Genetics and cardiovascular disease: Design and development of a DNA biobank

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Coronary artery disease (CAD) is a complex disease with environmental and genetic determinants. Many other cardiovascular (CV) conditions also have a genetic basis. A positive family history of CV disease in first-degree relatives is a strong independent risk factor for CAD as well as several other cardiac disorders. This genetic susceptibility to CV diseases will be understood more clearly when combined with genomics, proteomics and genotyping.

The Department of Cardiology at Gold Coast Hospital (Queensland, Australia) with the Faculty of Health, Science and Medicine at Bond University (Queensland, Australia) established the Gold Coast Cardiovascular DNA bank in 2006. The dataset on each individual volunteer includes coronary angiograms, clinical information (including a coronary risk factor profile), biochemical (including cardiac biomarkers) and hematological parameters, and electrocardiograms and echocardiograms. The establishment of the DNA biobank was associated with several key challenges, both technical and logistic.

Given the comprehensive nature of the information gathered, the present study has the added potential of identifying genes associated with nonischemic cardiomyopathies, valvular heart disease, congenital heart diseases and other cardiomyopathies. Pooling data from results obtained here with multiple existing DNA biobanks and registries will help in finding answers to the genetic conundrum in CV diseases. The present DNA biobank will serve as a resource well into the future as the technology and science of medical genetics evolve.

The most frequent pathology encountered in the biobank is CAD. The significance of the familial occurrence of CAD has been the focus of research for at least 50 years, with a positive family history of CV disease emerging as an independent predictor of risk in the development of CAD. By applying the knowledge learned from studies on CV genetics together with the data from the DNA biobank, scientists may be able to effectively prevent and treat CV diseases in the future.

Key Words: *Cardiovascular diseases; Genetic biobank; Single nucleotide polymorphisms*

Coronary artery disease (CAD) is the leading cause of death and disability in the western world. It is a complex disease with environmental and genetic determinants. The risk of developing CAD is two to 12 times higher among individuals with a first-degree family history of cardiovascular (CV) disease (1). The risk is highest among those with affected family members who have had early-onset CAD and among those with a greater number of affected first-degree relatives (2,3).

Despite years of research, the genetic basis of CAD remains to be fully elucidated. Many of the classic risk factors are under genetic control (blood pressure, lipids, obesity), but they account for only a portion of the familial aggregation of coronary heart disease (1). The genetic basis of CAD is multifactorial and highly dependent on environmental triggers on most occasions (4-9).

Common genomic variants that have a non-neutral impact should be investigated for the purpose of rapidly translating genomic knowledge into diagnostics and therapeutics of cardiovascular (CV) disease (10). Since the identification and mapping of approximately four million single nucleotide polymorphisms (SNPs) by the Human Genome Project and the SNP Consortium, the emphasis has been to identify patterns of SNP groups called haplotypes or haploblocks (11). Haploblocks are areas of strong linkage disequilibrium (LD), in which blocks of SNPs are inherited together (12). The power of whole genome association studies has been greatly increased by the identification of the size and location of haploblocks (13).

The extent of LD is variable. It can extend for hundreds of kilobases and can be population specific. The significant feature of these haplotypes is that one SNP in such a region suffices for analysis of an entire haplotype block. Thus, the use of these 'tag SNPs', which serve as proxies for nearby SNPs in LD, dramatically increases the power of SNP association studies and simplifies the design of multiplex assays for particular traits (14). Therefore, the availability of sets of 500,000 tag SNPs greatly enhances the strength of such an analysis (15). Several recent studies (14) have successfully demonstrated the increased power of tag SNPs for whole genome association studies of complex polygenic traits.

However, recent developments indicate that the focus on SNPs alone will not capture the full range of meaningful human genomic variation, such as a newly characterized and annotated form called copy number variation (10).

Over the past decade, there has been a 400% increase in citations referencing genotype or genetic polymorphism. Within this group, genetic association studies have increased 1000% during the same time frame (16). These numbers far exceed the increase in the citations referencing other more traditional coronary risk factors.

HUMAN GENES LINKED WITH CAD

A large array of genetic associations of CAD and other cardiovascular conditions have been described (Table 1). The following findings identify some important associations in the genes of subjects with CV disease.

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TABLE 1
Chromosomal association studies in selected populations

Chromosome loci	Study population	Association
Chromosome 9p21.3 (17)	Wellcome Trust Case Control Consortium	Coronary artery disease
	German Myocardial Infarction Family Study	Coronary artery disease
Thrombospondins and plasminogen activator inhibitor-2 (18)	Gene Quest	Coronary artery disease
Thrombospondin-4 (18)	Gene Quest	Coronary artery disease
Thrombospondin-2 (18)	Gene Quest	Coronary artery disease
Plasminogen activator inhibitor-2 (18)	Gene Quest	Coronary artery disease
15q26 *MEF2A* (19)		Coronary artery disease
2q21.1–22 (22)	Finns	Premature coronary artery disease
Xq23–26 (22)	Finns	Premature coronary artery disease
16p13.3 (23)	Indo-Mauritians	
14q11.2–12 (24)	Western European	
6p21.3 (28)		Coronary artery calcification
10q21.3 (28)		Coronary artery calcification

Chromosome 9p21.3

Pooled data from the Wellcome Trust Case Control Consortium and the German Myocardial Infarction Family Study identified chromosome region 9p21.3 (SNP, rs1333049) to have the strongest association with CAD – ($P=1.80\times10^{-14}$ and $P=3.40\times10^{-6}$, respectively (17). Four other loci of significance identified in this study are chromosomes 1p13.3 (rs599839), 1q41 (rs17465637), 10q11.21 (rs501120) and 15q22.33 (rs17228212).

Thrombospondins and plasminogen activator inhibitor-2

In a study (18) from the Gene Quest population, significant associations were identified with the polymorphisms in thrombospondin-4, thrombospondin-2 and plasminogen activator inhibitor-2, the strongest being with the A387P variant in thrombospondin-4 (P=0.002).

There are 10 genetic polymorphisms with significant ($P<0.05$) associations that include *ACE*, *APOE*, *F7*, *FGB*, *GP1BA*, *IL1RN*, *LRP1*, *MTHFR*, *SELP* and *THPO* genes. For five of these genes, the polymorphisms associated are novel and may be explained by possible LD. However it is also possible that these associations are not real.

Myocyte enhancer factor 2A

A significant linkage to chromosome 15q26 is associated with a dominant form of inherited CAD that results in acute myocardial infarction (MI). Chromosome 15q26 contains approximately 93 genes, of which 43 are known. One of the genes is myocyte enhancer factor 2A (*MEF2A*), which encodes a member of the *MEF2A* transcription factors that has been associated with heritable CAD (19).

To date, evidence for an association between mutations in *MEF2A* gene and heritable CAD has been inconsistent. Future studies will be necessary to determine the true prevalence of CAD and MI in association with *MEF2A* mutations. A large study (20) conducted among families with apparent Mendelian inheritance of CAD and populations presenting with sporadic MI failed to display any significant association between the mutations Pro279Leu variant in exon 7 or the (CAG)n repeat in exon 11 of the *MEF2A* gene and CAD or MI, thus suggesting its possible rarity.

The genetic basis of atherosclerosis and that of MI are two distinct phenomena. The former has many aspects that interplay, including the genetic basis of coronary risk factors. However, it is important to note that of those who have established atherosclerosis, only a minority sustain MIs (21).

OTHER LOCI LINKED TO CV DISEASE

Chromosome 2q21.1–22

To date, there have been only a few other published studies that have identified loci linked to CV disease and MI. Two loci have been linked to premature CAD in Finns: one on chromosome 2q21.1–22 and the other on Xq23–26 (22). A susceptibility locus for CAD on chromosome 16p13.3 was identified in Indo-Mauritians (23). A whole genome scan in 513 western European families found evidence for linkage to MI in a region on chromosome 14q11.2–12 (24). Acute coronary syndrome has been linked to 2q36–q37.3 (25).

Coronary artery calcification and its progression are heritable, and an association has been described with loci 6p21.3 and 10q21.3 (26). Identification of specific genes associated with increased progression of coronary artery calcification may provide insights into molecular mechanisms of atherosclerosis and possibly lead to blood tests for early detection of susceptible individuals.

THE DNA BIOBANK

Biobanks usually collect and store biological material, including DNA, that may provide valuable information into the pathogenicity and pathophysiology as well as the genetic basis of diseases. These biobanks may include material from the diseased subjects, their first- and second-degree relatives, as well the healthy population controls. The biological material may take the form of buccal smears, whole blood or other tissue material. DNA biobanking involves the extraction and storage of genetic material from the harvested cells, especially the white blood cells.

Many large-scale prospective, randomized and nonrandomized studies and registries have had genetic material collected for substudy analysis. Several workers have collected genetic material from isolated communities, such as those that live on islands, for population genotyping. Some countries have commenced massive scale ambitious programs to collect and type genetic material of entire populations. For meaningful associations to be observed, a wide array of clinical and nonclinical information should be collected and stored in addition to the DNA. These comprehensive collectives of genetic, phenotypic, environmental and other nongenetic data may help in the subsequent analyses to determine the genetic basis of the pathology of different disease conditions. It may also help to derive environmental triggers of phenotypic manifestation of genetic disorders.

The collection and storage of genetic material can be contentious, and as such, quite challenging. The ethical and

confidentiality boundaries could be possibly breached and the population's anxiety related to this issue may make the challenge of setting up a biobank even more difficult. Sensitive ethical issues and ownership issues related to the biological material as well as the intellectual property would create regulatory hurdles and legal implications.

Researchers engaged in many large studies, such as the Framingham Heart Study, have collected thousands of DNA samples from subjects, along with an array of data on their clinical and laboratory status. Technological developments now permit high throughput testing of the several hundred thousand individual sequence variants necessary to provide adequate coverage of all the DNA blocks in humans to ensure that if a variant associated with a disease is present, it will be found. By coupling the genotypic data with epidemiological data that include many covariates, one is theoretically able to identify genes or gene-environment interactions that predispose to both normal trait variation and disease processes (27,28).

Guidelines on the design and methodology of studies that describe associations between DNA polymorphisms and disease have been proposed (29). Simultaneously, multiple gene banks (Table 2) have been set up across the globe, which are providing data for these association studies. The recent development of high-density genotyping arrays provides clear resolution for whole genome assessment of variants associated with common diseases.

TABLE 2
Established DNA biobanks

Biobank	Sample size	Study population
Generation Scotland	50,000	From Scotland's population to study genetic association with heart disease, diabetes mellitus, cancer and mental illness (30)
Estonian	1,000,000	$200 million dollar plan to store genetic codes of at least two-thirds of Estonia's population (31)
Cleveland Clinic Heart Centre		Carrying out two large-scale cardiovascular system studies – GeneBank and Gene Quest. In 2003, discovered *MEF2A* as a cause of coronary artery disease (32)
Alfred and Baker	5000	Venture between The Alfred and Baker Heart Research Institute (33)
Framingham Genomics	5000	Data from Framingham Heart Study, which has data on two generations of residents for more than 57 years (34)
deCODE Genetics		Database from Iceland's Genealogy Genotype Phenotype Resource, which has collected data for more than 15 years (35)
UK Biobank	500,000	From volunteers aged 40 to 69 years to identify genetic components of heart disease, cancer, diabetes and dementia (36)
Uman Genomics	100,000	Operated by Umea University and Vasterbotten County Council to study cardiovascular diseases and diabetes (37,38)

THE GOLD COAST DNA BIOBANK – A UNIQUE DATASET

Methods

The Department of Cardiology at Gold Coast Hospital (Queensland, Australia) with the Faculty of Health, Science and Medicine at Bond University (Queensland, Australia) established the Gold Coast Cardiovascular DNA bank in 2006. This project has collected DNA from more than 3000 individuals who underwent coronary angiography for a clinical indication or presented for percutaneous closure of structural cardiac abnormalities such as a patent foramen ovale, atrial septal defect or ventricular septal defect. The DNA samples are collected from consenting patients. In an angiography cohort, the study uses the results of the angiogram to define the presence or absence of CAD, and if present, its extent and severity. Donors with normal angiograms (approximately 30% of the angiograms in contemporary practice) function as the control population in this study. This overcomes the limitations of most other similar studies in which there is no definite exclusion of macrovascular CAD in the control group.

Clinical dataset

A comprehensive dataset on each individual donor is collected. The pertinent information comprising this dataset includes age, sex, ethnicity, body mass index, birth country and country of residence; comprehensive medical history that includes the coronary risk factor profile and cardiovascular medical history; resting electrocardiogram; resting echocardiogram defining the left ventricular ejection fraction as well as detailed cardiac anatomy and function; biochemical parameters including serum electrolyte profile, renal function indexes, fasting cholesterol level, blood sugar level, cardiac biomarkers; hematological parameters that include the full blood count and the differential count, white blood cell count, platelet count and coagulation profile; and definitive CV diagnosis – in this study, the CV diagnoses of the donor cohort include stable CAD, unstable angina, MI, congestive cardiac failure, idopathic dilated cardiomyopathy, alcoholic cardiomyopathy, hypertrophic obstructive cardiomyopathy, isolated valvular disease (especially degenerative aortic stenosis and mitral regurgitation), cryptogenic stroke (with or without migraine headaches), etc. Of note is the inclusion of Australian Aboriginals and Islander ethnics in this study who have a significantly higher proclivity to develop severe CAD at a much younger age.

Age distribution among the donors in the biobank is between 20 and 94 years.

Method of specimen collection

Blood is collected in two EDTA tubes. DNA is extracted from the blood using the QIAGEN BioRobot EZ1 Workstation using EZ1 DNA Blood 350 µL Kits as per the manufacturer's instructions (QIAGEN Pty Ltd, Australia), and stored at –20°C and –70°C. The final DNA yield ranges from 4 µg to 9 µg. The remaining blood sample is also stored at –20°C and –70°C. Differences in the genotypes of candidate gene polymorphisms in diseased patients and the control group are assessed for an

association with CV disease and for a potential role in the causation of the disease. Plasma from the second sample can be used for RNA expression analyses and proteomic analyses.

Methods of genomic analysis

The DNA is used for genomic analyses investigating genotypic and phenotypic associations with CV pathologies.

One application of the DNA databank is for association studies of SNP candidate genes. This will require SNP genotyping of the target SNPs in the chosen genes. Many SNP assay technologies have been developed, and these are based on methods of allelic discrimination using a variety of different detection platforms. There are four main approaches used for allele discrimination, namely primer extension, hybridization, ligation and enzymatic cleavage. Primer extension involves the incorporation of a labelled nucleotide at the SNP site on the DNA template, using enzyme specificity to achieve allelic discrimination. Hybridization methods use differences in the thermal stability of double-stranded DNA to distinguish perfectly matched from mismatched target-probe pairs. The length of the probe, sequence of the probe, location of the SNP within the probe and hybridization conditions all influence the effectiveness of this method. The specificity of ligase enzymes is used in the third approach. An allele-specific probe and an abutting common probe are hybridized to the DNA template. When the allele-specific probe is complementary to the SNP site, ligation to the adjacent common probe can occur, and detection of this longer molecule occurs after removal of unligated probes. The last strategy for allele discrimination involves enzymatic cleavage, which is based on the ability of certain enzymes to cleave DNA at specific sites. This last approach requires the SNP to lie within an enzyme recognition sequence.

CHALLENGES

Disclosure and privacy

The study has identified addressing of the key issues of consent, disclosure and privacy to be paramount in developing the DNA biobank. The project was scrutinized in detail and approved by the Gold Coast Health Services District Ethics Committee, which comprises medical professionals, legal practitioners, scientists, consumer and community representatives, and clergy. A detailed project disclosure statement was developed for donor information before consenting. Consenting was preceded by counselling of the donor by a medical officer in the team. The principal investigator and the chair of the ethics committee are available to the donors for consultation at all times. Clinical information, corresponding imaging study details, coronary angiogram and the specimen samples are de-identified and coded before archiving and storing. Principal investigators from the hospital and the university remain the custodians of the biobank on behalf of the research team.

Limitations

There are significant limitations in association studies. There is also a serious need for uniformity in the way that these studies are conducted and reported. Candidate genes should be chosen based on strong a priori evidence, and initial investigations should be validated on follow-up within independent datasets. The statistical methodology should be rigorous enough to avoid spurious associations. Statistical methods should also address the appropriate calculations for power, corrections for multiple testing and the accuracy of matching to account for population structure.

Most genetic associations of CAD described hitherto are outside the coding regions. Thus, even the future validation of their functional relevance may carry questionable clinical relevance due to their limited and very finite phenotypic effects. Many of the linkage studies reported in the present article describe quantitative trait loci that contain hundreds of genes and not specific or singular genes. Thus, understanding and defining the precise genetic associations of clinical and public health significance still remains a remote reality.

COLLABORATION

Beyond the single-site studies discussed here, there is a need for greater collaboration. Given what we know of complex diseases and the complexity of gene-gene interactions, the sample sizes needed for large-scale association studies will be far beyond what is currently available, and no single institution or entity alone will be able to provide a reasonable number of patients for the most complex analyses. Datasets capturing clinical and genetic information on hundreds of thousands of patients are technically feasible; the establishment of network collaborations would increase the capacity for patient ascertainment, overcome the sample size limitations of association studies, and greatly assist the study of complex disease.

Either traditional meta-analysis or pooling of data across a number of similar studies with a thorough analysis of the effects of criteria, confounding and interaction may identify subsets of individuals for whom a particular genetic marker may have the greatest impact on risk of CAD.

POSSIBILITES AND FUTURE DIRECTIONS

The Gold Coast DNA Biobank, through collaboration with both national and international registries in the field of cardiovascular research, will contribute to the exciting developments of the future. A common front will have to be forged to address the epidemic of CADs. Pooling data from multiple registries is the answer to the logistics in obtaining adequate power to reach certain conclusions. The anticipated outcomes include identification of culprit and protective genes in CAD and cardiomyopathies. Aggressive primary prevention would be the norm for people identified with the implicated genes but normal coronary vessels.

Gene banks need to be set up in countries such as China and India, which are huge resources for genetic studies and have a large migrant population worldwide. The gene-environment interaction resulting in cardiac morbidity can be best studied in these populations.

The genome belongs to the human race as a whole and is also unique to each individual. Notwithstanding various cultural, economic and geographical differences, universal protocols should be in place to overcome barriers. To ensure collaboration and maintain public trust, rules and methods for ethical and efficient conduct of genetic research have to be developed alongside the technical advances. These guidelines should continuously be assessed by experienced oversight committees that can contribute to ongoing consensus about proper actions. Above all, we should not forget that patients will

benefit from research based on DNA, because new knowledge affects diagnostic procedures, development of new therapies and targeting of therapies.

CONCLUSION

Genetic material together with a comprehensive complement of vital relevant data comprises the Gold Coast Cardiovascular DNA Biobank, which is a futuristic project aimed at contributing to the study of the genetic basis of heart disease. Inclusion of the gold standard test information in the way of coronary angiography makes this DNA biobank unique among the multiple such biobanks established in different parts of the world. Analysis of the information gathered in this biobank would provide resources well into the future to unravel the genetic mysteries and associations in heart disease. Collaboration with similar biobanks elsewhere in the world would facilitate large scale studies that are likely to have a higher and more significant yield. Unique challenges and ethical implications and issues related to confidentiality are encountered in the development of databases that include the storage of human genetic material. There is a pressing need for a global convention on protocols and guidelines on the creation of DNA biobanks and also for the analysis of the material and the data thus gathered. The ethical basis of a project of this nature remains a topic of great contention and one that lacks consensus hitherto in the scientific community.

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