

## Genomic Discoveries in Perioperative Medicine

### Educating the Audience

IN the summer of 2002, the reality television hit *American Idol* swept the world and instantly became a trend-setting phenomenon. In similar award-winning fashion, this issue of ANESTHESIOLOGY features a study by Fox (no relationship with the television network) *et al.*<sup>1</sup> that was orally presented at the 2008 American Society of Anesthesiologists Annual Meeting session "Best Abstracts of the Meeting: Anesthesiology Editor's Picks." The authors associated candidate gene variation in the natriuretic peptide system with cardiovascular phenotype, namely ventricular dysfunction (VnD) after coronary artery bypass grafting (CABG). The magnitude of this modern collaborative clinical trial is remarkable. These authors have previously written an excellent pharmacogenetics review in ANESTHESIOLOGY,<sup>2</sup> but the present study epitomizes the expanding emphasis on clinical genomics, a formative subject field in the anesthesiology literature.

Unfortunately, one may argue that the biggest challenge of disseminating this innovative research is the ability of the general audience to understand it. Blame-worthy of success, the mind-bending speed of genomic technology and discovery has outpaced genomic education in medical school and anesthesiology residency. Eventually, education in genomics will be essential for practitioners to translate genomic discoveries into clinical practice. In the meantime, the present article may generate fanfare somewhat short of *American Idol*, but pointed similarities between the two may improve our understanding of case-control genotype-phenotype association studies, as illustrated in figure 1.

In the beginning (2001), the network (institution) had the foresight to consider the impact of genetic variation on cardiovascular disease phenotypes. The *CABG Genomics Study* was created to prospectively collect blood samples for DNA analysis in 3000 patients undergoing CABG. By storing the DNA and

building a vast repository of clinical data, candidate genes could be sequenced and their relationships with disease-related phenotypes could be explored in perpetuity.

The judges (authors) searched the globe (genome) for genes that would be expected to become famous for their influence on heart function after CABG. Initially, thousands of genes waited in long lines to make fruitless and pathetic auditions, only to be dismissed as unimportant or irrelevant. The authors wisely narrowed the field to genes that encode or regulate well-accepted and readily obtainable biochemical markers of cardiac dysfunction. The working hypothesis was based on evidence that genetic variation in the natriuretic peptide system influences cardiovascular disease-related traits.<sup>3</sup> The experimental question was whether common allelic variations (single nucleotide polymorphisms or "SNPs") in these genes independently predict risk of VnD following CABG.

With genes as contestants, clinical phenotypes set the stage. All patients in the analysis underwent CABG surgery nonemergently, without the presence of factors such as preoperative anemia, inotropes, assist devices, creatinine greater than 3 mg/dl, missing B-type natriuretic peptide (BNP) and troponin values, or non-European ethnicity. This left 697 patients who were then categorized into 2 groups: (1) control: preserved ventricular function postCABG (No-VnD, n = 621); (2) case: presence of VnD postCABG defined by the need for at least 2 inotropes or placement of assist-devices (n = 76). From these patients, the authors genotyped 155 SNPs in seven candidate genes involved in the natriuretic peptide system. Some outlying SNPs were excluded for reasons such as rare incidence of the minor allele (less than 1%, technically labeling this allele a mutation rather than a SNP). As shown in the Supplemental Digital Content appendix, each SNP was presented by their genome-wide unique "rs" identifier, their chromosomal location, and their affiliated gene region.

The first round of competition determined the presence of each minor allele (generic "a") and major allele (generic "A") in the two groups. For instance, a significant SNP was rs549596 on chromosome 1 in the gene region that encodes the precursor protein for BNP (NPPB). Allele frequency in the No-VnD group was reported as aa/aA/AA = 124/306/191, meaning that 124 patients were homozygous for the minor allele, 306 were heterozygous, and 191 were homozygous for the major allele. Allele frequency in the VnD group was aa/aA/AA = 9/30/37.

◆ This Editorial View accompanies the following article: Fox AA, Collard CD, Sherman SK, Seidman CE, Seidman JG, Liu K-Y, Muehlschlegel JD, Perry TE, Aranki SF, Lange C, Herman DS, Meitinger T, Lichtner P, Body SC: Natriuretic peptide system gene variants are associated with ventricular dysfunction after coronary artery bypass grafting. ANESTHESIOLOGY 2009; 110:738-47.

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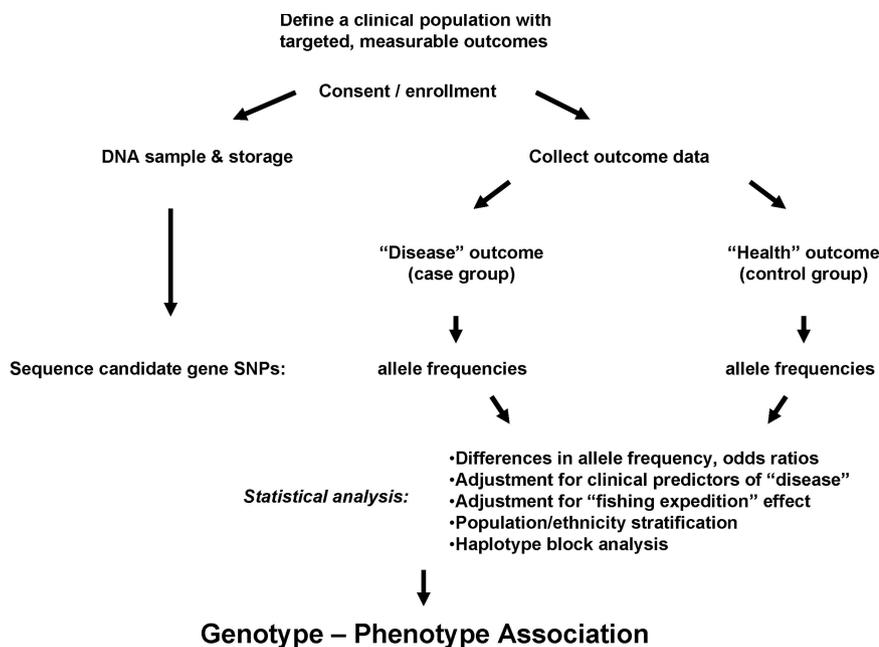


Fig. 1. General approach for case-control genotype-phenotype association studies examining identified single nucleotide polymorphisms (SNPs) within candidate genes.

For each SNP, the association of genotype with disease was analyzed by logistic regression in three models: (1) additive model, meaning “does the number of minor alleles in this SNP differ between groups?”; (2) dominant model, meaning “does the presence of at least one copy of the minor allele (compared to no copies) predict disease group?”; and (3) recessive model, meaning “does the presence of 2 copies of the minor allele (compared to 0 or 1 copy) predict disease group?” In our example, the rs549596 minor allele was significant in the additive and dominant model. The odds ratio and confidence interval were less than 1, indicating that this minor allele was more prevalent in the control group (No-VnD). The minor alleles of 13 SNPs in the NPPA/NPPB gene region were associated with No-VnD. The minor alleles of seven SNPs in the natriuretic peptide receptor type-3 gene (NPR3) were significant in the recessive model; the odds ratios and confidence intervals were greater than 1, thereby associating these minor alleles with the case group (VnD).

The next round of competition adjusted for multiple SNP comparisons, screening for the “fishing expedition” effect. This adjustment compensates for the fact that searching a large enough family of SNPs within a gene is likely to reveal some SNPs that are statistically associated with phenotype simply by chance. Hence, the authors performed a family-wise adjustment for total number of SNPs assessed in the gene region. As shown in table 3,<sup>1</sup> this reduced the field to 11 SNPs in the NPPA/NPPB gene and 6 SNPs in the NPR3 gene.

The next round scrutinized the SNPs by multivariable analysis, accounting for patient characteristics (*i.e.*, age and body mass index) and clinical predictors of VnD. The predictors of VnD were low ejection fraction preoperatively, increased BNP preoperatively,

and cardiopulmonary bypass time greater than 2 h. As an added challenge, SNPs who survived this cut also underwent adjustment for the family-wise “fishing” effect. As shown in table 4,<sup>1</sup> this reduced the field to seven finalists in the NPPA/NPPB gene region and four finalists in the NPR3 gene. Thus, these SNPs have evidence of an independent association with phenotype that could not be explained by clinical factors or the number of SNPs assessed.

The encore performance was the haplotype analysis. A haplotype is the collective genotype of a number of closely linked loci on a chromosome. In other words, how were these minor alleles in the SNPs inherited in relation to one another? To determine this relationship, we look for linkage disequilibrium, which represents the chance that two alleles from different sites on a chromosome are inherited together more often than chance alone. In the article,<sup>1</sup> figure 1 is a linkage disequilibrium plot of all SNPs studied in the NPPA/NPPB gene region listed topographically along chromosome 1. The analysis creates a strength-of-inherited relationship between each minor allele so that the block structure forms an inverted triangle. Intersections between alleles present a number and a color shade that increase (and darken) in proportion to the strength of linkage. Why is this important? Because there are an estimated 10 million SNPs in the human genome, and we cannot sequence them all for every study. The linkage disequilibrium relationships may allow future investigations to genotype fewer SNPs to define the majority of common genetic variation. Moreover, because one SNP is extremely unlikely to be a disease-causing locus, an understanding of linkage disequilibrium relationships aids the interpretation of the findings and may assist in determining candidate gene regions and their interactions that influence phenotype.

In the end, the finalists go on tour. Several steps will be required to translate these findings into clinical practice, including replication from additional studies in multiple ethnic and racial populations, functional analysis of these variable sites in animal and human tissue models, and feasible genotyping strategies in surgical patients. This study has potential implications for the prediction of postoperative morbidity, development of disease prevention strategies, and personalized pharmacological interventions that pertain to the natriuretic pathway. This study also provides a blueprint for future episodes. Stay tuned.

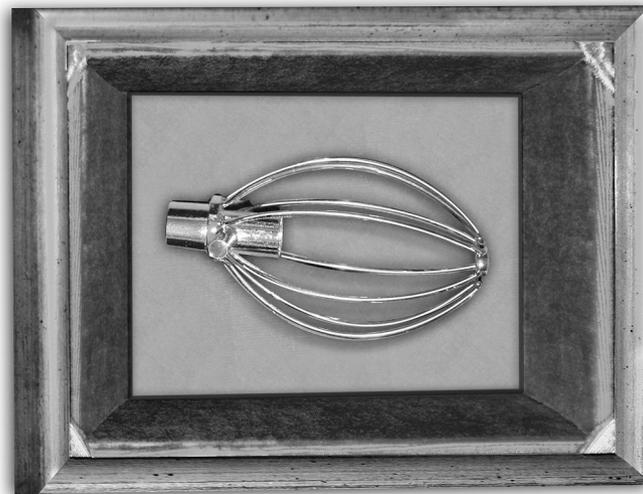
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## ■ ANESTHESIOLOGY REFLECTIONS

### Gold-plated “Pender Lemon” Ether Device



In 1943 Lt. John William Pender, M.D., U.S.N.R. (1912–2001) invented his “Pender Lemon,” which facilitated the open-drop use of ether on patients in unusual neurosurgical positions. He presented his gold-plated Pender Lemon (pictured here) to the Wood Library-Museum of Anesthesiology. For decades he and Dr. John Leahy encouraged the WLM’s videotaping of anesthesiologists interviewed by their peers. After retiring as Stanford University’s first Emeritus Clinical Professor of Anesthesiology, Dr. Pender bequeathed funding for the “Mayo Room” and for oral history at the WLM. Today, the WLM’s Robert D. Dripps Room houses the John W. Pender Collection of the Living History of Anesthesiology. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in the *Anesthesiology Reflections* online collection available at [www.anesthesiology.org](http://www.anesthesiology.org).)

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