Meeting report

Cardiovascular genomics: recent progress, current challenges, future promise
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In the next decade, it is anticipated that the Human Genome Project and related human and animal genome sequencing projects will exert a profound influence on our understanding and management of human disease. A complete sequence of the human genome and a first-pass map of common genetic variants (single-nucleotide polymorphisms, SNPs) is expected within the next few years. Thus, attention has now turned to the many disease areas for which there may be applications for genomics, and the related subfields of functional genomics, proteomics, and bioinformatics. One important area of focus will be cardiovascular disease, the leading cause of death of men and women in the developed world and soon to be in the developing world. Traditional genetics has taught us much about genes underlying rare familial cardiovascular diseases, such as hypertrophic cardiomyopathy. Hypertrophic cardiomyopathy is caused by a variety of mutations in genes encoding sarcomere proteins, the structural proteins in heart muscle. For ‘complex’ diseases, such as myocardial infarction and congestive heart failure, however, it remains unclear which genes are most responsible for disease onset, even though patients with these conditions often report a strong familial predisposition.

Expectations are high for the potential of cardiovascular genomics to lead to major advances in our understanding of normal cardiovascular functioning and of cardiovascular disease pathogenesis, of interactions of genes with environment, of strategies for disease prediction, and in drug development. While the major findings have been few to date, this meeting provided a broad window into how far the field of cardiovascular genomics has come and where it is going. Among the areas receiving attention were genetic epidemiology and the array of approaches for identifying candidate genes for disease, emerging strategies - particularly transcriptional profiling - for identifying novel molecular targets and pathways, animal models (including rat and mouse) for human disease, and gene therapy. There was particular interest in human cardiovascular diseases and conditions receiving much attention included atherosclerotic diseases, such as myocardial infarction, and its risk factors (for example, hyperlipidemia, hypertension, inflammation), congestive heart failure, and life-threatening ventricular arrhythmias.

A decade ago, the discipline of genetic epidemiology - which is concerned with the study of interactions of genetic and environmental factors in the etiology of disease - was obscure to most meeting attendees. As noted by the meeting chair, Howard Jacob (Medical College of Wisconsin), the ‘post-genomic’ era is now ushering in an acute demand for genetic epidemiologists to design and conduct human studies of the highest quality with the aim of successfully identifying which of the 80,000 to 100,000 human genes and their common sequence variants (on average two to four SNPs per gene) are implicated in cardiovascular health and disease. The early, simplistic assumption that gene discovery can proceed merely by simple comparisons of genotype frequencies in quickly ascertained cases of disease-containing versus disease-free controls (‘case-control studies’) is clearly incorrect. We now recognize that at least three formidable hurdles stand in the way of detecting causal associations rather than spurious findings from patient studies: the design of the studies themselves need to minimize bias; large sample sizes must be analyzed so as not to miss important effects; and detailed and accurate phenotyping must be carried out. Case-control studies are efficient and relatively inexpensive and have become extremely popular for the study of complex disease genetics, but they are severely
limited by the potential for 'confounding', particularly due to population stratification, as well as the potential for publication bias. (A confounding variable is a factor that is associated with the genotype and the outcome, so that the association between gene and outcome is not causal.) This point was emphasized by Alistair Hall (Institute for Cardiovascular Research, UK), who presented a meta-analysis of studies examining association of the angiotensin-converting enzyme (ACE) insertion/deletion polymorphism with coronary artery disease. Significantly elevated risks were suggested by several of the earlier, smaller studies; but, in subsequent large study populations (thousands of subjects) and in the totality of patients, little or no association is seen. These findings reinforce the point made repeatedly throughout the conference - that case-control designs are useful for generating hypotheses, whereas population-based cohorts and particularly population-based family studies are needed to provide the strongest level of evidence that disease phenotypes are associated with SNPs and other mutations. A number of case-control and cross-sectional studies of associations of specific genetic variants with disease were presented, with appropriate caveats regarding their conclusiveness, including studies by Giuseppe Sagnella (St George’s Hospital Medical School) regarding associations of the G-protein β-3 subunit with hypertension in Afro-Caribbean populations, and a presentation by Gordon Duff (Sheffield University) regarding a series of studies of the association of interleukin-1 gene polymorphisms with vascular disease.

Family-based studies may provide the strongest evidence for causality. In one such study (with which I am associated) - the Framingham Heart Study - there is the unique opportunity to test for both population-association and linkage in a single randomly ascertainment population containing hundreds of extended sibling relationships. Modest association of the candidate ACE locus with hypertension, confirmed by linkage analysis, was found in the Framingham Heart Study. Genome scanning using microsatellite markers in randomly selected families is a complementary gene-finding approach. An example of genome scanning was presented by James Hixon (Southwest Foundation for Medical Research), who described the identification of several cardiovascular disease quantitative trait loci, such as low density lipoprotein LDL size classes and the platelet adhesion marker P-selectin. Once a candidate region has been identified by association or genome-scan linkage, the next challenge is to identify the functional polymorphism(s) in the known positional candidate genes or in novel genes. Igor Splawski (University of Utah) presented a state of the art summary of the mutations and their functions in four genes for potassium channel subunits and one gene for a cardiac sodium channel, all of which are implicated in the rare Mendelian condition, the 'long QT' syndrome (a condition associated with elevated risks of sudden cardiac death). No data are yet available for functional mutations in complex-disease causing genes.

One of the more exciting current genome research applications is transcriptional profiling using DNA microarrays or 'chips'. The cDNAs from an increasing number of human and animal genes have been assembled onto microarrays. Hybridization analysis allows monitoring of gene expression changes in tissues that are in normal or abnormal states. Scientists both from academic institutions and from biotechnology companies have begun to utilize this technology to identify potential cardiovascular drug targets. In one such example, Thomas Henkel (MediGene AG) presented data regarding the simultaneous tracking of the expression of multiple genes in a cDNA library in normal and diseased human left ventricle. Richard Lawn (CV Therapeutics) presented data on the function of the ABC1 transmembrane transporter in control of cellular apolipoprotein-mediated lipid removal, using microarray expression analysis of cultured fibroblasts in addition to genetic mapping and biochemical studies of lipid efflux. From several fascinating presentations of expression profiling, it is apparent that the simultaneous tracking of expression of many or all genes presents formidable bioinformatic challenges for accurate assessment of the direction and magnitude of change in gene expression and for identifying patterns in clusters of genes. Choong-Chin Liew (University of Toronto) provided an update in an ambitious effort to collect a database of expression libraries for identification and classification of all cardiovascular genes [http:\\www.tcg.med.utoronto.ca]. Together, the microarray-based presentations provided a glimpse of the potential wealth of knowledge to accrue from this new technology. The bioinformatics tools for analyzing the data are still in development, however, in part because not all of the human genome is yet defined. The fidelity of expression libraries will require continued contributions from molecular biologists to classify the functional role of each individual gene in the various cardiovascular states (such as development, adulthood, response to stress and disease) and tissues (for example, myocardium and endothelium). Precise definition of the tissue phenotype being studied will remain critical.

A detailed session on the use of animal models for genetic analysis underscored the central importance that small animal research, primarily involving rats and mice, will play in cardiovascular functional genomics. Throughout the conference, questions were asked about how far animal data can be used to extrapolate to the human condition. Such concerns were addressed by several impressive presentations of sophisticated phenotyping, including the description of an expansive array of hypertension phenotypes in rats (Howard Jacob) and detailed electrophysiological and hemodynamic phenotypes in mice (Charles Berul, Children’s Hospital and Harvard Medical School). Jacob presented a detailed summary of the advantages of rats for use in functional genomics and the particularly strong track record in the study of hypertension phenotypes. Using sophisticated cluster analyses, novel patterns in functional responses are seen to
Figure 1
Cardiovascular genomics: the interdependence of biology and epidemiology. The successful coupling of basic biology with population studies will accrue benefits in the diagnosis and treatment of disease.

Various stimuli, and clustered responses may be useful for identifying novel pathways for design and application of therapeutics. Another clear advantage of animal (over human) models is the ability to conduct genetic crosses and manipulations for rapid gene discovery. George Cicila (Medical College of Ohio) presented data on his efforts to identify an interesting disease-causing gene in a rat model (the candidate gene is Cyp11b1,11 beta-hydroxylase) for blood pressure and left ventricular mass. These findings underlined the real challenges ahead for firmly identifying disease-causing genes in complex human diseases, and the important role that animal models may play. It was also clear from the rat and mouse models presented that the field of cardiovascular genomics will be propelled forward by the integrative thinking of biologists with expertise in multiple animal systems, not only mammalian, but also yeast and zebrafish.

Beyond the identification of novel targets for drug treatment, the evolving field of gene therapy - the targeting of increased gene expression into specific diseased tissues - was the focus of several presentations. A number of viral gene transfer strategies are being examined, including systems based on retroviruses, lentiviruses, and adenoviruses. Major variables for different vectors are the efficiency of gene transfer, duration of effect, and immunogenicity. Several examples of ongoing gene therapy research in animal models were presented, but with the safety concerns that have been raised in some ongoing clinical trials for non-cardiovascular applications of gene therapy, it is clear that questions remain regarding the appropriate timing for large-scale initiation of clinical trials in humans.

The meeting participants included biologists and molecular biologists, physiologists, molecular geneticists, cardiovascular and/or genetic epidemiologists, clinical cardiologists and other clinicians. Clearly, progress in this field will require a new level of interdependence between specialists broadly in the biological sciences and those in the population and clinical sciences (see Figure 1). The prospect of embarking on this voyage is at the same time daunting and exhilarating: researchers must rapidly ‘get up to speed’ on so many evolving new disciplines, but there is the opportunity to travel into uncharted territories to make discoveries at a great pace and on a large scale.