Genomics and Future Treatment Implications

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Learning Objectives:
1. Describe variability in the human genome and how it impacts human physiology
2. Discuss progress made in genomic technology
3. Describe clinical evidence supporting the role of genetic variation in clinical outcomes
4. Outline possible clinical approaches for utilizing genetic variability to maximize patient outcome

1) **Introduction**

a) Complications such as postoperative heart failure, myocardial infarction, arrhythmias, stroke and transplant rejection have important implications for health care costs, quality of life and postoperative survival. Improved understanding of how genes and environment interact in the perioperative environment may result in better prevention and treatment of these adverse postoperative events.

b) Genomics is the study of genes and their function.
   i) Structural genomics tries to enhance understanding of the structure of the genome (ie. mapping genes and sequencing chromosomal regions).
   ii) Functional genomics includes assessment of genes, gene mRNA transcripts, and ultimate translation to proteins.
   iii) **Perioperative genomics describes efforts to understand how genetic variants may influence patient outcomes with surgery.**
   iv) Pharmacogenomics describes efforts to define novel genetically influenced biology that can be the focus for developing new drugs and for using presently available drugs more effectively with fewer related side effects.

c) Molecular biology review
   i) DNA is double stranded helix that involves pairings of 4 types of nucleotides: adenine (A), guanine (G), thymine (T), and cytosine (C). Pairings are A to T and G to C
   ii) DNA serves as a template for mRNA transcription
   iii) A human gene is a DNA sequence at a specific location on either the 22 pairs of human non-sex (autosomal) chromosomes or on the 2 sex chromosomes. Each gene sequence codes for a specific protein
      (1) Promoter regions of genes are involved in the timing and amount of DNA gene transcription to mRNA
      (2) Exons are regions of the gene that ultimately code for the amino acid sequence of the corresponding protein
      (3) Introns are non-coding portions of the gene that are not transcribed into mRNA. Sometimes intronic regions have enhancer or promoter functions.

2) **Variability in the human genome and how it impacts human physiology**

a) Human genome project: a complete draft of the human genome was released in 2003; however novel variants within the genome are still being discovered/described.

b) Studies that link gene sequence variations to phenotypic changes (intermediate phenotypes such as biomarkers and other proteins as well as clinical disease phenotypes)
should expand knowledge regarding the molecular biology underlying complex common diseases such as coronary artery disease.

c) An allele refers to variation in base pair coding on two different chromosomes at the exact same chromosomal location within a gene.

d) While an allele may be “silent” resulting in no change in ultimate protein structure or function, allelic variation may also result in changes in protein structure and function that then lead to changes in a patient’s outward clinical presentation (ie. disease phenotype).

e) Minor allele refers to the variant that occurs less commonly, while the major allele refers to the variant that occurs more commonly.

f) Types of genetic variation

i) Single nucleotide polymorphisms (SNPs): most common allelic variations (over 13 million SNPs have been discovered to date; overall occur about every 1000 base pairs, although they are not evenly distributed across the genome)
  (1) Point mutation where nucleotides pairing at a single chromosomal position differ (ie. A instead of C at a position).
  (2) SNP frequencies (minor and major allele frequencies) often vary between racial groups (population admixture).
  (3) SNPs are typically biallelic, although tri- or tetra-allelic forms exist
  (4) Often silent in that the nucleic acid switch does not ultimately result in an amino acid change in the resulting protein.
  (5) SNPs in promoter regions can result in changes in transcription and related quantity of protein production
  (6) SNPs in exonal or coding regions can cause changes in actual protein structure and function
  (7) Copy number variants: duplications or deletions of segments of a gene or multiple genes within a chromosome
  (8) Frequently copy number variants increase related protein expression

3) Progress made in genomic technology

a) Genotyping of known SNPs

i) There has been significant effort devoted to developing accurate, rapid and affordable approaches/technologies for SNP analysis.

ii) Genotyping throughput has improved substantially in recent years because of advances in multiplexing capabilities. Using some genotyping technologies up to a million SNPs can be detected in parallel per assay.

iii) There are multiple technical approaches available for SNP identification. We will discuss techniques commonly referenced in gene association studies. Primer extension and hybridization techniques for SNP genotyping are both readily conducted at standard core genotyping laboratories.

(1) Primer extension techniques measure each allele of the SNP by binding different complementary DNA sequences to each of the alleles. The resulting sequences are then detected by mass spectrometry (different molecular weights for each primer) or fluorescence (different fluorescent labels for each primer).

(2) Hybridization techniques involve gene chip arrays containing many allele specific probes for SNPs (some available chip microarrays are designed to detect >900,000 SNPs in parallel for an individual DNA sample assay; genome-wide genotyping).
After DNA hybridization to the chip, SNP genotypes are inferred using fluorescence.

(3) Sequencing takes a portion of the genome and chips off each nucleotide in turn, measuring each one by fluorescence. SNPs are found by having mixed fluorescence at one position in the genome.

b) Technology for transcriptional profiling (gene expression analyses)
   i) Among other things, microarray technology allows simultaneous examination of genome-wide changes in expression of thousands of prespecified mRNA transcripts.
   ii) New high throughput sequencing techniques can now be used to analyze complete transcriptomes across the entire genome. Genome wide transcriptome analysis allows detection and quantification of novel as well as previously described transcripts.

c) Other evolving applications for genomic technologies

d) Some useful publicly available SNP databases are outlined in a review by Kim and Misra.

e) There have been advances in software for genotype calling (major allele homozygote, heterozygotes, minor allele homozygotes).

f) There have been advances in accessible statistical software to analyze large amounts of genotyping for case-control gene association studies. Such software addresses statistical power, quality control of genotyping data, and population structure.

g) Common statistical issues encountered in genome-wide association studies are nicely reviewed by Teo, Y (2008).

4) **Clinical evidence supporting the role of genetic variation in clinical outcomes**

a) Perioperative responses to major cardiovascular and thoracic surgeries and related stresses such as end-organ ischemia, anesthetic agents, hemodynamic fluctuations and inflammatory responses (particularly in patients requiring extracorporeal circulation or massive transfusion) contribute to adverse cardiovascular outcomes in some patients but not others.

b) Approximately 1.25 million postoperative cardiovascular complications occur each year.
Gene association studies (case:control studies) are increasing being conducted by perioperative investigators. Results of such studies conducted to date increasingly support that there are significant genetic influences on perioperative cardiovascular outcomes.

c) To date published studies of adverse perioperative cardiovascular outcomes have been candidate gene studies, meaning variants within select genes have been chosen *a priori* for assessment with outcomes based on potential biology related to those genes. Genome-wide association studies allow an unbiased assessment of up to a million SNPs in relation to outcome, yet these studies require large numbers of patients, are more costly than candidate gene studies and have to date been published with regards to ambulatory cohorts.

d) Results of several gene association studies will be reviewed in order to provide a sense for what some prior cardiovascular research has shown, what approaches have been taken to assess outcomes related to cardiovascular disease and to suggest future directions for perioperative genomic research. There are numerous excellent genome-wide association and candidate gene studies in the literature, and it cannot be overemphasized that the list below does not include many of these excellent papers.
   i) Genome wide association study of atrial fibrillation in ambulatory cohorts
   ii) Candidate gene study of postoperative atrial fibrillation
   iii) Gene expression studies and heart transplant rejection

5) **Possible clinical approaches for utilizing genetic variability to maximize patient outcome:**

*Future directions and implications*
a) Preoperative knowledge of patients with genetic profiles that render them “at risk” for adverse surgical outcomes should allow for improved perioperative risk stratification and related anesthetic and surgical planning.

b) Unbiased genome-wide association studies should identify genetic regions with high association with specific adverse postoperative outcomes. Identification of such novel associations should lead to better understanding of the biologic mechanisms underlying perioperative events and should allow targeted prevention and treatment breakthroughs.

c) Establishment of multiple large surgical patient databases and blood repositories will enhance ability to perform and replicate findings from genome-wide association studies.

d) Establishment of surgical tissue repositories will enhance efforts to understand the biology underlying gene association findings by enabling gene expression and proteomic studies.

e) It may be that perioperative stresses unmask genetic predisposition to adverse cardiovascular events that are also relevant to the same adverse cardiovascular events in ambulatory populations. Findings from perioperative gene association studies should also be assessed in large and well phenotyped ambulatory cohorts.
References

7. Teo YY: Common statistical issues in genome-wide association studies: a review on power, data quality control, genotype calling and population structure. Curr Opin Lipidol 2008; 19: 133-43