate sponsors also worry that the inclusion of a "genetic" test within the context of a clinical trial will cause many potential participants to be unwilling to participate for fear that identification of a "genetic" marker might put them or their family members at risk when they apply for health insurance or life insurance. Assessing genotype in a patient trial might also preclude the use of subjects from a diverse array of ethnic and racial backgrounds. For example, clinical trials are increasingly being carried out in India and Asia because the cost and regulatory impediments are far less. However, these countries might not be representative of the response in a U.S. population if the prevalence of important genetic variants is substantially different. Thus, there is an inherent bias on part of the pharmaceutical and device industries to avoid the topic of "pharmacogenetics" for as long as possible and to focus more on the phenotype of the populations that are studied. (Figure 1)

Genetic Factors

Genetic differences are observed between humans at both the individual and the population level. There may be multiple variants of any given gene in the human population. In humans, two alleles make up the individual's genotype. Variation in these alleles is referred to as a polymorphism. Genetic polymorphisms are monogenic variations that exist in the normal

population in a frequency of more than 1%. Any two individuals differ by approximately 1–10 million base pairs which is <1% of the 3.2 billion base pairs of the haploid genome. There are a large number of genetic variants that can alter the identity of an allele. Common variants include the “insertion” of a fragment of DNA in the coding region of the gene that results in production of a larger than normal protein, the “deletion” of a fragment in the coding region of the DNA resulting in transcription and translation of a longer protein, or the substitution of a single nucleotide – A,T,C, or G - on one or both alleles of the gene which is referred to as a “single nucleotide polymorphism” or a SNP. (Figure 2)

The extent of inherited variations became apparent with the completion of the HGP. More than 12 million single nucleotide polymorphisms (SNPs) have been identified in the human genome. [13,14] SNPs occur approximately once every 300–3000 base pairs [15]. Almost all common SNPs have only two alleles. Single-nucleotide polymorphisms may be insertions or deletions. [16] They can fall within coding sequences of genes or non-coding regions of genes. SNPs within a coding sequence will not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code. A SNP in which both forms lead to the same polypeptide sequence is termed synonymous (silent mutation) and nonsynonymous if a different polypeptide sequence is produced. Nonsynonymous change may either be missense or nonsense, where a missense change results in a different amino acid, while a nonsense change results in a premature stop codon. SNPs that are not in protein-coding regions may still have consequences for gene splicing, transcription factor binding, or the sequence of non-coding RNA. This is true with alternative splicing or intron variants which can lead to a different protein formation during transcription. Variants in promoter areas can also lead to major changes as they control expression of DNA. This may lead to changes in protein expression and phenotype. Variable number of tandem repeats (VNTR) is another large class of polymorphisms. It includes variable numbers of nucleotide repeats that might fall anywhere in the genome coding, noncoding or promoter regions [17]. More recently, investigators have begun to study the influence of changes in the sequence of the 3' un-translated region of genes. Mutations in this region can alter the confirmation of the 3'tail of the transcribed mRNA or inhibit or facilitate the binding of regulatory proteins thereby altering the stability of the mRNA and in turn altering translation of the protein product.[18]

Until recently, analyzing the genome of one cell was thought to provide information representing all the other cells. However, tissue specific effects such as tissue methylation, histone modification or micro-RNA expression that can significantly and constantly change the pattern of gene expression have been identified. Epigenetics is the study of these heritable changes to DNA structure.[19] However, tumors can also harbor mutations in selected genes that result in the expression of pro-oncogenic proteins and these pro-oncogenes are not found in normal cells.

Linkage analysis was one of the first methods for mapping genes responsible for some human diseases like Huntington disease[20], familial adenomatous polyposis [21] and many others as mendelian patterns of inheritance made it possible to segregate a single major gene. Linkage analysis is based on the fact that genetic loci on the same chromosome are physically connected and tend to stay together during meiosis and are thus genetically linked. By contrast, alleles for genes on different chromosomes are not linked and sort independently during meiosis. When chromosomes segregate during the early phase of meiosis sections of matched chromosomes are exchanged and when the two chromosomes break apart the new chromosomes may not be identical to either parent chromosome. Thus the genes have recombined to produce an offspring that has a combination of both maternal and paternal traits that differs from either parent. The probability of this happening is greater if the alleles are far apart on the chromosome than if they are close together. Thus, if one identifies the location of two genetic traits in an individual, the higher the percentage of descendants that do not show

both traits the farther apart the two genes are on the chromosome. Using a genetic map or a linkage map, geneticists can identify which markers a mutation is close to and thus identify a mutation's location on the linkage map. Linkage analysis was initially tedious and labor intensive and required a relatively large number of both affected and non-affected members in the same family and careful phenotyping to unequivocally identify those family members with the disease. More recently, high throughput sequencing technology and silicon chip technology has dramatically enhanced the time it takes to sequence and analyze samples. However, studies have demonstrated that a large number of mutations within a single gene or mutations in a number of different genes can result in the same phenotype. For example, we will see how wide array of mutations have been associated with hypertrophic cardiomyopathy. In addition, for Multifactorial diseases with more complex patterns of inheritance, the assumption of a single major gene may be incorrect and genetic heterogeneity and sporadic mutations may be present.

A second analytical approach to identifying genetic mutations that cause human disease or alter a patient's response to therapy is the use of association studies. Association studies start by identifying the presence of mutations in a candidate gene and then testing to assess whether that particular mutation is associated with the development of disease or with a specific response to medical therapy in a case control study[22]. This approach entails collecting samples from a large number of normal patients and from a large number of patients with a specific phenotype. The gene of interest is then sequenced in order to identify the presence of variants within the gene and the percentage of patients with the mutation is assessed in both normal patients and patients with disease. These initial studies are only hypothesis generating as studies must then be undertaken in normal populations to ascertain the percentage of normal patients with the mutation who will eventually develop the disease as compared with patients without the mutation.. Association studies can also be useful in identifying whether the presence or absence of a particular genetic variant results in an altered response to a therapeutic intervention. For example, recent studies have demonstrated that a mutation in the gene encoding cytochrome P-450 isoform CYP2C19 which leads to reduced function of the enzyme results in lower levels of the active metabolite of the drug clopidogrel. As a result, patients harboring the mutation and receiving the drug at the time of treatment for an acute coronary syndrome had diminished platelet inhibition, a higher rate of major adverse cardiovascular events and a higher incidence of stent thrombosis when compared with patients who did not have the CYP2C19 variant. [23]

The ability to carry out association studies has been substantially influenced by the development of rapid, low cost and efficient methods using silicon-based technology to examine specific genes for abnormal nucleic acid sequence. However, association studies are limited given the complexity of multifactorial and polygenic variations that affect many disease processes. Furthermore, many association studies are not adequately powered too assess the role of a single variant – much less the role of multiple variants. As a result, some journals now require that association studies be repeated in two separate and distinct populations to qualify for publication. A second limitation of association studies is that they have often looked at a single variant in a selected gene without ascertaining whether there are other mutations in the same gene that might have similar or contrasting effects on an individual's response to a drug or device. Similarly, association studies do not take into consideration the potential role of variants in other genes that might have an important affect on an individual's response.

Since many genes are likely to influence responses, millions of SNPs must be identified and analyzed to determine their involvement (if any) in disease processes and drug response. As the field of Pharmacogenetics made its transformation into pharmacogenomics with the completion of Human Genome project, Genome wide association studies (GWAS) became very important particularly for complex diseases with polygenic variations. The Genome-wide

association study is an approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease. Once new genetic associations are identified, researchers can use the information to develop better strategies to detect, treat and prevent disease. Such studies are particularly useful in finding genetic variations that contribute to common, complex diseases, such as asthma, cancer, diabetes, heart disease and mental illnesses. With the completion of the Human Genome Project in 2003[15] and the International Hap Map Project in 2005[24], researchers now have a set of research tools that make it possible to find the genetic contributions to common diseases. The tools include computerized databases that contain the reference human genome sequence, a map of human genetic variation and a set of new technologies that can quickly and accurately analyze whole-genome samples for genetic variations that contribute to the onset of a disease in less time and effort. Many high-throughput genomic and proteomic survey have been developed. However, even GWAS has limitations. First, in order to achieve statistical power, GWAS must collect DNA from thousands of normal volunteers as well as from thousands of affected individuals. Second, the technology that is currently available can analyze between 1 and 2 million markers. As there are between 10 and 15 million SNPs in any human genome, the current technology has the potential to “miss” a large number of potential variants. Furthermore, the GWAS has not fulfilled its promise as to date only a handful of disease-causing mutations have been identified using GWAS. Clearly, the research is on the right track – but additional technologic advances will be necessary for GWAS to fulfill its full potential and promise.

Among the important techniques used in the field of pharmacogenomics are Polymerase Chain Reaction (PCR), Denaturing High Performance Liquid Chromatography (DHPLC) and DNA microarray technology. Polymerase chain reaction (PCR) developed in 1984 by Kary Mullis, is a technique to amplify DNA and can generate millions of copies of a specific DNA sequence. The method relies on thermal cycling with repeated heating and cooling of DNA during which enzymatic replication of DNA takes place. As PCR progresses, the new DNA generated is used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR starts with denaturing DNA and separating the double helix into two strands under high temperatures. Each strand is used as a template for DNA synthesis. At lower temperatures short DNA fragments containing sequences complementary to the target region of DNA (Primers) anneals to the DNA strand and initiate DNA synthesis by a heat stable polymerase. DNA polymerase enzymatically assembling nucleotides elongating the segment and producing a complementary DNA strand, by using the original single-strand of DNA as a template. Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target are used. The primers give PCR its selectivity and ability to amplify specific sequences. PCR can be extensively modified to perform a wide array of genetic manipulations. PCR is widely used in the isolation, amplification and quantification of DNA. Currently many variations on the basic technique are used with different conditions and targets in almost all current pharmacogenetic analysis. [19]. Real time PCR (RT-PCR) detects PCR products as they form while the PCR reaction proceeds, using fluorescence energy transfer techniques for genotyping of SNPs in many samples. PCR is used routinely in the early diagnosis of malignant disease such as leukemia and lymphomas[25], and in quantifying viral loads and in many other areas of medicine.

High-performance liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry to separate, identify, and quantify compounds. HPLC is consistent of a stationary phase which utilizes a column that holds chromatographic packing material, a mobile phase that is moved through the column with a pump, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvents used. In denaturing high performance liquid chromatography (DHPLC) the solid phase has differential affinity for

single and double-stranded DNA. DNA fragments are denatured by heating and then allowed to reanneal. The melting temperature of the reannealed DNA fragments determines the length of time they are retained in the column[26]. Using PCR, two fragments are generated; target DNA containing the SNP polymorphic site and an allele-specific DNA sequence, referred to as the normal DNA fragment. This normal fragment is identical to the target DNA except at the SNP polymorphic site, which is unknown in the target DNA. The fragments are denatured and then allowed to gradually reanneal. The reannealed products are added to the DHPLC column. If the SNP allele in the target DNA matches the normal DNA fragment, only identical homoduplexes will form during the reannealing step. If the target DNA contains a different SNP allele than the normal DNA fragment, heteroduplexes of the target DNA and normal DNA containing a mismatched polymorphic site will form in addition to homoduplexes. The mismatched heteroduplexes will have a different melting temperature than the homoduplexes and will not be retained in the column as long. This generates a chromatograph pattern that is distinctive from the pattern that would be generated if the target DNA fragment and normal DNA fragments were identical. The eluted DNA is detected by UV absorption [26] DHPLC is easily automated as no labeling or purification of the DNA fragments is needed. The method is also relatively fast and has a high specificity.[26]. Lai et al, used DHPLC to screen patients with atrial fibrillation for the KvLQT1 gene which has been reported to be responsible for autosomal dominant hereditary atrial fibrillation [27].

DNA microarrays consist of a collection of microscopic DNA spots attached to a solid surface, such as glass, plastic or a silicon bio chip. The affixed DNA segments are called probes. Thousands of them can be placed in known locations on a single DNA microarray. Probes are used to hybridize with a target sample to detect SNPs, RNA expression levels (cDNA microarrays) or in resequencing mutant genomes. Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in a short period of time. Therefore arrays have dramatically accelerated many types of investigation. Microarray technology, allows rapid visualization of molecular targets in thousands of tissue specimens at a time, either at the DNA, RNA or protein level. Microarrays are ideally suitable for genomics-based diagnostic and drug target discovery since it facilitates rapid translation of molecular discoveries to clinical applications by identifying location, prevalence, and clinical significance of candidate genes. By using microarray technology, the speed of molecular analyses is increased by more than 100-fold, and a large number of molecular targets can be analyzed simultaneously[28].

Given its amazing potential, pharmacogenetics gained considerable interest in the biotechnology industry. New genome sequencing systems for a comprehensive and economical analysis of individual genomes are being developed in preparation for the implementation of personalized medicine in the near future[29]. Small biotechnology companies have risen based on this new technology that makes genotyping less time consuming and commercially available. For example, the commercial development of Pharmacogenetics started with one company in 1997 and reached 200 collaborations in 2003. In addition, companies have begun to market the ability to sequence an individual's entire genome as well as to market the ability to provide rapid turnaround when medical diagnosis and/or treatment requires a knowledge of the genotype of a tumor or a patient's genome.

Pharmacogenetics and Heart Failure

Like many diseases, an understanding of the pathobiology and pharmacology of heart failure will be greatly influenced by the field of Pharmacogenetics. Heart failure remains a disease of epidemic proportions in the U.S. and despite recent advances in the management of patients with heart failure, morbidity and mortality rates remain high with an estimated 5-year mortality rate of 50%. [30] Over the past 10 years, the rate of hospitalizations has increased by >150%.

[31] An estimated 5 million Americans have CHF and each year, there are an estimated 400,000 new cases and it is expected that this number will increase substantially by 2020. Since the 1960's, investigators have examined pathophysiologic changes that contribute to the progression of heart failure (HF) as a mean to develop pharmacologic interventions that might reverse or halt the vicious cycle of HF. The biologic pathways that were known to protect the heart during acute dysfunction were found to be the same pathways that cause progressive deleterious effects with chronic activation: the sympathetic nervous system and neurohormonal activation. As a result, the neurohumoral system has provided key targets for pharmacologic therapy in patients with heart failure.

Clinical trials assessing the efficacy of drugs used in the treatment of heart failure have suggested that different populations react differently to the same drug. For example, heart failure outcomes were found to differ by gender, race, etiology and environment. [32,33] According to the American Heart Association in 2005, HF death rates for Caucasian men were 19.4 compared to 21.9 for African American men and 18.2 for Caucasian women compared with 19.4 for African American women. [34] A pooled analysis of five randomized control trials by Frazier et al, found women to have more severe heart failure symptoms when compared to men, but better survival across different heart failure etiologies. [32,33] M-HART and ENRICHED looked at the effects of psychosocial interventions, depression and low perceived social support on clinical events after myocardial infarction (MI). [35–38] Psychosocial interventions were found to be harmful in women but not in men being treated post MI. Taken together, all these factors lead to the hypothesis that an individual's phenotype could predict their response to a drug. In addition, in the classical study of the use of beta blockers in the treatment of heart failure, patients treated outside of the U.S. had a far more robust response to the drug than did the North American treatment group. [39]

However, as we will see in subsequent chapters of this text, each of the targets of current pharmacologic therapy have important genetic variations that alter the function of the receptor-mediated signaling pathway both under physiologic circumstances and during disease. While some drugs have been shown to markedly alter outcome in selected populations, their effects have only been seen in a subset of the study population that cannot be defined by typical phenotypic variables. Furthermore, many of the currently used agents for the treatment of heart failure have demonstrated very different effects in different ethnic or racial groups. [32,33] This is particularly true in the use and evaluation of agents that act by modulating the function of the adrenergic receptor-G protein complex – a complex that has been found to have a significant number of genetic variants that alter the biology of the pathway during both normal physiology and during disease. Thus, it is not surprising that in the subsequent chapters of this text we will see that genotype has an important influence on the effects of therapy and prognosis in patients with heart failure. It is likely that in the near future, physicians will evaluate an individual's genotype before prescribing the appropriate medical therapy. In so doing they will be able to build a therapeutic regimen for each patient that optimizes the efficacy of the therapy while at the same time limiting potentially adverse or toxic side effects.

References

1. John Meletis KK. Favism - from the "avoid fava beans" of Pythagoras to the present 2004;7(1):17–21.
2. Luisada L. Favism: Singular disease affecting chiefly red blood cells. *Medicine* 1941;20:229–250.
3. Sansone, GaSG. Sensitivity to broad beans. *Lancet* 1957;2:295.
4. DAE. N-acetyltransferase. *Pharmacology & Therapeutics* 1989;42(2):157–234. [PubMed: 2664821]
5. HL, EWaM. Pharmacogenomics – Drug Disposition, Drug Targets, and Side Effects. *New Engl J Med* 2003;348:358–349.

6. Wilkinson, GR. The Pharmacological Basis of Therapeutics. Hardman, JG.; Limbird, LE.; Gilman, AG., editors. Vol. 3–29. New York: McGraw-Hill Medical; 2001.
7. Gross RT, Hurwitz RE, Marks. An hereditary enzymatic defect in erythrocyte metabolism: Glucose-6-phosphate dehydrogenase deficiency. *J Clin Invest* 1958;37:1176–1184. [PubMed: 13563647]
8. Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. *Blood Rev* 2007;21(5):267–83. [PubMed: 17611006]
9. Lander, ESea. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860–921. [PubMed: 11237011]
10. Venter, JCea. The sequence of the human genome. *Science* 2001;291:1304–1351. [PubMed: 11181995]
11. Meyer UA, Gut J. Genomics and the prediction of xenobiotic toxicity. *Toxicology* 2002;181–182:463–6.
12. Lazarou J, Pomeranz BH, Corey PN. Incidence of Adverse Drug Reactions in Hospitalized Patients: A meta-analysis of prospective studies. *JAMA* 1998;279:1200–1205. [PubMed: 9555760]
13. Lobmeyer MT, et al. Synergistic polymorphisms of beta1 and alpha2C-adrenergic receptors and the influence on left ventricular ejection fraction response to beta-blocker therapy in heart failure. *Pharmacogenet Genomics* 2007;17(4):277–82. [PubMed: 17496726]
14. Lindblad-Toh K, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 2005;438(7069):803–19. [PubMed: 16341006]
15. Sachidanandam R, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001;409(6822):928–33. [PubMed: 11237013]
16. Rigat B, et al. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86(4):1343–6. [PubMed: 1976655]
17. Redon R, et al. Global variation in copy number in the human genome. *Nature* 2006;444(7118):444–54. [PubMed: 17122850]
18. Young LESS, Bemis-Standoli K, Pena EA, Price RL, Dixon DA. The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. *Gastroenterology* 2009 May;136(5):1669–79. [PubMed: 19208339] Epub 2009 Jan 15
19. Brockmoller J, Tzvetkov MV. Pharmacogenetics: data, concepts and tools to improve drug discovery and drug treatment. *Eur J Clin Pharmacol* 2008;64(2):133–57. [PubMed: 18224312]
20. Gusella JF, et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 1983;306(5940):234–8. [PubMed: 6316146]
21. Bodmer WF, et al. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987;328(6131):614–6. [PubMed: 3039373]
22. Schaid DJ, SSS. Genotype Relative Risks: Methods for Design and Analysis of Candidate-Gene Association Studies. *Am J Human Genetics* 1993;53:1114–1126. [PubMed: 8213835]
23. Mega JL, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009;360(4):354–62. [PubMed: 19106084]
24. [hapmap.org], T.I.H.P.W.S. *The International HapMap Project Web Site*. [cited.
25. Day PJ. Miniaturized PCR systems for cancer diagnosis. *Biochem Soc Trans* 2009;37(Pt 2):424–6. [PubMed: 19290874]
26. Oefner PJ, Underhill PA. Comparative DNA sequencing by denaturing high-performance liquid chromatography (DHPLC). *Am J Hum Genet* 1995;57 [Suppl.]:A266.
27. Lai LP, et al. Denaturing high-performance liquid chromatography screening of the long QT syndrome-related cardiac sodium and potassium channel genes and identification of novel mutations and single nucleotide polymorphisms. *J Hum Genet* 2005;50(9):490–6. [PubMed: 16155735]
28. Kallioniemi OP, et al. Tissue microarray technology for high-throughput molecular profiling of cancer. *Hum Mol Genet* 2001;10(7):657–62. [PubMed: 11257096]
29. Webster A, et al. Integrating pharmacogenetics into society: in search of a model. *Nat Rev Genet* 2004;5(9):663–9. [PubMed: 15372088]
30. Ho K, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure: the Framingham Study. *J Am Coll Cardiol* 1993;22:6A. [PubMed: 8509564]

31. Jessup MBS. Heart Failure. *N Engl J Med* 2003;348:2007–2018. [PubMed: 12748317]
32. Frazier CG, et al. Associations of gender and etiology with outcomes in heart failure with systolic dysfunction: a pooled analysis of 5 randomized control trials. *J Am Coll Cardiol* 2007;49(13):1450–8. [PubMed: 17397674]
33. Rumsfeld JS, Masoudi FA. Sex differences: implications for heart failure care. *Eur Heart J* 2004;25(2):101–3. [PubMed: 14720524]
34. Hussey LC, Hardin S. Comparison of characteristics of heart failure by race and gender. *Dimens Crit Care Nurs* 2005;24(1):41–6. [PubMed: 15714073]
35. Mitchell PH, et al. A short social support measure for patients recovering from myocardial infarction: the ENRICHD Social Support Inventory. *J Cardiopulm Rehabil* 2003;23(6):398–403. [PubMed: 14646785]
36. Carney RM, et al. Depression as a risk factor for mortality after acute myocardial infarction. *Am J Cardiol* 2003;92(11):1277–81. [PubMed: 14636903]
37. Berkman LF, et al. Effects of treating depression and low perceived social support on clinical events after myocardial infarction: the Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Randomized Trial. *Jama* 2003;289(23):3106–16. [PubMed: 12813116]
38. Frasure-Smith N, et al. Randomised trial of home-based psychosocial nursing intervention for patients recovering from myocardial infarction. *Lancet* 1997;350(9076):473–9. [PubMed: 9274583]
39. Design of the cardiac insufficiency bisoprolol study II (CIBIS II). The CIBIS II Scientific Committee. *Fundam Clin Pharmacol* 1997;11(2):138–42. [PubMed: 9107560]

Figure 1

Figure 1.
Pharmacogenetics and Environmental Variables

Types of Genetic Variation – Effects on Gene Product (protein)

Intron variants resulting in **alternative splicing**

Promoter variants effecting gene transcription

Variants in the **3' UTR** influencing mRNA stability

Figure 2.
Representative Genetic Variants

Table 1

Terminology

Genotype	A person's genetic makeup, as reflected by his or her DNA sequence.
Phenotype	The clinical presentation or expression of a specific gene or genes, environmental factors, or both.
Heterozygous	Having two different alleles at a specific gene locus.
Homozygous	Having two identical alleles at a specific gene locus.
Allele	Different variations of the same gene.
Haplotype	A combination of alleles or sequence variations on the same chromosome.
Codon	A three-base sequence of DNA or RNA that specifies a single amino acid.
Exon	A region of a gene that codes for a protein.
Intron	A region of a gene that does not code for a protein.
Penetrance	The likelihood that a person carrying a particular mutant gene will have an altered phenotype.
Genetic Polymorphism	Monogenic variations that exist in the normal population in a frequency of more than 1%
Single-nucleotide polymorphism (SNP)	A common variant in the genome sequence; the human genome contains about 10 million SNPs.
Nonsynonymous	A mutation that produces a protein that takes on a new or enhanced function.
Synonymous	A mutation that produces the same ^{protein}
Alternative splicing	A regulatory mechanism by which variations in the incorporation of a gene's exons, or coding regions, into messenger RNA lead to the production of more than one related protein, or isoform
Nonsense mutation	Substitution of a single DNA base that results in a stop codon, thus leading to the truncation of a protein.
Regulatory mutation-	A mutation in a region of the genome that does not encode a protein but affects the expression of a gene- promoter region
Single-nucleotide polymorphism (SNP)	A common variant in the genome sequence; the human genome contains about 10 million SNPs.
Point mutation	The substitution of a single DNA base in the normal DNA sequence.
VNTR	Variable number tandem repeats - a stretch of DNA bases that occurs in the genome in multiple identical or closely related copies
Linkage disequilibrium	The non-random association in a population of alleles at nearby loci.
Epigenetics	The study of heritable changes to DNA structure but not the sequence. DNA methylation and histone modifications are examples of these modifications
Genomics	The study of the functions and interactions of all the genes in the genome, including their interactions with environmental factors.
Monogenic	Caused by a mutation in a single gene.
Polygenic	Caused by a mutation in more than one gene.
Multifactorial	Caused by the interaction of multiple genetic and environmental factors.
Proteomics	The study of an organism's complete complement of proteins
GWAS	Genome-wide association study
HGP	Human Genome Project
Microarray Technology	High through put sequencing of DNA, RNA and protein.
PCR	Polymerase chain reaction- amplification of DNA
qRT-PCR	Quantitative reverse transcriptase PCR- used to quantify amounts of transcripts (RNA) in a sample.
DHPLC	Denaturing high performance liquid chromatography