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An update on genetic and perioperative patient management

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An update on genetic and perioperative patient management

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LEARNING OBJECTIVES

1. Be able to describe genetic polymorphisms and how they may change proteins
2. Be able to describe the difference between background genetic changes and those that may have clinical impact
3. Describe how to begin planning for a genetic outcome study in the perioperative period

INTRODUCTION

Genetic principles are increasingly being incorporated into every day medicine. In spite of this, confusion exists among physicians and other health care providers regarding how to use this new information most accurately. Furthermore, since common complex medical diseases are often multigenic and little is know on the exact genes involved for most diseases to date, where does a physician scientist start in examining whether genetics plays a role in a particular disease or patient outcome? Answers to these questions are addressed in this tutorial, but first a review of basic concepts important in genetic medicine is important.

GENETIC VARIABILITY

Anesthesiologists have long recognized that response to drug administration or stress depends on the individual. In fact, a bell shaped curve of responses to various environmental perturbations (drug administration, hemodynamic challenge, inflammatory response to stress of surgery, etc.) demonstrates that while most patients respond in predictable patterns, others respond either more/less vigorously (Figure 1). In fact, much of the "art" of anesthesiology is the astute clinician prepared to deal with "outliers." Increasingly clinicians are appreciating that an individual patient's response to stress may alter perioperative outcomes such as incidence of respiratory distress syndrome, perioperative myocardial infarction, survival, and response to pain management. But what are the mechanisms underlying variability to pharmacodynamic and physiologic stress? The answer(s) to this complex question include understanding how the unique genetic background an individual brings to the operating room affects his/her surgical outcome. This lecture reviews basic genetic information and then explores how some genetic variants have been shown to alter specific patient diseases and therefore may be important to consider during the perioperative period.

BASIC GENETIC VARIABILITY

Genetics has revolutionized medicine. Sequencing 3 million nucleotides of human DNA as part of the human genome project has been hailed as one of the greatest achievements of our time. Most DNA is identical between individuals, as evidenced by the fact that humans are usually easily differentiated as Homo sapiens rather than other mammalian species! However, variation in exact DNA sequence does exist between individuals, making each person unique in terms of hair/eye color, body habitus, fingerprint, mood, and approach to life. In the context of medicine such genetic variability may have important implications (Figure 2). For example, a single nucleotide change in DNA in the hemoglobin gene results in an altered amino acid in the encoded protein leading to enhanced hemoglobin sickling upon exposure to hypoxia or acidosis; sickle cell anemia is the resulting disease. While most classically inherited genetic diseases tend to be rare, DNA variation may have more subtle effects in complex diseases where multiple genes are thought to interact to produce the final clinical outcome (Figure 3). In such a situation (e.g. diabetes, hypertension, atherosclerosis), rather than directly causing the disease, genetic variability may contribute to disease onset or progression. Even in biologically important proteins, not all genetic variants result in alterations in function. In order to characterize the functional

consequence(s) of altered DNA sequence, researchers often resort to examining intermediate biologic endpoints. For example, genetic variants of P450 metabolizing enzyme genes may result in altered enzyme activity and altered drug levels, or no change at all. Furthermore, specific variants may alter drug metabolism to differing degrees. Sometimes the effects of genetic variability are not as easily quantitated since they result in altered biological function only during stress (e.g. receptor variants with enhanced desensitization properties upon agonist stimulation). Such genetic variability may have significant clinical implications during surgery or trauma that cannot be predicted at this point with anything other than identification of the DNA sequence variant and its association with perioperative outcome. While more difficult to elucidate, such investigations (which require the combination of detailed genetic and clinical databases) may lead to some of the most important discoveries in clinical medicine over the next 5-10 years. The broad field examining effects of genetic variability on protein function or clinical outcome defines the brave new world of pharmacogenetics and functional genomics.

BIOLOGIC CONSEQUENCES OF GENETIC VARIABILITY-MOUSE MODELS

One way scientists have evaluated biological consequences of genetic variability is to over express or eliminate genes using mouse models (transgenic/knockout mice, respectively) (Figure 4). Many important discoveries and mechanisms have been elucidated using these approaches. For example, the fact that receptors exist in dynamic equilibrium between inactive (R) and active (R*) forms was first discovered in transgenic mice over expressing β_2-adrenergic receptors (β_{2A}Rs). If 1% of receptors are in the active (R*) state in normal mice, then it

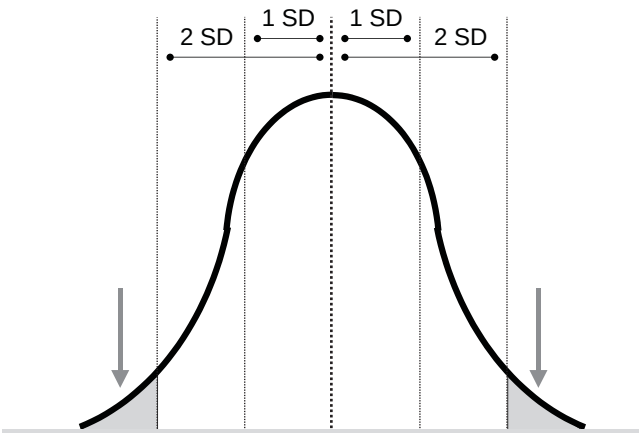

Figure 1. Curve of responses to various environmental perturbations.

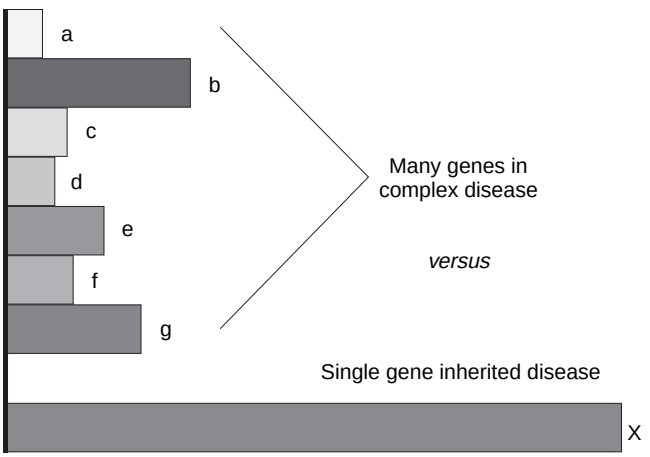

Figure 3. Complex diseases are usually multi-factorial.

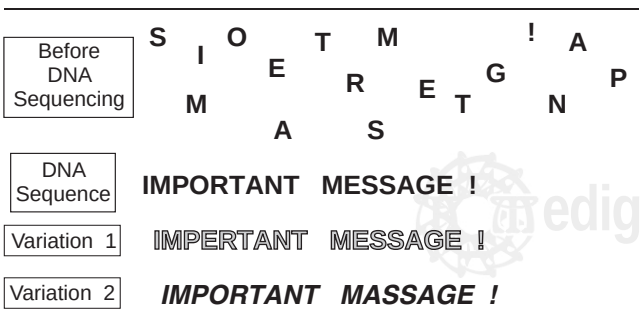

Figure 2. When are genetic polymorphisms important?

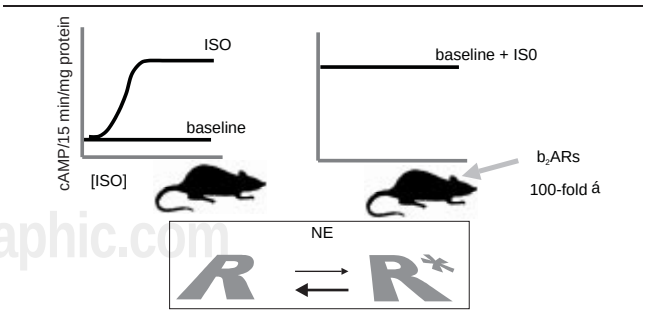

Figure 4. Testing biologic significance: Transgenic/knockout mice.

will take agonist isoproterenol (ISO) to activate the receptor. However when the receptor is over expressed dramatically, 1% becomes a very large number, leading to maximal activity in the absence of agonist (Figure 4). The discovery of an elevated baseline in the absence of agonist was a surprising discovery that led theoreticians back to the concept that agonists stabilize R* rather than causing the shift from R to R*. In spite of these elegant mechanistic discoveries, lingering questions remain regarding the ultimate usefulness of murine (mouse) models since not all signaling molecules are the same in mice and men. Hence, the rest of this review will focus on genetic variability in humans.

GENETIC VARIABILITY IN HUMANS

Some genetic variability in humans can be considered "background," leading to distinctive personal traits, but having no biologic consequence. Other genetic variants, however, are important clinically since they may be linked to patient outcome. Indeed, β_2AR genetic polymorphisms have been shown to be clinically relevant in diseases such as hypertension, asthma, and congestive heart failure (Figure 5). In the β_2AR gene, genetic alterations in the upstream leader sequence (an introductory regulatory sequence occurring immediately upstream from where the protein coding sequence begins) result in enhanced β_2AR expression. Resultant increased airway β_2ARs have been shown to be protective against methylcholine-induced broncho-constriction. Another β_2AR genetic variant (Arg16) enhances downregulation, or dampening of receptor function. Since β_2ARs mediate vasodilation, it is not surprising that this dampened variant results in increased blood pressure. In fact this variant is associated with hypertension. It is important to note at this point that the 16Gly, 19Arg, and 27Glu variants travel together (Figure 5), as do the more common Arg16, Cys19, and Gln27; in other words they define a haplotype. This means that if an individual has the 16Gly variant, he/she will most likely also have the other 2 variants since this "chunk" of DNA is inherited together in most individuals. Finally, a very rare, but clinically important b_{2A}R variant is the Thr164Ile; this variant appears to have no clinical cardiac effects until patients experience congestive heart failure (CHF), often later in life. Once CHF appears, however, patients with 164Ile have a more rapid downhill course clinically. If such an individual is considered for cardiac transplantation, it may be prudent to them toward the top of the list. It is important to note that most often genetic variants alter understood physiologic pathways. Hence β_2AR variants make sense clinically. However, since we do not understand fully all of the pathways necessary for normal cell function, naturally occurring genetic variants often give surprising insights. This suggests that until all human biochemical pathways are understood in detail, it will be important to continue to use a combination of genome-wide scans, targeted candidate gene studies, and intensive resequencing of genes already known to be important in given diseases in order to elucidate how genetic variants affect complex clinical outcomes in humans.

GENETIC HAPLOTYPES

Up until this point, we have described mutations that are causative. That is, the genetic variant described is responsible for disease mechanistically. However, in clinical medicine, it may not be necessary to identify precisely the genetic mutant involved/associated with a disease. Because genetic variability is most often the result of chromosomal crossovers over generations, this means that regions of each chromosome travel together (see example described above for β_2ARs). The ability to categorize human chromosomes by "chunks" of chromosomes or haplotypes is convenient (Figure 6). This fact suggests that clinical outcomes may be able to be predicted simply from the presence of a known genetic marker, since it should predict presence/absence of a disease-altering genetic variant downstream on the chromosome. From a practical laboratory perspective, often one genetic variant is easier to discern than another given its chemical properties.

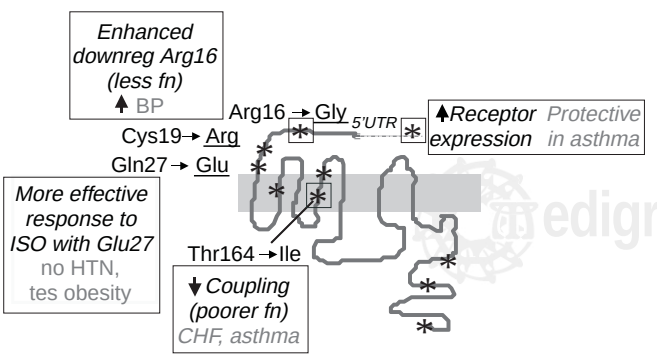

Figure 5. Genetic variability.

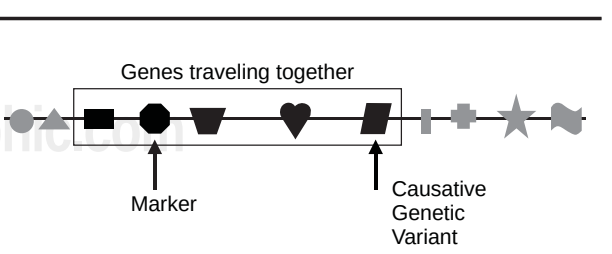

Figure 6. Haplotypes: Genes traveling together.

Therefore clinical tests may be able to be designed more easily for a marker downstream than for the causative variant. It is the ability to know which genes travel together that enables genetic markers to be valuable in identifying individuals at risk for a given disease. This is particularly relevant since this fact enables us to design predictive clinical tests for disease now while scientists work out the details of precisely which genetic variant actually mechanistically causes disease or alters its progression.

PERIOPERATIVE GENETIC PREDICTIONS

Perioperative pharmacologists currently incorporate genetic tools into their studies in order to identify factors that influence surgical outcome. In the future, rather than simply tabulating patient risk factors (e.g. age, race, sex, history of hypertension, congestive heart failure, chronic obstructive pulmonary disease), a more detailed genetic history will need to be taken into account. It is not hard to imagine use in the near future of a preoperative "gene chip" designed to highlight the most notable genetic variants thought important in bleeding, inflammatory, and neurologic responses to perioperative stress. At that point in medical history, we as perioperative physicians will have far more robust information for use in designing the most appropriate and safest anesthetic plan for a given patient. Thus "designer anesthesia" is not far away in this new pharmacogenomic era. But how can this brave new world be accomplished? A brief overview is given below.

PRACTICAL ISSUES IN PERIOPERATIVE GENETIC ASSOCIATION STUDIES

In order to determine whether a genetic variant is important to perioperative outcome or disease, variants must be tested to see if they are enriched in the population with the outcome to be studied. This approach is called an association study because it associates the genetic variant with a disease or outcome. The most common association study methodology used today is the candidate gene approach. In this method, the researcher identifies physiologic pathways known to be important in a given disease or end organ, and then examines the literature for evidence that genetic variants are present in proteins in these particular pathways. The problem with this approach is that it presumes that all physiologic pathways important in a disease are known and that all genetic variants have been discovered; these presumptions are most often not true. Therefore more "unbiased" approaches to determining candidate genes have been introduced recently; tools such as microarray or proteomic analysis (which measure how mRNA and protein levels [and/or phosphorylation state for proteins] are altered between "healthy" and "diseased" tissues. But once specific molecules (proteins or genes) are identified using these more unbiased approaches, then a search for variants begins (known variants via the literature or identification of new variants via large-scale resequencing). The next step is to identify the best patient population to study and then collect detailed, reproducible, carefully defined clinical endpoints. Since the design of an association study can be complicated, it is wise to involve a statistical geneticist from the beginning. Obtaining informed consent for genetic studies is somewhat more involved than a standard clinical trial, since there are ethic issues important to communicate with the patient. Once patients are enrolled, collection of blood is fairly straightforward, but careful processing of the sample and information is critical. Bar coding is important! Accurate genotyping is also essential. In complex disease many genes may interact to give a particular clinical description (or phenotype) of a disease, so finding an association is difficult for a given single gene under the very best circumstances. Any mistakes at a genotyping level decrease the power of the study dramatically. Once all databases are aligned and genotyping completed, there are various strategies statistical geneticists use to determine whether association exists and whether that association is reproducible within the initial data set and/or in other populations. Several possible approaches to data analysis will be discussed during the tutorial.

CONCLUSION

Genetics is the wave of the future. Carefully crafted and well-completed perioperative genetic studies have the potential to contribute to our understanding of genetics and medicine, with impact far beyond the operating room.

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