Deciphering Cardiovascular Genomics and How They Apply to Cardiovascular Disease Prevention

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6.1 Why Is Genomics Important?

Genomics, or the study of genomes, is concerned with understanding how the deoxyribonucleic acid (DNA) of which genomes are constituted contributes to making an organism unique. Accordingly, human genomics focuses on how DNA sequences produce individuals' traits, e.g., skin color and cholesterol levels, and contribute to diseases, e.g., myocardial infarction and diabetes mellitus. The last decade has witnessed a remarkable leap forward in the use of genomics technology to understand human traits and diseases, to the point that new discoveries regarding what makes each person unique are being widely reported in the press and advertised by companies to the lay public. Although *currently practical use of genomics is limited*, there are high expectations that it will be clinically useful in the near future. Discussions with patients of the implications of genomics – whether it is in the form of genetic testing for disease risk, pharmacogenomics, or personalized medicine – will be unavoidable for primary care providers. This chapter seeks to (1) explain the basic biology underlying genomics technology; (2) describe the potential future uses of genomics to improve patient care, particularly in cardiovascular medicine; and (3) set realistic expectations for the utility of genomics and explore the ethical implications of the technology.

6.2 A Brief Introduction to Molecular Biology

Deoxyribonucleic acid (DNA) is a molecule with two strands that are wrapped around each other in a helical formation, hence its description as a "double helix." The outer part of the helix contains the sugar and phosphate "backbone" of the DNA, and the inner part contains the "coding" portion of the molecule with four types of bases – adenine (A), cytosine (C), guanine (G), and thymine (T). An organism's genetic information is determined by the order of the sequence of the bases – with four bases available; the number of potential sequences is almost endless. The versatility of DNA results from the obligatory pairing of bases in the two strands. An adenine in one strand is always matched up with a thymine in the other strand, and cytosine is always paired with guanine. Thus, the two strands contain redundant information, and each can serve as a template on which a new complementary strand can be synthesized. This allows for easy duplication of the DNA so that when a cell divides into two, each descendant cell receives the same genetic information as the original cell.

An organism's DNA is organized into superlong strands that are packaged by a large complex of supporting proteins into chromosomes. Humans have 23 pairs of chromosomes, including the pair that determines gender, which in females comprises two X chromosomes, and in men, one X and one Y chromosome. For each chromosome pair, one was inherited from the mother and one from the father. The full set of chromosomes is collectively called the genome. The human genome is contained within the nucleus of each cell, where it is separated from the rest of the cell's functions.

In general, the genome is characterized by vast stretches of "noncoding" DNA sequence punctuated by small areas of "coding" DNA, also called genes, that represent the instructions needed by cells to perform their functions. Coding DNA is "transcribed" into a single-stranded molecule called ribonucleic acid (RNA) by a transcription enzyme complex. RNA is structurally similar to a DNA strand and also contains four types of bases, including adenine, cytosine, and guanine [in RNA, uracil (U) is substituted for DNA's thymine (T)]. The transcription enzymes have proofreading functions that ensure that the sequence of the RNA molecule perfectly matches the sequence of the DNA template from which it was synthesized. RNA is more flexible and mobile than DNA and is transported out of the nucleus of the cell

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into the outer compartment, the cytoplasm. Thus, RNA production is the mechanism by which genetic information is "expressed" and relayed from the central repository (DNA) to the rest of the cell, where it directs cellular functions.

While some RNAs have specialized functions – e.g., serving as structural components of certain parts of the cell – most RNAs take the form of "messenger" RNAs (mRNAs) that are "translated" by ribosomes into proteins. The ribosome reads from the beginning of the mRNA and uses it as a coding template with which to build proteins, with each nonoverlapping set of three consecutive bases ("codons") serving to specify a particular amino acid. With four available types of bases, there are 64 possible codon combinations; with some redundancy, these codons are translated into any of 20 different amino acids or into a "stop" signal. In this way the RNA sequence is converted into an amino acid sequence until a stop signal is reached that prompts the ribosome to finish and release the protein. The protein is then processed by the cell and then deployed to serve its purpose (as an enzyme, as a secreted factor, etc.).

This highly organized progression from DNA, to transcribed RNA, to translated protein is known as the "central dogma" of molecular biology (Fig. 6.1), and while there are exceptions to this sequence of events, the central dogma explains the vast majority of cellular processes. By and large, in humans these processes combine with environmental influences to determine each person's individual characteristics, susceptibility to diseases, and responses to medications. The technology is now available to study the cellular processes at any step of the central dogma. When the investigation occurs at the level of DNA, it is termed "genomics"; when at the level of mRNAs, "transcriptomics"; and when at the level of proteins, "proteomics." Processed proteins or other products of enzymatic reactions are called metabolites, the study of which is termed "metabolomics." The study of structural modifications to the chromosomes, which can have effects on the transcription of DNA, is termed "epigenomics."

6.3 The Principles of Human Genomics

The human genome is roughly 6 billion DNA bases in size, spanning the 23 chromosome pairs, and represents the complete list of coded instructions needed to make a person. There are an estimated 20,000–25,000 genes in the human genome, most of which encode proteins or components of proteins. What makes each person unique is a large number of DNA variations distributed throughout the genome. Some people have particular genetic variations that can predispose to heart disease; some of these variants require the presence of environmental factors (such as smoking and obesity) to trigger heart disease. Less commonly, certain variations have such a strong effect that they can cause heart disease outright. Other variations may determine how well patients respond to particular medications.

One reason some people are more susceptible to getting a disease than other people or respond differently to medications is that their DNA variants affect the function of genes. There are rare variants that have a large effect on a gene's function, either by significantly increasing or decreasing the gene's activity; these are the kind of variants that cause disease in many members of a single family and are also known as "mutations." There are common variants (>1% of the general population) that have a small effect on a gene's function. These variants do not change gene activity enough to cause disease by themselves but, instead, need to be combined with other gene variants or with environmental factors in order for

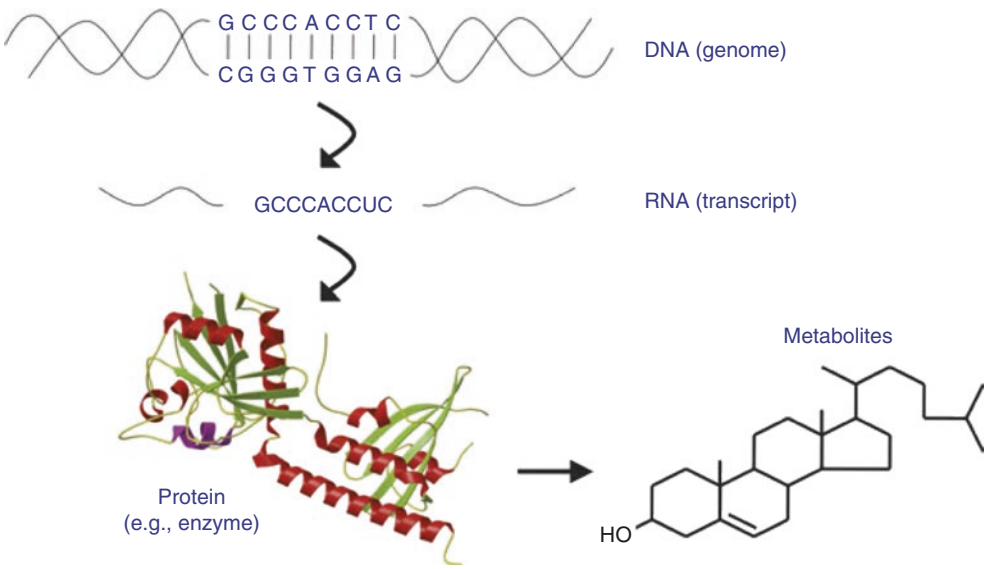

Fig. 6.1 Decoding and implementation of genetic information. Also known as the "central dogma," the cellular pathway begins with deoxyribonucleic acid (DNA) and proceeds with transcription of DNA into ribonucleic acid (RNA) transcripts, followed by translation of RNA into proteins (e.g., enzymes), which in turn produce metabolites

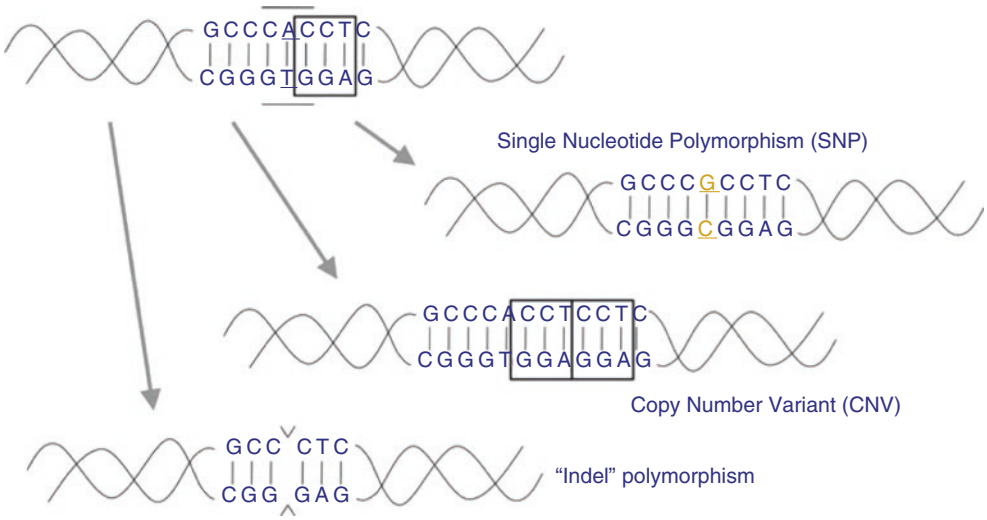

Fig. 6.2 Three types of polymorphisms. Variations in DNA sequence from person to person, or polymorphisms, can take the form of single nucleotide polymorphisms (SNPs), copy number variants (CNVs), and insertion–deletion variants ("indels")

disease to occur. This is the case with most cardiovascular diseases where there are many contributing factors (e.g., hypercholesterolemia, myocardial infarction). Conversely, there are common variants that have the opposite effect – they offer modest protection against disease.

All of these differences at the DNA level are called "polymorphisms," of which there are several types (Fig. 6.2). The best characterized to date are single nucleotide polymorphisms (SNPs) in which a single base in the DNA differs from the usual base at that position. A copy number variant (CNV) is a polymorphism in which the number of repeats of a DNA sequence at a location varies from person to person. An "indel" (short for insertion–deletion) is a polymorphism in which a DNA sequence is either present or absent at a location, varying from person to person. SNPs are the most common and best understood of the polymorphisms, with tens of millions of SNPs having been identified across the human genome.

"Locus" is one of the several terms used to describe a local area on a chromosome around an SNP. In most cases, each person has two copies of each locus because of the pairing of chromosomes; the exceptions are loci on the X or Y chromosome in men, who have only one of each. A person's "genotype" at an SNP is the identity of the base position for each of the two copies – also called "alleles" – of the SNP on paired chromosomes; thus, a genotype is typically two letters. A "haplotype" is a combination of SNPs at multiple linked loci – often adjacent to each other – that are usually transmitted as a group from parent to child (Fig. 6.3).

Some SNPs lie in genes and affect the genes' function. Most SNPs lie outside genes, in the large stretches of noncoding DNA between genes, and do not directly affect the genes. Groups of SNPs near genes tend to stay together with the genes from generation to generation, over thousands of years, in what are called "linkage disequilibrium" blocks that

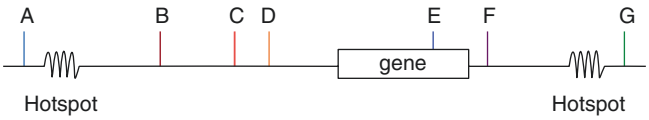

Fig. 6.3 Linkage disequilibrium. SNPs in proximity to a gene tend to stay together with that gene through many generations, a phenomenon known as linkage disequilibrium. In this example, only E is in the gene and directly affects its function. Genotypes at B, C, D, E, and F will stay together on a chromosome as it is passed from parents to offspring. In contrast, A and G are separated from the gene and the other SNPs by recombination hotspots, and thus they may not stay together on a chromosome through many generations – they will not be in linkage disequilibrium. Being linked, B through F make up a haplotype. Knowledge of any one of the five SNPs gives information on – acts as a "tag" for – the other four SNPs. Thus, genotyping B (or C or D or F) will indirectly yield information about the gene, even though the SNP is not in the gene

are separated by chromosomal recombination hotspots (for a more detailed explanation of this phenomenon, please see [1]). Thus, even if it is not known which polymorphism in a gene causes a disease (which is usually the case), one can use a SNP that is not in the gene but is in linkage disequilibrium with the gene – as a "tag" for that disease-causing variant of the gene (Fig. 6.3).

The technology is now available to decode millions of "tag" SNPs in a person's DNA all at once using "gene chips" or "arrays" or "panels." By applying the gene chips to thousands of individuals, some with a disease and some without the disease, researchers are able to identify tag SNPs that are associated with disease (though the association is typically not perfect nor do associations imply causality). These studies are termed "genome-wide association studies" or "GWAS."

As an example of how this technology might be used, consider GWAS performed for myocardial infarction. The study design would entail collecting DNA samples from

thousands of patients who have suffered heart attacks and thousands of control individuals (who have not have had heart attacks but are otherwise similar to the patients). A gene chip is used to determine the genotype for more than 1 million SNPs in each of the study subjects. Despite having a massive amount of information (1 million genotypes for several thousand people or billions of pieces of data), the statistical methods to analyze the information are relatively simple. The investigators set up computer software to analyze each SNP and ask: Does allele "A" versus allele "B" of this SNP occur in equal proportions in the myocardial infarction patients and the control individuals? In the vast majority of cases, there will be no difference in proportions; for a particular SNP, however, there may be a significant difference in the proportions (Fig. 6.4). Because the SNP "tags" any nearby genes, the implication is that there is a variant affecting the function of one of the nearby genes in such a way as to modify the risk of myocardial infarction (presumably through involvement in a pathophysiological process).

Several GWAS with precisely this design have been performed for myocardial infarction and coronary artery disease. These studies all found SNPs in a locus on chromosome 9p21 to be highly associated with coronary disease, with weaker associations seen for SNPs in other chromosomes [2–9]. (At the time of this writing, it remains unclear which gene near the 9p21 locus contributes to myocardial infarction.) Other studies have identified SNPs associated with atrial fibrillation [10–16], lipid levels [17–25], diabetes mellitus [26–41], electrocardiographic QT interval [42–46], abdominal aortic aneurysm [47–52], and statin-induced myopathy [53–56].

Recently, genome-wide approaches have been expanded to also study the relationship of physical modifications to the structure of chromosomes (epigenome-wide association studies) [57] and gene expression levels (transcriptome-wide association studies) [58] in relevant tissues to cardiovascular traits. Such studies are still in their early phases and have been applied to some of the traits mentioned above, but they have the potential to further establish the relationship of common DNA compositional and expression differences to disease when applied to larger populations, tissue types, and specific disorders and clinical outcomes.

In parallel with GWAS, which rely on testing the association of *common* variants one-by-one with a trait or disease being considered, great progress has been made in methods to discover *rare* variants as they relate to cardiovascular diseases and traits. Among these approaches are deep medical resequencing of candidate genes, whole-exome sequencing (WES), and exome-wide genotyping. All of these approaches rely on the notion that (1) the genetic variation that is most likely to significantly impact the function of a gene is that which disrupts the protein encoded by the gene and thus may exist in the coding regions of the gene ("exons") and (2) such variation underlying an extreme trait or disease may be rare in the population but enriched in subsets with a high burden of disease.

Deep medical resequencing involves choosing candidate genes for sequencing on the basis of their known role in a particular trait or disease. The exons of an entire gene or set of genes are resequenced. Variants identified in the candidate genes can then be ascertained for their functional

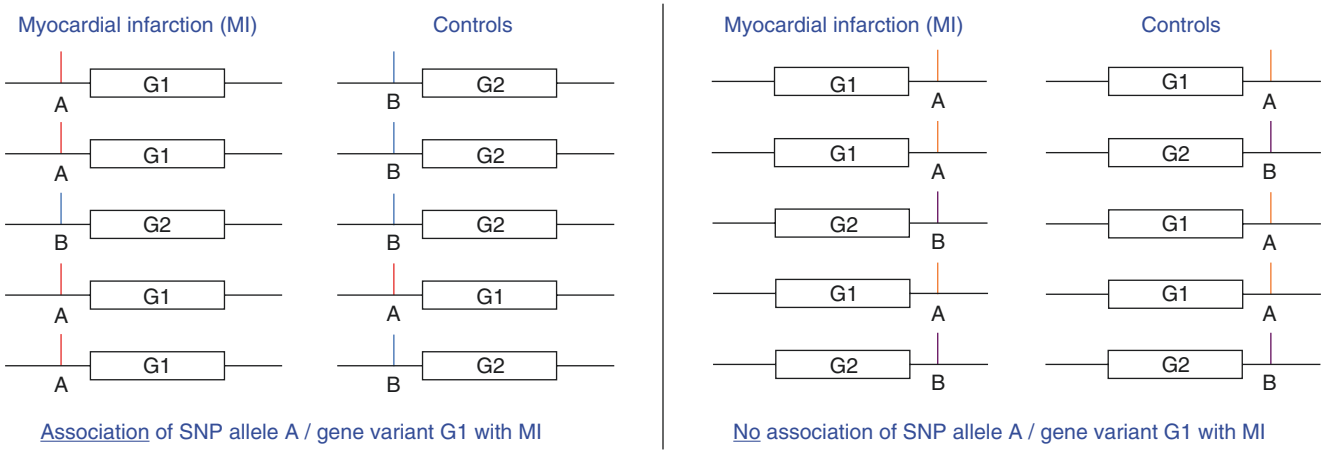

Fig. 6.4 General strategy for genome-wide association studies. For each of millions of SNPs distributed across the genome, the genotypes at the SNP are determined for both cases (myocardial infarction in this example) and controls. As shown on the *left*, an allele of the SNP may be seen in higher proportions in cases than controls. This SNP is therefore associated with the disease, and the strength of the association (*P* value) and exact increase in disease risk can be calculated using biostatistics. Even if this SNP is not in the causal gene (as in this example), it may be in linkage disequilibrium with a polymorphism in the gene, explaining the association with disease. On the *right*, the SNP alleles are present in the same proportions in cases and controls; this SNP is not associated with disease. Typically, out of hundreds of thousands of SNPs, only a few (if any) show a statistically robust association with disease

effects on the encoded proteins as well as their potential to cause the observed trait or disease. Such variants are notable when they are identified in multiple individuals harboring the trait or disease but absent in those who are unaffected. Similarly, when candidate mutations are identified in families and are present in affected members but not in unaffected members, this supports the possibility that the mutation is directly causing the trait or disease. Targeted sequencing gene panels are currently being developed, primarily for research purposes, to identify variants in genes known to contribute to cardiovascular traits and diseases [59–67]; however, their applicability for clinical diagnostics and risk prediction are still limited because it is challenging to interpret whether the identified rare variants are "neutral" (i.e., are of no consequence) or pathogenic [68].

WES applies the principle described for deep medical resequencing across all the regions of the genome that encode proteins (the "exome"). In addition to having applications similar to those for candidate gene deep resequencing, WES allows the ability to identify novel heritable causes underlying traits and diseases. As an example, the first application of WES to a clinical cardiovascular phenotype was its use to identify the underlying cause of a newly identified syndrome of low plasma levels of all the major lipid traits (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides), a disorder called familial combined hypolipidemia [69]. The authors performed WES in two siblings with this disorder and found that both harbored two novel protein-truncating variants in the *ANGPTL3* gene, which encodes a protein that delays the turnover of triglycerides and HDL in experimental models. The authors found no other mutations in other genes that could account for the condition and that were present in both of the affected siblings, and they were thus able to conclude that loss of *ANGPTL3* function was the cause of the dyslipidemia. This example highlights the potential power of WES in identifying new heritable causes of rare or poorly understood clinical traits. Additional potential clinical applications of WES will be discussed further below.

Exome-wide genotyping combines approaches similar to GWAS and WES together to assess protein-coding variation in the genome as it relates to traits and diseases. This method uses SNP panels similar to those used in GWAS but that cover only protein-coding variants for genotyping. These panels include both common and rare coding variants to allow for their combined assessment for association with traits of interest [70–72]. The utility of this approach may be in its ability to capture known rare variants and to assess their burden in particular populations [73] and test their associations with a broad range of traits across large cohorts of patients [74, 75] in a less expensive and more scalable manner than current WES approaches allow.

6.4 Practical Uses of Genomics Studies

GWAS allow for the mapping of diseases (e.g., myocardial infarction) and clinical traits (e.g., cholesterol levels) to specific regions on chromosomes. They narrow the resolution from 3 billion bases (the entire human genome) to around 100,000 bases (chromosomal locus) surrounding a tag SNP. In principle, the tag SNP can then be used for disease risk prediction or for pharmacogenomics (see below). The tag SNP can also be used to pinpoint causal genes underlying the disease or trait or response to therapy. Subsequent studies on those genes can give important insights into basic biology as well as facilitate the development of new therapies that target the genes (Fig. 6.5).

Similarly, sequencing to uncover rare variants has identified multiple putative targets for drug therapies for cardiovascular diseases. A notable example is the discovery of both loss-of-function and gain-of-function protein-coding variants in the *PCSK9* gene. In 2003, rare variants in the *PCSK9* gene were identified that caused extremely high LDL cholesterol levels [76]. Subsequent studies in humans confirmed that these variants were likely gain-of-function mutations that increased *PCSK9* function [77–79], and additional work in mice demonstrated that indeed *PCSK9* increased LDL cholesterol levels [80, 81]. Following this work, sequencing of human subjects with extremely low LDL cholesterol levels identified common loss-of-function *PCSK9* mutations [82]. These mutations result in up to 88% reduction of risk for coronary disease [82, 83]. Additional studies further established the causal and direct relationship of LDL cholesterol levels to coronary disease [84, 85] and paved the way for the development of therapies targeting PCSK9 [86–94]. In 2015, two PCSK9-inhibiting monoclonal antibodies were approved for clinical use to treat extreme forms of hypercholesterolemia [95]. This marked the success of a bench-to-bedside journey that had started only 12 years earlier.

Subsequent large-scale WES efforts in patients with coronary artery disease or early-onset myocardial infarction have also identified cholesterol-related targets of therapeutic rele-

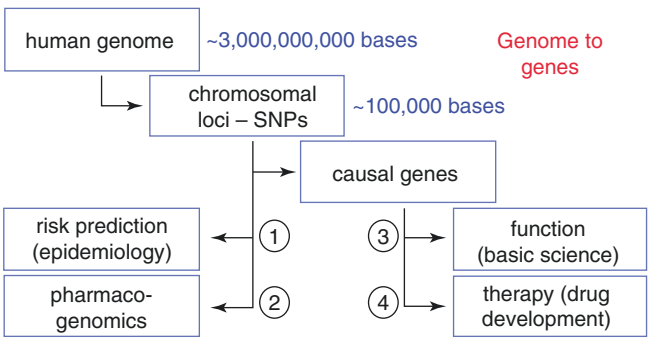

Fig. 6.5 Potential uses of information learned from genome-wide association studies

vance. These include the *LDLR* gene, the indirect target of statin therapy and also the gene responsible for many cases of familial hypercholesterolemia [96]; the *NPC1L1* gene, the target of the cholesterol absorption antagonist ezetimibe [97]; and the *LPA* gene, which encodes the defining protein component of lipoprotein(a) [Lp(a)], a strong coronary disease risk factor [98].

Genomics has also been useful in assessing whether biomarkers for coronary disease are truly causal for disease. In this regard, the recent application of genomics to study the impact of HDL cholesterol and triglycerides to cardiovascular risk has been particularly informative. In the case of HDL cholesterol, the failure of several HDL-raising therapies such as nicotinic acid [99–103] and CETP inhibitors [99, 100, 104–106] was almost simultaneous with the finding that genetic variants that raise HDL cholesterol do not reduce the risk of coronary disease [99, 100, 107–109]. For example, exome-wide genotyping and deep resequencing of the *SCARB1* gene identified carriers of a protein-coding loss-of-function variant in this gene who had extremely high levels of HDL cholesterol but, unexpectedly, had a moderately increased risk of disease, casting doubt on the "protective" role of HDL cholesterol [110]. These and other studies have fueled interest in identifying the physiological functions of HDL beyond their cholesterol content as possible mechanisms by which HDL may still confer protection from cardiovascular diseases [99, 100, 102, 111–113].

In contrast, GWAS and other approaches to studying common variants affecting triglyceride levels have shown that variants associated with decreased triglycerides are also associated with decreased risk of coronary disease [25, 114]. Additional studies of rare protein-coding variants have further established that the lipoprotein lipase (LPL) pathway of circulating triglyceride clearance is protective against coronary disease [115–120]. In particular, loss-of-function mutations in two genes encoding inhibitors of LPL, the *APOC3* gene [116–118, 120] and the *ANGPTL4* gene [115, 119], are protective against coronary disease, making them prime targets for the development of novel therapies to reduce cardiovascular risk [119, 121–123]. A third inhibitor of this pathway, *ANGPTL3*, is also being explored as a therapeutic target [124, 125].

6.5 Genetic Testing and Disease Risk Prediction

After identifying a number of SNPs – in different chromosomal loci across the genome – that are associated with a disease of interest, one can use these SNPs to calculate a genetic risk score for the disease (Fig. 6.6). One simple example entails cataloging for each SNP: Does the patient have two copies of the lower-risk variant of the SNP, two

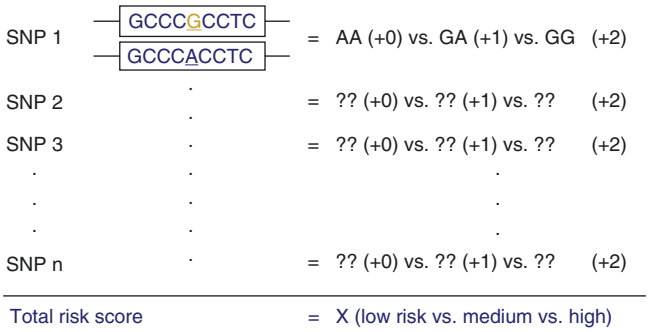

Fig. 6.6 Calculation of a genetic risk score

copies of the higher-risk variant of the SNP, or one copy each of the lower-risk and the higher-risk variant? Risk "points" are assigned depending on the genotype at the SNP. These points are added up for all of the SNPs, yielding a total risk score. This risk score, especially when combined with a traditional risk score (e.g., Framingham risk estimate) that accounts for endogenous (blood pressure, serum lipids, age) and environmental factors (e.g., cigarette smoking), might be useful in predicting the likelihood of developing the disease. Eventually, clinicians would be able to order this panel of SNPs as a blood test and get back a risk score that would help guide patient management.

One of the first published reports of a genetic risk score for cardiovascular disease, in early 2008, demonstrates the potential usefulness of a risk score [126]. The investigators calculated a lipid-based genetic risk score using nine SNPs associated with LDL cholesterol or HDL cholesterol (score from 0 to 18) and found that the score is associated with cardiovascular disease. The higher the risk score, the more likelihood the individual had of developing cardiovascular disease during the study period. However, when this particular genetic risk score was added to a traditional risk prediction model, it did not improve overall risk prediction. After adjustment for traditional risk factors, the relative risk between individuals with high genetic risk scores and those with low genetic risk scores was 1.63, a modest difference [126]. Although the degree of risk discrimination is likely to improve as additional SNPs discovered to be associated with cardiovascular disease are added to the genetic risk score, it remains to be seen whether it will be enough to significantly improve on current risk prediction strategies.

For a healthcare provider presented with this type of genetic information, it will be a challenge to meaningfully integrate it into clinical practice. This is especially true when the relative risks associated with SNP variants are in the 1.0–2.0 range – i.e., the at-risk genotype confers between one and two times the risk of developing the disease – as seems to be the case with most disease-associated genotypes. Providers must already ponder the utility of novel biomarkers, such as high-sensitivity C-reactive protein, that are only modestly

predictive of cardiovascular disease and do not reclassify large proportions of patients into new risk categories [127]. To date, genetic risk scores do not appear to be any more predictive than these biomarkers. Indeed, it remains unclear in the absence of any clinical trials whether a genetic risk score will prove more useful than simply asking the question: "Do you have a family history of heart disease?"

Nevertheless, several companies see significant commercial potential in these types of risk scores and have already started marketing SNP panels to the general public, charging hundreds to thousands of US dollars. The implication of the advertising for these panels is that they will let patients know if they are at higher risk for particular diseases. None of these panels has yet been shown to add value to traditional risk factor algorithms, and they should not be recommended to patients at this time for that purpose.

There are other important limitations of these SNP panels. They do not include rare variants that cause disease (these include the mutations that are unique to one person, or to one family, and so are not going to be found on the SNP panels). So while the patient may learn from an SNP panel that she has a variant of a common SNP that modestly decreases the risk of a particular disease, e.g., breast cancer, she may unknowingly harbor a mutation – not found by the SNP panel – that dramatically increases her breast cancer risk. In this case, having only partial genetic information would give false reassurance and may even be harmful if the patient chooses to forego screening with mammography.

Furthermore, because the initial series of GWAS were performed in Caucasian populations of European ancestry, the first generation of SNP panels may not be relevant to individuals of other ethnic or racial backgrounds. For now, non-Caucasian individuals will benefit less than Caucasians from the recent advances in genomics, although this situation should change as more GWAS are performed in a wider variety of racial and ethnic groups.

When asked about SNP panels by patients, it is appropriate to say that the tests are experimental – they may eventually prove to be useful, but they may also prove to be a waste of money. It is also appropriate to point out that many old-fashioned preventative health practices – good diet, weight control, exercise, and smoking cessation – can have a far larger impact on one's risk of getting a disease than any genetic influences that one may learn about from genetic testing.

6.6 Pharmacogenomics

The field of pharmacogenomics – the use of human genomic variation to predict efficacy and toxicity of drug therapy – is a promising area for the clinical application of genomic information. Commonly used medications such as lipid-lowering therapy, antihypertensive drugs, antiarrhythmic drugs, and anticoagulants have differential effects depending on variation in certain genes. The ultimate objective of pharmacogenomics is to deliver the "right drug for the right patient" by accurately predicting both therapeutic response and safety before a drug is prescribed.

One scenario for the practical application for pharmacogenomics is the use of a screening test to identify patients who are at risk for adverse side effects from medications or who are unlikely to respond to a therapy (Fig. 6.7). A patient presenting to medical attention with a particular condition would undergo the screening test, which would identify the genotype of a relevant polymorphism or set of polymorphisms. The genotype information would be used to determine whether the patient's condition is likely to improve from the treatment, whether the treatment poses a risk and should be avoided altogether, or how much of the treatment should be given – i.e., tailoring the dose to the patient.

When associations between genotype and drug sensitivity have been identified, as in the case of INR response to warfarin therapy on the basis of *CYP2C9* genotypes and *VKORC1* haplotypes, trials must be conducted to evaluate the clinical efficacy of the gene-based prescribing strategy and determine whether the increment in efficacy or safety warrants the cost of genetic testing [128]. An initial trial reported in 2007 assessed an algorithm that used a patient's specific *CYP2C9* and *VKORC1* SNPs to calculate an ideal starting warfarin dose for anticoagulation. When compared to the usual practice (i.e., providers picking a starting dose using best judgment), this specific algorithm did not improve the safety of warfarin initiation (out-of-range INR measurements were not reduced compared to traditional dosing), although it did reduce the number of dosing changes needed [128]. A subsequent study using six additional algorithms for calculating warfarin dose based on *CYP2C9* genotype versus a nongenetically determined dosing strategy found a significantly higher percentage of genotype-dosed patients with INR >2 5 days after initiation relative to the non-genotype-based dosing cohort [129]. More research studies are underway to see whether genetic dosing of warfarin will be clinically useful in broader practice.

Just as GWAS are being used to characterize disease risk, a similar strategy can be used to characterize appropriate or

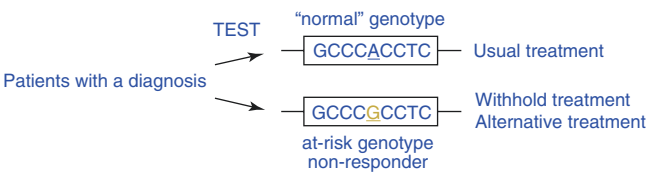

Fig. 6.7 The general strategy of pharmacogenomics

adverse responses to therapy. A GWAS published in 2008 showed that individuals with one genotype at an SNP in the *SLCO1B1* gene have 17 times the risk of statin-induced myopathy than individuals with another genotype [53]. This dramatic difference in relative risk (though not absolute risk, given the overall rarity of statin-induced myopathy) suggests that a genetic test for this SNP could be helpful in predicting which patients are at risk of getting myopathy before they are started on statins. A *SLCO1B1* SNP test might be particularly useful for patients in whom there is already a clinical suspicion for risk of myopathy (e.g., family history, history of myalgias on statin therapy). As with all genetic findings to date, however, this strategy needs to be tested in a clinical trial before it can be recommended for general use.

Another potential application of genetics to predicting response to therapy involves the antiplatelet agent clopidogrel, which has become a mainstay of post-acute coronary syndrome (ACS) patient management, particularly after percutaneous coronary intervention (PCI). Clopidogrel is converted into its active metabolite in the liver by the cytochrome P-450 2C19 enzyme. In three large studies of post-ACS patients on clopidogrel therapy (TRITON-TIMI 38, FAST-MI, and AFIJI), the *CYP2C19* gene encoding this enzyme was genotyped, with identification of at least one reduced-function allele in ~30% of individuals. In all of the three studies, carriers of reduced-function *CYP2C19* alleles suffered significantly higher rates of cardiovascular death, myocardial infarction, and stroke [130–132]. This is consistent with the finding in TRITON-TIMI 38 that reduced-function allele carriers had lower plasma levels of the active metabolite of clopidogrel [131].

However, further studies have called into question the value of using *CYP2C19* genotype to guide post-ACS clopidogrel dosing. One study compared data from clinical trials examining effects of clopidogrel vs. placebo on outcomes and observed a comparable impact on risk between the two groups [133]. Another study in patients who largely underwent PCI with stenting found that carriers of reduced-function *CYP2C19* alleles had a higher rate of adverse events within 30 days of initiating treatment [134]. Larger meta-analyses of patients undergoing PCI have had mixed findings, with one study finding that reduced-function allele carriers had a higher rate of in-stent thrombosis and other adverse cardiovascular events than non-carriers [135]; however, these conclusions were not supported by other meta-analyses in lower-risk patients [136–138]. To date, there are still no published reports from large clinical trials assessing the utility of prospective *CYP2C19* genotyping in improving clinical outcomes. Such studies will be needed to determine whether routine post-ACS genotyping of *CYP2C19* will be of any merit in reducing poor outcomes.

6.7 Risks of Genetic Testing

Although some "early adopter" patients may take the initiative to avail themselves of commercial SNP genotyping services and then bring genetic information to providers for interpretation, others will approach their providers first and ask whether genetic testing is advisable. It may seem harmless for a patient to undergo SNP genotyping – typically involving only a swabbing of the inside of a cheek or a drawing of a blood sample – but there are important potential consequences to consider. As mentioned above, it is not yet clear how physicians should best interpret the results of genetic testing, since few clinical trials have been done. Furthermore, in the "Google era," there is the danger of patients overinterpreting the results of their tests based on misleading information available on the Internet.

One worrisome possibility is that a patient may be falsely reassured by hearing that his genetic risk score is low. He may not be vigorous about lifestyle changes that, if enacted, would reduce his risk of disease even more than the protection offered by his favorable genetic profile. Conversely, a high genetic risk score may cause undue worry and even strain family relations. For example, a person may learn that the spouse is more likely to develop a serious illness, and this may impact their relationship as well as relationships with parents and potential offspring. Arranging for a patient and family members to meet a genetic counselor is recommended if this type of situation should arise.

Finally, privacy issues should be seriously considered prior to the use of genetic tests. It remains to be seen what insurance companies will do if they obtain access to genetic data. The US Congress has acted to prohibit discrimination by employers and health insurers on the basis of genetic testing with the Genetic Information Nondiscrimination Act (GINA), but further ethical safeguards will undoubtedly be needed as the social implications of genomics become clearer.

6.8 Conclusion

Although genomics offers great promise for the improvement of cardiovascular medicine, applications of the technology are still being demonstrated and validated, and the clinical utility of genomics for diagnosis and intervention is in its infancy. Yet with the enormous publicity surrounding genomics discoveries, it will be natural for patients to seek advice about genetic testing from their providers. These inquiries should be welcomed, since they reflect patients taking an active interest in their own health, and they are opportunities for providers not only to educate patients about genomics – to highlight the present uncertainty of the clinical

usefulness of the tests, as well as the potential hazards of obtaining the information – but also to reinforce old-fashioned preventive messages, good diet, weight control, exercise, and smoking cessation, as well.

6.9 Case Study 1

A 57-year-old Caucasian man presents to your clinic for the first time. He is eager to talk to you about the results of his "gene tests." Upon hearing about a commercial "personal genome service" that reads more than 500,000 locations in the genome and offers information on more than 100 diseases, he immediately signed up for the service. He has printed out all the results of the tests and brought them to you so you can read them and keep them in his medical record. He is particularly concerned because the tests reveal that he has an increased risk of having a heart attack. When you look at the specific information in the printouts, you see that on the basis of several SNP genotypes, his relative risk of myocardial infarction is estimated to be 1.6 times that of the general population.

On physical examination, the patient is overweight and moderately hypertensive. He admits that he does not regularly exercise, smokes half a pack of cigarettes a day, and has not been taking the cholesterol medication prescribed to him by a physician 3 years ago. He asks how concerned he should be about the results of his genetic testing.

Answer: You can advise the patient that although his genetic testing may suggest a modestly increased risk of heart attack, the information is not useful at the present time because there have been no clinical trials testing whether this type of information is valid. You should point out that he has several traditional risk factors for myocardial infarction – high blood pressure, high cholesterol, and tobacco use – all of which make it much more likely that he will get a heart attack in comparison to his putative 1.6-fold risk from his SNP genotypes. Importantly, he can do something about those risk factors – improve his diet, exercise regularly, take his prescribed medications, and stop smoking – while he cannot do anything about his genetics.

Given the potential privacy issues, keeping the results of nonclinical genetic testing in the medical record is not advisable at this time.

6.10 Case Study 2

You are seeing in your clinic a 63-year-old woman whom you have been following for several years. She suffered a myocardial infarction 2 years ago, after which she was appropriately prescribed a statin drug for secondary prevention. She stopped taking the statin because she developed severe muscle aches, and she was switched to ezetimibe instead. On a fasting lipid profile taken several weeks ago in anticipation of today's visit, her LDL cholesterol remains quite elevated – 135 mg/dL – far above the optimal goal of 70 mg/dL. You advise her that she really should be on a statin drug, and you can prescribe her a different statin than the one she took before in the hope of avoiding her prior symptoms. She is hesitant to proceed; she has learned that her father developed bad "muscle disease" when he was taking a statin 10 years ago, requiring hospitalization, and both her brother and sister have experienced muscle aches when taking statins.

Is there a role for genetic testing in this patient's management?

Answer: A SNP in the *SLCO1B1* gene has recently been reported to be strongly associated with myopathy [53]. Individuals with the at-risk genotype have 17 times the risk of developing myopathy compared to other individuals. There is now a commercial test for this *SLCO1B1* variant available. Given this patient's prior symptoms and her strong family history, she appears to be at increased risk of statin-induced myopathy. Determining if she has the at-risk *SLCO1B1* genotype could be helpful in her management; if she does have the genotype, it would be prudent to avoid statin therapy altogether. If she does not have the genotype, one might be encouraged to cautiously start her on a different statin.

Recommended Reading

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