Genomic Medicine Why Do “Similar” Patients Have Different Outcomes?

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ABSTRACT
Genomic variation is an important factor in why supposedly “similar” patients react differently to drugs, have different disease course(s), and varying clinical outcomes. This review provides an update on concepts in modern genomic medicine with emphasis on clinically relevant study approaches, disease/drug pathway analysis, and recent pharmacogenomic findings. The application of genomic medicine and its importance for rapid diagnosis of disease causing agents, as well as its clinical application in human disease diagnosis/treatment and in cardiovascular disease are discussed. In addition to direct clinical applications, modern genomic approaches also play an important role in elucidating new mechanisms of disease. Finally, the role of the NIH national pharmacogenomics research network (PGRN) in codifying “bench to bedside” translation of genetic results that impact drug therapy will also be discussed.

INTRODUCTION
Although clinical genetics has been incorporated into many fields of medicine, its effect in surgical patients is somewhat less investigated. Having said this, anesthesiologists have long recognized that the response of apparently similar patients to drugs and surgery/interventions can be highly variable. Indeed, a drug given at the same relative concentration to an array of patients results in varying physiologic responses, creating a classic bell-shaped effect curve (or more precisely a Gaussian distribution) of response. Today it is widely recognized that variation in both pharmacokinetic and pharmacodynamic response to drugs can be explained, at least in part, by genetic differences between individuals. Therefore this review aims to update the reader on new concepts in genomic medicine and how they might be relevant to the perioperative patient and the field of anesthesiology.

GENERAL DEFINITIONS
DNA, RNA, protein, and metabolites: Genetic material that controls composition of each individual human being, from cell to entire organism, is contained in the form of double stranded deoxyribonucleic acid (DNA) in the cell nucleus in the form of chromosome pairs (23 total pairs including sex-determining chromosomes). Genes are stretches of DNA that ultimately encode a specific protein; encoded protein segments are called exons and long stretches of DNA sequence that appear before or in between exons are called 5′-regulatory or 5′-untranslated regions, and introns, respectively. While DNA is compacted by being wound tightly around histones, this tight packing intermittently unwinds so that transcription factors can bind to 5′-regulatory regions of DNA to initiate/modulate transcription of specific genes into single stranded ribonucleic acid (RNA). RNA is then processed (spliced, polyadenylated, degraded) and transcribed to amino acids (3 nucleotides encode an amino acid), and ultimately assembled into strings of amino acids, or proteins. After various cellular modifications of proteins, which provide their spectrum of activity, protein action ultimately produces small molecules, or metabolites in the cell. Metabolites form the milieu in which chemical and biologic reactions occur – such small molecules are a measure of activation/inhibition of final physiological pathways in cells.

“Omics”: After sequencing the entire human DNA of a few individuals in 2000, scientists turned to massively producing DNA sequences from individual patients with and without disease. Such massive screening of DNA is termed “genomics” and studies using these large-scale efforts, clinical genomics. The next large scale tool to be added to the genomics toolbox were large arrays consisting of thousands of single stranded RNA molecules, or fragments of such RNA, from cells or animal/human tissues. By comparing before/after conditions, changes in RNA quantities could be examined. Large-scale protein analysis has been more difficult technically since it involves predominantly the use of mass spectroscopy which is more labor intensive; this field is called proteomics. Following suit, identification of hundreds of small molecules and metabolites in cells, predominantly by old-fashioned biochemistry methodologies, is called metabolomics. Since DNA analysis is by far the easiest and cheapest of these methods, many studies using DNA sequencing surfaced first, with RNA microarrays running a close second historically. From these 2 methods, fingerprints of the genomics of tumors and diseases in patients have begun to be derived. Ironically, however, the most logical way to examine diseases would be to start with metabolomics, since this is the milieu that is most often changed with disease or acute insults. By understanding alterations in proteins and metabolites, a true signature of biomarkers is obtained. RNA microarrays can then be used to determine the mechanism and/or pathway by which such diseases occurred (rather than being primarily a diagnostic tool itself), particularly given the unstable nature of RNA in general. DNA alterations can ultimately be used as an inexpensive screening tool once such variation is linked with protein/metabolite change.
DNA sequence variation: Variation in DNA sequences may lead to alterations in protein sequence and function, and therefore form the basis of variability in disease expression and therapeutic efficacy. DNA variation can consist of single nucleotide polymorphisms (SNPs) which are alterations of a single base, or it can result from shortened (deletions) or extended repeat sequences (insertions) within DNA itself. Genome-wide association studies (GWAS) are performed routinely now and consist of sequencing thousands of short DNA sequences (markers) found throughout the entire human genome. Since there are approximately 23,000-30,000 genes in the human genome and much more regulatory DNA, even thousands of DNA fragments represent only a small fraction of total DNA. Fortunately DNA cross-overs, where 2 paired chromosomes exchange DNA inherited from mother and father, occur in fairly large fragments of DNA/chromosomes. This creates stretches of DNA that travel together, called haplotypes. Because of this fact, once the human genome was sequenced, the next step was creating a haplotype map (hap map) since SNPs within a haplotype block are often able to predict the presence or absence of other genetic variants. The field is now beginning to move beyond inferred DNA sequence changes using haplotypes, to directly re-sequencing all exon sequences known to exist to refine DNA sequence variation important in disease versus controls. Such studies are the cutting edge methods being used today and are called “exomics,” and even more extensive sequencing in numerous individuals is called “deep sequencing.”

Mitochondrial DNA: Thus far we have been discussing only genomic DNA. It is interesting to note that mitochondria, the powerhouses of cells, contain their own DNA. Mitochondrial DNA encodes only 13 genes, is circular, single-stranded, and is inherited from maternal mitochondrial DNA (as opposed to genomic double-stranded DNA inherited from both mother and father), although >1000 proteins are related to mitochondrial DNA. It is interesting to note that proteins required for development of intact mitochondria are a mixture of protein products from genomic DNA as well as the 13 genes in mitochondrial DNA. Because of the importance of mitochondria in producing free radicals with ischemia/reperfusion injury, it is increasingly apparent that variation in genomic and mitochondrial DNA is critical in determining how an individual patient may respond to injury. This is a burgeoning field and will be increasingly important in both understanding mechanisms of disease as well as using genetic variability as predictors to outcomes after surgery.

MicroRNA: Adding complexity to gene regulation is the recent discovery of microRNAs (miRNAs) and other longer non-coding RNAs. miRNAs are small 18–25 nucleotide long non-coding RNAs that modulate gene expression levels in a sequence-specific manner via the binding of mature miRNAs to complementary mRNAs. This binding negatively regulates expression of specific genes by either degrading the bound target mRNA or directly inhibiting translation. Specific miRNAs have been implicated in cell differentiation, cell apoptosis/death, ischemia/reperfusion responses, fat metabolism, and carcinogenesis in various species. Presence/absence of specific miRNAs in tumors has been hypothesized to potentially predict clinical outcome with tumor resection/treatment and ultimate clinical outcome, although one recent study in non-small cell lung cancer suggests no predictive ability. miRNAs also play a critical role in controlling cardiac stress responses that lead to transcriptional and translational alterations in gene expression. Over-expression of various miRNAs in cardiomyocytes in vitro induces cardiac hypertrophy and overexpression of miR-195, a known stress-inducible miRNA, resulting in abnormal cardiac remodeling and heart failure in transgenic mice. These findings suggest that miRNAs are important regulators of cardiac function and represent potential therapeutic targets for heart disease.

**UPDATE ON CLINICAL GENOMIC STUDY METHODS**

Candidate gene association studies: The historical standard for clinical genetics studies is the association study, where incidence of DNA genetic variants (predominantly SNPs in a few candidate genes) is examined between groups of individuals with and without a disease. Such studies require careful matching for clinical co-variants such as presence/absence of chronic disease, active medications, population stratification (race, country of origin), age, sex, clinical intervention details, etc. While such studies have been powerful, they are notoriously difficult to replicate, requiring large numbers of patients and crisp definitions of clinical outcomes (which are sometimes difficult to assure from medical records alone). In addition, even when SNPs from several genes are examined, and interactions considered, ultimately investigators “guess” which genes may be most important in a disease and use those as the starting point. As has been pointed out by many, this introduces bias in that only “known” genes/pathways are considered rather than all possible mechanisms. As a result, targeted candidate association studies alone are increasingly hard to publish unless replication in a separate group of individuals and/or associated biologic changes can be reported in the same study.

GWAS studies: Genome wide association studies (GWAS; described initially above) also examine groups of patients and control healthy individuals. But rather than examining targeted SNPs from a selected group of genes, GWAS specifically takes an unbiased approach by using thousands of GWAS markers spread across the entire genome. The theoretical advantage of such an approach is that novel pathways/genes can be elucidated that may be important in either predicting disease or providing mechanistic insights. As with targeted association studies, large populations of patients must be studied, both cases and controls. This has been difficult since GWAS panels containing...
thousands of genes per patient are quite expensive. Also, even though thousands of SNPs are examined, this still means that potentially only 1 per 10,000 DNA nucleotides is studied. Since not all genetic variations are present in haplotypes with a study marker, or related to the marker SNP by linkage disequilibrium, important genetic variability can be missed. Hence this approach should be considered a first “low hanging fruit” approach where a positive may be meaningful for common genetic variants, but a negative result may not be helpful. Indeed, some have argued that large GWAS studies in hypertension, even those with >30,000 individuals studied, have neither illuminated key genes with significant biologic effects nor unlocked the genetic basis of the disease. One conclusion from these studies is that rare genetic variants may play a bigger role in “common” disease than was originally thought.

Whole exon sequencing: In order to study both common and rare SNPs in an unbiased way, recent studies have begun to resequence all known exons across the genome. While whole genome sequencing is rapidly decreasing in price, these studies remain extremely expensive. As a result, what is often done is to identify populations of patients with a range of quantitative phenotypes (clinical expression of disease) and examine the top and bottom 10% for comparison. For example, if blood pressure is to be studied, perhaps 30 patients with the highest blood pressures and 30 with the lowest blood pressure might be examined. A major advantage of resequencing exons is that all forms of genetic variation in a given gene can be elucidated. Interestingly, genes encoding proteins known to be important in a given disease may have multiple ways they can become dysfunctional. Therefore a wide-range of rare SNPs may represent various ways to mediate dysfunction of the same gene product (protein), but would technically be considered rare SNPs rather than common SNPs due to the percent occurrence individually. Because of this phenomenon, whole exon sequencing may help the entire field of clinical genetics redefine common and rare variants over the next few years.

Importance of genetic controls for any clinical study: One important consideration that has come out of recent genetics trials is the concept of genetic controls. For example, if a trial is designed to examine the efficacy of a drug in a specific clinical setting, then it is important to ensure that genetic variability in drug metabolizing enzymes is controlled within the trial. Otherwise efficacy of a drug might be mistakenly enhanced in patients who are less able to metabolize the active drug, and hence its concentration stays higher and longer. The opposite is true for drug side-effects; they would be more common in patients unable to rapidly and effectively metabolize a given drug.

DIAGNOSING PRESENCE OF DISEASE CAUSING AGENTS

One area where medicine and anesthesiology have benefited dramatically from genomic medicine advances is in diagnosis of pathogens causing disease. This is especially true in the intensive care unit where presence of bacteria and viruses can be identified rapidly, including identification of specific strains. This is possible using diagnostic amplification of small fragments of DNA from these invading organisms. While normal flora must be taken into account, drug-resistant and highly virulent strains of bacteria can be identified now fairly rapidly, enabling treatment to be definitively initiated within hours of specimen testing. Diagnostic cultures often take several days, and can still be used for confirmation, but in many cases a more definitive anti-microbial agent can be started immediately. This decreases drug resistance within hospitals (by decreasing the use of broad-spectrum antibacterial agents) and helps to track strains present within outbreaks.

In the outpatient setting, diagnosis of sexually transmitted diseases has also been greatly enhanced using molecular genetic approaches to diagnose presence and virulence of specific strains. Recent discoveries suggest a new mechanism of sexually transmitted disease may be infection by non-viral Trichomonas vaginalis which may itself be infected with up to 4 distinct strains of viral DNA, complicating overall disease expression. This type of information is crucial for modern day public health tracking and interventions.

Chronic disease patients also benefit from examination of pathologic infectious agents. For example, patients with cystic fibrosis often have gram negative lung infections since they have difficulty clearing their thick mucous secretions. A recent study examined the role of specific strains of Pseudomonas Aeruginosa in patients with cystic fibrosis and demonstrated that a common strain (Liverpool epidemic strain) is associated in England, Australia, and Canada with worse lung function, death and/or need for lung transplantation in this vulnerable population of patients. This information then provides the opportunity to intervene in such patients more rigorously.

CURRENT CLINICAL HUMAN DISEASE APPLICATIONS

Tumor diagnosis and treatment: Traditionally, tumor diagnosis has been accomplished using histology and pathologic methods. Such approaches have increasingly relied on antibodies capable of identifying tumor markers, which generally are proteins uniquely expressed in tumor cells and not in host tissue cells. However, since the genomic revolution, it has been recognized that genetic abnormalities in cells that ultimately go on to become cancerous can be harnessed for diagnosis and prediction of treatment options and efficacy. This has been true for childhood cancers for almost 2 decades since isolation of tumor cells in blood is rather easily available. However, it is a harder prospect for solid tumors. Hence new molecular findings relating molecular markers (predominantly DNA deletions and mutations) for specific brain tumors (gliomas) are encouraging since they appear to
facilitate diagnosis, management, and predict outcome in low-grade gliomas. In addition, in other studies involving neuroblastoma, the important prognostic role of the ABCC1 (ATP-binding cassetted sub-family C member1) gene for patient outcome has recently been suggested. Another example is breast cancer where BRCA gene mutations are well known to increase risk of breast cancer in a subpopulation of patients, yet the majority of breast cancers without BRCA mutations remain difficult to categorize and, in the cases, treat. Molecular genetics of tumors is an important growth area in medicine and may be able to finally unlock adult solid tumors to the point of having better response to therapeutic intervention and ultimately better outcomes.

Cardiovascular disease: Many aspects of cardiovascular disease have a genetic component, ranging from coronary disease to familial peripheral arterial calcification, blood coagulation, and cardiovascular drug action. Even chronic inflammation, known to be important in the acquisition and progression of cardiovascular disease, has been examined in terms of “inflammasome-mediated disease”. In this review we highlight one example of a commonly used clinical genetics approach to two types of anti-coagulation.

One of the more thoroughly investigated areas where genomic approaches have real impact on clinical practice is in the area of coagulation, specifically prediction of starting dose for highly toxic drugs such as warfarin (coumadin) and use/efficacy of anti-platelet drugs such as clopidogrel. In these settings, genetic testing can reveal opposite situations. For warfarin, genotypes for warfarin metabolism and vitamin K (e.g. genotype variants of Cytochrome P450 metabolizing enzymes CYP2C9 and CYP4F2, as well as the vitamin K activating enzyme VKORC1 which requires less warfarin for inhibition) have been shown to be important in improving prediction of therapeutic warfarin dose and overall anticoagulation management versus standard clinical approaches. Because the improved prediction has great potential to limit warfarin side-effects such as excessive bleeding and emergency room visits, genetic testing is becoming more routine as warfarin is initiated. For clopidogrel, an antiplatelet drug, it is usually therapeutic efficacity, rather than side-effects, that is tested. Interestingly, clopidogrel is a pro-drug, so individuals with specific genetic variants cannot metabolize the pro-drug to active drug and hence do not respond with the expected anti-platelet activity. This results in lack of protection from myocardial infarction in the setting of unstable angina or interventions such as coronary artery stent placement. This risk is considered so high, and clopidogrel so common in this important clinical setting, that the FDA recently put a black box warning so that clinicians would be aware to prescribe alternative anti-platelet drugs to the subset of patients who are non-responders. Only recently has genetic variability of the enzymes regulating metabolism of the active drug been also investigated as another source of variable clinical outcomes.

Transplantation: Because genetic variation exists in molecules regulating innate and adaptive immunity, organ transplantation has become an area where genetic approaches are becoming increasingly considered. Genetic variants in this setting can have important effects in both organ preservation (e.g. sufficient immunosuppression to prevent rejection) and drug side effects (e.g. limiting immunosuppression side-effects such as infection, metabolic derangements, and renal injury). These effects include immune system modulation as well as drug metabolism pathways (e.g. CYP3A5 for tacrolimus dosing). Taken together, effects on acute rejection, delayed graft function, long-term allograft dysfunction and mortality, post-transplant metabolic complications, and recurrent disease are affected by many known genetic variants, specific for each phase of transplantation long-term success. Genetic variants and mRNA profiling that can be used for screening purposes, as well as future visions for how genomics can add value in this unique area of medicine, have been summarized in several recent reviews.

Translation of genomic findings from “bench to bedside”: It is difficult for the average clinician to keep up-to-date with new genetic information, specifically what genetic variants should be taken into account in drug therapies used to treat common diseases. With this in mind, almost 10 years ago the National Institutes of Health recognized the need to have researchers create and collate data on genetic variants important for drug action. They created a group of researchers called the Pharmacogenomics Research Network (PGRN), located at multiple sites across the U.S., who participate in clinical genetics trials in various common, complex human diseases. Their findings are located on the PGRN website at the NIH (http://www.pharmgkb.org/) which is helpful is the pharmacogenetics knowledge base (PharmGKB; http://www.pharmgkb.org/) which is frequently updated based on new results from PGRN investigators’ clinical trial findings. Data is annotated so clinicians can understand strength of results and recommendations. Although this site is not meant to be used as sole criteria for dosing a patient clinically, it does provide education, references, and definitive guidelines by the manufacturers, as well as results of interactions between various genetic variant combinations that might be present in a given patient. A specific subgroup of the PGRN is called the Clinical Pharmacogenetics Implementation Consortium (CPIC), which is currently a group of 6 medical centers who are in the process of implementing at least one (and often several) common genetic variants into their electronic ordering system in order to give every clinician at their institution expert advice at the point of drug ordering.
GENETIC VARIANTS REVEAL NEW MECHANISMS OF DISEASE

Naturally occurring human genetic variants can also provide insights into disease mechanism. An example of this can be seen in alpha1-adrenergic receptors (α1ARs), which are G protein-coupled transmembrane receptors that mediate actions of the sympathetic nervous system through binding of endogenous catecholamines epinephrine and norepinephrine (NE). Among the 3 α1AR subtypes, 1αARs predominate in human vascular smooth muscle, particularly in resistant vessels. Vasoconstriction and vascular remodeling are precipitating factors in human hypertension, a major cardiovascular risk factor for developing heart disease and stroke. Stress-induced development of hypertrophy is characterized by changes in the structure of both blood vessels and heart. Recently it has been found that a genetic variant present in the 3rd intracellular loop of the human 1αAR constitutively couples to a distinct biochemical pathway with enhanced cellular growth effects. Such findings suggest that by discovering new pathways activated by genetic variants in physiological pathways, entirely new drug classes may be considered in the treatment of common diseases such as hypertension.

CONCLUSION

Clinical genetics has become part of mainstream medicine in many settings relevant to anesthesiologists. This brief review has highlighted key areas of medicine where genetic testing is routinely used for diagnosis, prediction of treatment efficacy, or elucidating more fundamental mechanisms of disease.

REFERENCES

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