Dr. Robert Roberts, ('£0 of Ottawa Heart Institute and farmer chairman of cardiology at B$_{ay}$lor College of Medicine, responded admirably to my suggestion that he offer his view of how the science of cardiovascular genetics may alter the practice of medicine in the future. His perspective provides a glimpse of many exciting years to come for those involved in the cardiovascular sciences. His manuscript is published here with my deepest appreciation.

- William L. Winters, Jr., MD, Editor-in-Chief journal of the Methodist DeBak$_{ey}$ Heart Center

PERSONALIZED MEDICINE: AN IDEA WHOSE TIME IS APPROACHING

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INTRODUCTION

The field of Cardiology has seen major advancements in the past 50 years, including the advent of cardiac catheterization, bypass surgery, angioplasty, defibrillators and ablation. Basic research and clinical trials have defined the efficacy and safety of a range of treatments such as statin therapy for coronary artery disease and ACE inhibitors for heart failure. As a result, this "evidence-based medicine" has lead to the development and widespread use of clinical guidelines that have helped reduce cardiac mortality by more than 50% over the past 30 years.

The 21st century is poised to implement an even higher standard of evidence-based medicine - namely, therapy personalized to an individual's genetic variants. The gene is the most fundamental biological unit of the human body, responsible for generating and regulating proteins that perform the body's functions. Genes and their regulators determine which, when and how many proteins are synthesized in response to a host of signals, which makes genetics a major factor in many diseases. In fact, it is well recognized that more than 50% of one's predisposition to coronary artery disease is genetic. While coronary artery disease (CAD) is still the number-one killer in the Western world, the availability of the DNA sequence of human genes could mean its demise in the 21st century.

THE GENETIC COMPONENT OF CORONARY ARTERY DISEASE

The goal of eradicating heart disease can only be achieved if it is prevented before it arises. Prevention is already underway through an assault on major risk factors including hypercholesterolemia, obesity, hypertension and diabetes. However, researchers must identify the generic components of these risk factors, as well as other unknown genetic risk factors, before they can implement comprehensive screening and prevention.

The role of genetics is amply demonstrated by the influence of a family hiscory of CAD:

- A scudy of premature CAD found char only 38% had abnormal lipid values.[1]
- In Utah, 14% of the population has a family history of CAD, yet char 14% accounts for 72% of early CAD cases (< 50 years of age) and 48% of all CAD events at any age. Similarly, those 11% with a family history of stroke account for 86% of all early strokes in rhe Utah populacion.2
- In rhe Framingham Study, a family history of CAD, cerebral vascular accidents or peripheral arterial disease was associated with a 2.4-fold increased risk of CAD in men and 2.2 in women.
- In the Interheart Scudy, a family history of CAD was associated with a 1.55 increased risk and 1.45 after correction for other risk factors.3
- In the Procam Study, a family history of MI was an independent risk factor of CAD.4

While more than half of one's predisposition to CAD is genetic, the quantification of genetic versus environmental faccors awaits more precise definition. However, the recent introduction of SOOK DNA markers on a microarray chip and the mulci-slic:e Fast CT char allows noninvasive coronary angiograms are helping researchers in their pursuit to identify chose genes responsible for coronary artery disease.

ESSENTIALS OF THE HUMAN GENOME

Humans have an esrimated25,000 genes in their DNA, and one can reasonably expect chat most of these genes will be identified, sequenced and, in large pare, their function determined within the next 10-15 years. The human genome consists of 46 chromosomes, with each chromosome being a DNA molecule made up of repeating units of the four bases - adenine, guanine, thymine and cytosine. The total number of bases, sometimes referred to as nucleotides, consists of just over three billion. Yet the difference in the DNA sequence across all human beings is about one tench of one percent, meaning char 99.9% of human DNA sequence is identical across all humans. Nevertheless, this fraction of a percent still leaves

three million bases chat are unique co each individual. Mose of che differences contained in chose three million bases are substitutions of a single nucleotide, referred to as single nucleotide polymorphism (SNP), with probably about 10% being deletions, additions or inversions. These SNPs are fairly evenly dispersed throughout che genome, averaging one SNP per 1,000 base pairs. It is scriking co realize that all of our individual variations, including susceptibility co disease, are due co these three million base pairs. A major objective for biology and medicine over the next 10 to 15 years will be co identify these SNPs and determine their function. Clinicians will then be able to perform genetic screening and implement personalized therapy based on each individual's genetic variants.

PERSONALIZED MEDICINE: CURRENT AND FUTURE APPLICATIONS

A person's individual genetic variants determine their immune response. In 2004, for example, anaphylactic reactions were responsible for more than 100,000 U.S. deaths and more than two million hospitalizacions.[5] Genetic screening for these immune responses can eliminate such death and morbidity. Genetics also play a major role in the body's response co diec.[6-8] For example, 40% of the variation in L D L cholesterol levels in response co a low saturated-far diet is attributed co a familial traic.9 In the recent Decode study, in which 10,000 individuals with hypercholesterolemia were created with a scacin, only 80% had a significant decrease in their cholescerol.[10]

Increasing knowledge of pharmacogenetics is indicating chat che variable response to drug therapy is in large pare genetically determined_[11] An estimated 20% of individuals are resistant to aspirin.12 Warfarin, a drug used to prevent thrombosis, exhibits marked variation in the required dose, and 25% of the variation depends on che gene that encodes for vitamin K epoxide reductase (VKOR). There are 10 forms of chis gene chat, in the near future, could be screened to determine the dose. In the meantime, genetic screening co assess the ethnic profile of che different forms of V K O R has discovered that African Americans require a high dose, Asian Americans a lower dose, and European Americans a medium dose.13

A large clinical trial assessing the effect of Bucindolol in heart failure found chat a significant percentage did not respond. It was subsequencly shown that there was a significant reduction in mortality and hospitalization if the beta 1 Adrenergic Recepcor genotype was homozygosicy at residue 389 for Arginine (Arginine/Arginine), but if the genotype was Arginine/Gl$_{y\ c}$ine or Glycine/Glycine, no significant reduction in mortality or hospitalization was observed. Thus, in the future, one would prefer genotyping prior to administering Bucindolol.14

Multiple clinical trials with cancer patients are genotyping rhe patient prior to administering chemotherapy. A striking example is found in breast cancer patients, in which 20% exhibit the gene chat encodes for HER2 protein. The therapy Herceptin is given to block HER2 protein, which will be ineffective if the protein is not present. Thus, genocyping can determine if it is appropriate to administer the drug. The power of genetic variants and their role in determining therapy was vigorously enforced by the recent FDA approval of Bidil for heart failure only in African Americans. This drug was shown to exhibit a 43% reduction in mortality and hospitalization in African Americans with heart failure but had no effect in the Caucasian populacion.15

The alcohol dehydrogenase gene has 3 alleles, with type 3 allele associated with a slow rate of ethanol oxidation (RR;0_65) and a decreased risk of myocardial infarction. Those homozygous for allele 3 and who had at least one alcoholic drink a d$_{a\ y}$ had a greater reduction in risk for myocardial infarction (RR=0.14) and che highest H D L levels.[16]

GENETIC SCREENING: A PREREQUISITE FOR PREVENTING SUDDEN CARDIAC DEATH

An estimated 64% of total cardiac deaths are sudden cardiac deaths (SCD)_ The prevalence of cardiovascular disease in the United States (13 million people[17]) suggests chat 5% of che middle-aged U.S. population is significancly predisposed to SCD. This may in part explain why rhe incidence of S O C in the United States (more rhan 400,000 people annually) and in Canada (50,000 annually) has not changed in the past four decades despite a 50% reduction in overall cardiac morcalicy during chis interval.18•19

Ir is well recognized char S C D in chose younger than age 35 is due moscly to familial diseases, with more than 40% due to hyperrrophic cardiomyopathy followed by familial arrhythmias such as Long Q T syndrome or Brugada syndrome_[20] S C D below the age of 35 usually occurs without warning in otherwise asymptomatic individuals.[20,21] Genetic screening and prevention is the only hope for these individuals. S C D over che age of 35 is predominancly due co coronary artery disease and usually occurs within the first 60 minutes of symptoms, often precluding the availability of medical help. Unless genetic screening is available co target chose individuals for early preventive therapy, it is unlikely therapy will have a significant impact.

The treatment of choice for S C D is a cardiac defibrillator, since drug therapy for arrhythmias is relatively ineffective and has serious side effects. S C D accounts for more than 50% of deaths in patients with heart failure.[18] However, it is still undetermined which 50% of patients are vulnerable to S C D and would benefit from a defibrillator. In the C A D group, one approach would be to determine if genetic predisposition plays a role in SCD. The technology co pursue such genetic studies is now available. It is evident chat chose screened at an early age and found to have a genetic

predisposition would benefit most from preventive strategies.

THE SUDDEN DEATH PHENOTYPE EXHIBITS GENETIC PREDISPOSITION

There are several examples of a genetic predisposition to SCD. First, evidence of generic mechanisms responsible for sudden cardiac death comes from extensive studies showing mutations responsible for familial arrhythmias such as Long QT syndrome, Brugada syndrome, Shore QT syndrome, Wolff-Parkinson-White syndrome and atrial fibrillation.[20-22] Similarly, hundreds of mutations have been identified in the sarcomeric proteins responsible for cardiomyopathies and SCD. Second is the increased risk associated with a family history. Friedlander ec al.[23] and Jouvin et al.24 have shown a 1.6- to 1.8-fold increase in SCD susceptibility among offspring of parents who died from SCD. Although rhe sample size is small, the relative risk in offspring from families in which both parents experienced SCD was increased nine-fold. Third, variations in SNPs of the hepatic P450 clearance pathways increases the risk of ventricular (Torsades de pointes) arrhythmias.4 Fourth, a single SNP variant in the SCN5A sodium channel gene found in African Americans is associated with an increased incidence of arrhythmias, particularly in those receiving proarrhythmic drugs char prolong che QT incerval. This genetic varianc is presenc in more chan four million African Americans.25 This is just the beginning, but over the next 10 years one can expect several predisposing SNPs co be identified.

WHY HAS RESEARCH BEEN DELAYED IN IDENTIFYING GENES FOR CAD?

The application of molecular genetics to inherited cardiovascular disorders has met with great success,[26,27] mainly in the field of single gene disorders in which a single gene is sufficienc ro induce che phenocype. More than 2,000 of the estimated 6,000 single gene disorders have been identified. The first in cardiology was that of hypertrophic cardiomyopathy,[28] followed by multiple ocher genes for the cardiomyopathies,26 the long QT syndrome,29 WPW,[30-20] Brugada syndrome,[20,31] atrial fibrillation,[32,33] and others.[20,22] More than 1,200 mutations are considered responsible for single gene disorders char induce cardiovascular disease.

Like other complex disorders, CAD exists because susceptibility is conferred by multiple genes chat each contributes only mild to moderate risk. Unlike single gene disorders, in CAD one expects hundreds of SNPs to account for the phenotype. In genome-wide studies, searching for these SNPs would require a DNA marker every 6,000 base pairs, amounting to 500,000 genotyped markers for each DNA sample.34 Such high-throughput genoryping has until recently been prohibitive. In addition to genotyping 500,000 markers per individual, it would also require several thousand individuals to detect a SNP chat contributes only 5 to 10% risk. Thus, che prohibitive cost stymied the search for genes predisposing to complex diseases such as CAD. Recent reviews in Nature indicate chat none of che CAD studies have been adequace.[35,36] Genome-wide scans co date have utilized thousands of markers rather than the hundreds of thousands required, resulting in an inadequate sample size.[36]

A 500,000 SNP MICROARRAY ENABLES GENOME-WIDE GENOTYPING

The most sensitive and appropriate technique to idencify genes for CAD has been through case-control association studies. In contrast to single gene disorders, one collects a sample size of thousands of unrelated individuals with CAD and thousands of controls without CAD and compares the SNP frequency in both groups. A decade of publications[34,35,37] have assessed genetic predisposition co CAD using the candidate gene approach. However, because of an inadequate sample size or lack of replication in an independent population, essentially none of these studies have provided robust candidates chat can be used in the clinical management of CAD.[35,36] It is now recognized chat genome-wide genoryping for an association provides greater sensitivity and is che preferred method. This unprejudiced approach makes no presumptions and is all inclusive.

Once researchers realized that SNPs are distributed throughout the human genome an average of one per 1,000 bps (3 million per genome), SNPs were used as markers for dense genome-wide genoryping (also called scans). This culminated in a commercially available 500,000 SNP microarray chip and, more recencly, a I million SNP microarray chat provide on average a marker at intervals of 6,000 bps or 3,000 bps respectively. Studies by Hinds et al.34 and the International HapMap project[38,39] indicate that a minimum of 375,000 properly placed markers are required to genotype an American-European population.

Several parameters must be selected ro estimate the sample size for such studies. It is reasonable co assume any gene that increases the risk for CAD of 30% or more over that of controls would be clinically significant. Currencly identified risk factors increase risk by at least one-fold.[37] Similarly, it would be reasonable to power the study to detect genes with a frequency of 5%.37 Third, to obtain high sensitivity, one should power the study to detect a size difference between concrols and cases of 0.2.

With chis in mind, planning commenced for the Ottawa Heart Genomics Study. A sample size was calculated assuming 90% power, a gene frequency of 5%, odds ratio of 1.3, and 0.2 size differences between controls and cases. The initial population, estimated to be 2,000 {1,000 cases and 1,000 controls), would be

genotyped with the 500K marker sec to detect association having a p-value of 0.001 or more significant. Those markers showing an association (p = 0.001) in che initial population would be genotyped in a second independent population to ascertain che degree of replication. We estimated a second sample size of 12,000 (8,000 cases and 4,000 controls) to provide the power necessary to detect SNPs showing a stronger association at p-values, such as 10-8 or more significant. Using this sample size and the 500K microarray, the Ottawa Heart Genomics Study was initiated at the University of Ottawa Heart Institute.40 To our knowledge, chis is the first study utilizing the SOOK as a genome-wide scan for CAD with the latter documented by coronary aneriography.

RESULTS OF OTTAWA HEART GENOMICS STUDY

The Canadian Cardiovascular Genetics Centre, established in 2004 at the University of Ottawa Heart Institute, is equipped with high-throughput DNA extraction for high-throughput genotyping, including SNP detection with the Affymecrix platform utilizing rhe 500K chip for genome-wide scans. The centre currently can perform 48 million genotypes per day and has the sequencing power of abouc 20 million bases per day. The heart institute also acquired a 64-Slice Fast CT to perform non-invasive coronary angiograms. This technology and the existing four catheterization laboratories yield more than 10,000 angiograms per year to provide the necessary high-throughput phenotyping, and the institute has more than 100,000 coronary angiograms on individuals followed in our outpatient clinics.

The Ottawa Heart Genomics Scudy, which is pursuing genome-wide case control association studies to identify genes responsible for CAD, launched in August 2005 using the 500K scan and a sample size of 14,000 individuals (9,000 affected and 5,000 controls). We have completed more than 900 million genotypes on 1,800 individuals and expect to complete Phase I (n = 2,000) shordy. Unfiltered analysis of the first 500 controls and 500 affected cases indicates several thousand SNPs with p-values of 0.001 or more significant and more than 130 clusters with p-values ranging from 10-3 to 10-12. Analysis coupled with further customized SNP genocyping will be perfurmed in the second population (n = 12,000) to determine replication. (We recognize many of these associations are false positives and are not expected to be confirmed in the replication analysis.)

Completion of the initial phase will provide for a well-phenotyped and genotyped population with an all-inclusive set of SNPs exhibiting strong associations to the CAD phenotype. It will require extensive effons to confirm or exclude these genes as causative, and we hope to collaborate with investigators in Canada and other countries to identify and functionally analyze those genes contributing the most risk for CAD. After completing the replication studies, it will be possible to compare these genes to those in specific risk cohorts such as hypertension, obesity or hyperlipidemia.

le is a unique opportunity to provide the armamencarium fur comprehensive genetic screening and prevention of the number-one killer in the Western world.

REFERENCES

1. *Genestff, Martin-Munley 55, McNamara JR, Ordovas JM, Jennerj, M_yers RH, et al. Familial lipoprotein disorders in patients with premature coronary artery disease. Circulation. 1992 Jun;85(6):2025-33.*
2. *Hunt SC, Gwinn M, Adams TD. Family history assessment: strategies for prevention of cardiovascular disease. Am J Preu Med. 2003 Feb;24(2):136-42.*
3. *Yusuf 5, Hawken 5, Ounpuu 5, Dans T. Avezum A, Lanas F, et al. INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004 Sept 11-17;364:937-52.*
4. *Marinucci P. Risk of torsades de pointes with non-cardiac drugs: G_{rap}efruit juice is source of potentially life threatening adverse drug reactions. Bil.ff. 2001 fan 6;322(7277);47.*
5. *Couzin J Dmg safety. Gaps in the Safety Net. Science. 2005fan 14;307(5707):196-8.*
6. *Tall A, Welch C, Applebaum-Bow/kn D, WassefM. Interaction of diet and genes in atherogenesis. Report of an NHLBI working group. Arterioscler Thromb Vase Biol. 1997 Nov; 17(11):3326-31.*
7. *Ordoi,as JM. The genetics of serum lipid responsiveness to dietary interventions. Proc Nutr Soc. 1999 Feb;58(J):171-87.*
8. *McCombs Rf, Marca.dis DE, Ellis J, Weinberg RB. Attenuated hyperchol.esterolemic response to a high-cholesterol diet in subjects heterozygous for the apolipoprotein A-IV-2 allele. N Engl J Med. 1994 Sept 15;331(11):706-10.*
9. *Denke MA, Adams-Huet B, Nguyen AT. Individual cholesterol variation in response to a margarine- or butter-based diet: A study in families. JAMA. 2000 Dec 6;284(21):2740-7.*
10. *Helgadottir A, Manolescu A, Thorleifoon G, Gretarsdottir 5, Jonsdottir H, Thorsteinsdottir U et al. The gene encoding 5-lipoxygenase activati$_{n\ g}$ protein confers risk of myocardial infarction and stroke. Nat Genet. 2004 Mar;36(3):233-9.*
11. *Weinshi/boum RM. Inheritance and Drug Response. N Engl J Med 2003 Feb 6;348(6):529-37.*
12. *Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the isme of dmg resistance. Arterioscl.er Thromb Vase Biol. 2004 Nov;24(11):1980-7.*
13. *Service RF. Pharmacogenomics. Going from genome to pill. Science. 2005 Jun 24;308(5730):1858-60.*
14. *Mason DA, Moore JD, Green SA, Liggett SB. A gain-offanction pofrmorphism in a G-protein coupling domain of the human betal-adrenergic receptor. J Biol Chem. 1999 Apr 30;274(18):12670-4.*
15. *Holden C. Race and Medicine. Science 2003 Oct 24;302(5645):594-6.*
16. *Hines LM, Stampfer MJ, Ma J, Gaziano*

JM, Ridker PM, Hankinson SE, et al. Genetic variation in alcohol dehydrogenase and the beneficial effect o fmoderate alcohol consumption on myocardial infarction. N Engl] Med. 2001 Feb 22;344(8):549-55.

17. NHLBI fact book, fiscal year 2002. Bethesda, MD: National Heart, Lung, and Blood lmtitute; 2003 Feb. Ai•ailable at: www.nhlbi.nih.gov/about/factpdfhtm.
18. Bristow MR, O'Connell JB. Myocardial Diseases. In: Kelley WN, Watanabe AM, editors. Textbook o f Internal Medicine. Philadelphia: J B. Lippincott Co.; 1992. p. 150-7
19. Zipes DP, Wellem HJ. Sudden Cardiac Death. Circulation. 1998 Nov 24;98(21):2334-51.
20. Roberts R. Genomics and cardiac arrhythmias. J Am Coll Cardiol. 2006 Jan 3:47(1):9-21.
21. Marian A]. Roberts R. Molecular basis of hypemophic and dilated cardiomyopathy. Tex Heart Inst J 1994;21(1}:6-15.
22. Roberts R. Mechanisms of disease: Genetic mechanisms of atrial fibrillation. Nat Clin Pract Cardiovasc Med. 2006 May,-3(5):276-82.
23. Friedlander Y, Siscovick DS, Weinmann S, Austin MA. Psaty BM, Lemaitre RN, et al. Family history as a risk factor for primary cardiac arrest. Circulation. 1998 Jan 20;97(2):155-160.
24. Jouven X, Demos M, Guerot C., Ducimetiere P. Predicti$_{ng}$ sudden death in the population: the Paris Prospective Study 1 Circulation. 1999 Apr 20;99(15):1978-83.
25. Splawski I, Timothy AW, Tateyama M, Clancy CE, Malhotra A, Beggr AH, et al. Variant of SCN5A sodium channel implicated in risk o fcardiac arrhythmia. Science 2002 Aug 23:297(5585):1333-6.
26. Marian A], Roberts R. The molecular genetic basis for hypertrophic cardiomyopathy. J Mo! Cell Cardiol 2001 Apr;33(4):655-70.
27. Geisterfer-LowranceAA, ChristeM, Conner DA, IngwallJS, Schoen Ff, Seidman CE, et al. A mouse model offamilial hypertrophic cardiomyopathy. Science 1996 May 3;272(5262):731-4.
28. Geisterfer-Lowrance AA, Kass S, Tanigau•a G, Vosberg HP, Mc&nna W, Seidman CE, et al. A molecular basis for familial hypertrophic cardiom_yopathy: a beta cardiac myosium heavy chain missense mutation. Cell. 1990 Sept 7;62(5):999-1006
29. Keating /lf, Atkinson D, Dunn C. Timothy K. Vincent GM, Leppert M. Linkage o fa cardiac arrhythmia, the long Q T syndrome, and the Harvey ras-1 gene. Science. 1991 May 3;252(5006):704-6.
30. Gollob MH, Green MS, Tang AS, Gollob T. Karibe A, Ali Hassan AS, et al. Identification o fa gene respomibk for familial Wolff-Parkinson-White syndrome. New Engl J Med. 2001 jun 14;344(24):1823-31.
31. Brugada R, Roberts R. Brugada syndrome: why are there multiple questions to a simple amwer? Circulation. 2001 Dec 18;104(25):3017-9.
32. Gollob MH, Jones DL, Krahn AD. Danis L, Gong XQ, Shao Q, et al. Somatic mutations in the connexin 40 gene (G}A5) in atrialfibrillation. N EnglJ Med. 2006Jun 22,-354(25):2677-88.
33. Brugada R, Tapscott T, Czernuszewicz GZ, Marian A]. Igksias A, Mont L, et al. Identification o fa genetic locw for familial atrial fibrillation. N Engl J Med. 1997 Mar 27.-336(13):905-ll.
34. Hinds DA, Stuve LL, Nilren GB, Halperin E, Eskin E, Balli$_{nge}$r DG, et al. Whole-genome patterns o fcommon DNA variation in three human populatiom. Science. 2005 Feb 18;307(5712):1072-9.
35. Wang WY, Barratt BJ. Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. Nat Rev Genet. 2005 Feb,-6(2):109-18.
36. Altshuler D, Hirschhorn JN, Klannemark M, Lind$_{gr}$en CM, Vohl MC. Nemesh]. et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000 Sept;26(1):76-80.
37. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. Nat Rev Genet. 2005 Feb;6{2):95-J08.
38. The International HapA!fap Project. Nature. 2003 Dec 18;426(6968):789-96.
39. Altshukr D, Brooks LD, Chakravarti A, Collins FS, Daly .MJ, Donnelly P. A haplorype map of the human genome. The International HapMap Comortium. Nature. 2005 Oct 27:437(7063):1299-320.
40. Roberts R, Stewart AF. Personalized genomic medicine: a future prerequisite for the prevention o fcoronary artery disease. Am Heart Hosp J 2006 Summer;4(3):222-7.