Genetics of cardiovascular disease

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Summary

Linkage studies and genome-wide linkage analyses, which use polymorphic DNA markers throughout the genome, provide a useful method for identifying genes related to cardiovascular disease (CVD). Many genome-wide linkage studies have contributed to identify quantitative genetic loci influencing variables involved in the pathogenesis of CVD.
Meta-analyses of genetic studies provide the measure of association studies, so contributing to identify candidate genes which might influence the susceptibility to the disease. Really, candidate genes have been investigated, in relation to lipid metabolism (APOE), fibrinolytic protein (PAI-1), renin-angiotensyn system (ACE) and homocysteine metabolism (MTHFR). Recently, genome-wide panels of common single nucleotide polymorphisms (SNPs) based on the use of SNPs spread throughout the genome are also becoming available. This approach contributes to finely investigate the gene-gene and gene-environment interactions in CVD, and to look for the involvement of genetic polymorphisms in drug response.

KEY WORDS: linkage studies, genome-wide association studies, genetic predisposition

Cardiovascular disease (CVD), which represents a major health problem around the world and involves the heart, brain, and peripheral circulation, is a complex genetic trait with multiple genetic and environmental components contributing to the observed phenotype. Several challenges exist in identifying the genetic determinants of common diseases; of interest, challenges may be the phenotypic and genetic heterogeneity as well as gene-gene and gene-environmental interactions, given the multiple causal pathway leading to CVD.
The search of genes predisposing to CVD has led to identify common types of DNA human variations, which may be considered analogous to the already known CVD risk factors, thus contributing to assess the risk profile and to adopt preventive or therapeutic measures. Nevertheless, the majority of these polymorphisms are located in non-functional regions of the genome and have no phenotypic impact (neutral markers), those variations occurring within coding or regulatory regions may affect the protein sequence or the level of gene expression, thus affecting phenotype. Actually, common polymorphisms represent the object of most genetic studies performed in order to evaluate the risk of complex diseases.

Linkage studies

CVD may cluster in families. Twin and family studies have demonstrated that CVD aggregates in families, really a family history of early onset of CVD has long been considered a risk factor for the disease (1), and contributes to increase the risk independently of the well known risk factors (2, 3).
The genetic variants predisposing to CVD span from rare and deleterious mutations responsible for Mendelian diseases, such as familial hypercholesterolemia, to common polymorphisms that modulate the predisposition to complex diseases with a weak effect at individual level. Linkage studies, which require enrolment of families with several affected members carriers of phenotype of interest over different generations, are performed by using DNA markers throughout the genome, in order to identify genes related to CVD. The strategy uses genetic markers and tests whether particular alleles are co-transmitted with the disease at a higher frequency than expected. The advantage of linkage studies is related to the availability of phenotypically well-characterized families including a sufficient large number of informative subjects. The utility of linkage analysis lies in identifying new genes for coronary heart disease (CHD) and for stroke, as demonstrated by Helgadottir A et al. (4, 5), thus possibly providing a new tool for drug therapy. Moreover, genome-wide linkage studies evaluating quantitative markers of atherosclerosis, such as coronary artery calcium levels, carotid intima-media thickness and ankle-brachial index, have been performed, but candidate genes have still to be identified (6-8). Several genome-wide linkage studies have been reported concerning myocardial infarction and coronary artery disease. The British Heart Foundation Family Heart Study (9), a large study performed on 4,175 subjects with CHD from 1,933 families recruited throughout the UK, did not obtain a statistically significant LOD score, despite the large sample size. Alike, a LOD score of 2.70 was found for both CAD in 1,698 families with age at onset of 56 years or less, and myocardial infarction (LOD score 2.1) in 801 families with age of at onset 59 years or less. Data concerning the genomic locus for CHD were replicated by Farrall M et al. (10) by performing a linkage analysis in two independent samples of European whites, and founded evidence of replication for a locus on chromosome 17 (at 69cM).
Many genome-wide linkage studies have been performed concerning cholesterol and lipids with significant results (Table I). Of interest, genome scans carried out on 330 Framingham pedigrees of the Genetic Analysis Workshop 13 data (11),

Table I - Linkage studies.

Study	Population	Study sample (number of participants)	Chromosome	Location, cM	Nearest marker score	LOD	Phenotype
Samani NJ et al. (9)	United Kingdom	1,933 families	2	149	D2S347-D2S112	1.98	CAD
Samani NJ et al. (9)	United Kingdom	1,933 families	2	119.3	D2S2216	1.48	MI
Samani NJ et al. (9)	United Kingdom	1,698 families ≤ 56 yrs	2	140.5	D2S2271	2.7	CAD
Samani NJ et al. (9)	United Kingdom	801 families ≤ 59 yrs	2	119.3	D2S2216	2.1	MI
Farrall M et al. (10)	European	2,658 affected sibling pairs	17	69	D17S921-D17S787	2.68	CHD
Lin JP (11)	American	330 Framingham pedigrees	6	148	GATA184A08	3.1	HDLc
Coon H et al. (12)	American	649 White sibling pairs	5	48.2		2.74	HDLc
Arya R et al. (13)	American	330 Framingham pedigrees	6	152	D6S1009- GATA184A08	6.24	BMI, HDLc
Peacock JM et al. (14)	American	101 families from the NHLBI Family Heart Study	13	27.5	D13S149	2.36	HDLc

showed strong evidence for a quantitative trait locus on chromosome 6 near a marker influencing the variation of high density lipoprotein cholesterol and triglycerides in the Framingham population. Genotyping for 391 markers has been performed in 622 African American and 649 white sibling pairs in the Hypertension Genetic Epidemiology Network (HyperGEN) Blood Pressure Study (12) for qualitative lipid measurements. Although no score >3.0 were obtained, positive scores were found in several regions, such as on chromosome 5 a score of 2.74 for HDL-cholesterol in white sibling pairs. By using Framingham Heart Study data related to 1,702 subjects distributed across 330 pedigrees, substantial evidence for a quantitative trait locus with pleiotropic effects appearing to influence both BMI and HDL-C phenotypes has been found (13). Finally, a genome-wide linkage scan for quantitative trait loci influencing HDL-cholesterol concentration in 1,027 whites from 101 families in the NHLBI Family Heart Study (14), demonstrated suggestive linkage of a gene near a marker on chromosome 13 (LOD=2.36) influencing interindividual variation in HDL cholesterol.

Association studies

Because complex diseases do not follow a clear pattern of Mendelian inheritance, polymorphisms in candidate genes can be tested to search if they associate with disease by using association studies. This approach uses a case-control design, which compares allele frequencies in unrelated cases and controls in order to evaluate the contribution of genetic variants to phenotype (15). Association analysis, which uses DNA polymorphisms, is an epidemiological tool that may help to highlight the genetic basis of human traits, such as disease predisposition, drug response and aging. The major advantage of this approach lies in its simplicity and flexibility, easily allowing investigation of gene-gene and gene-environment interactions that constitute the underlying substrate of complex traits. On the other hand, several disadvantages may affect the strength of the results. In particular, to obtain statistically robust results the use of large samples and a stringent threshold for statistical significance is required. Moreover, the replication of association between common polymorphisms and disease is crucial for validating results; replication may be obtained by excluding potentially confounding populations and genotyping "neutral" markers throughout genome, and by dividing the study populations into a "test" group and a "validation" group. Finally, the stratification should be avoided.

Different genetic markers consist of DNA sequences located in genes encoding for components involved in the pathophysiology of the diseases, and a large number of candidate gene association studies have been performed for CVD. Meta-analyses have been reported for polymorphisms in a very large number of independent studies, and the results have not been homogeneous across the studies, with a weak association for these common polymorphisms. Candidate genes have been investigated, such as genes involved in the lipid metabolism (*APOE*) (16), fibrinolytic proteins (*PAI-1*) (17), angiotensin-converting enzyme (*ACE*) (18), and homocysteine metabolism (*MTHFR*) (19). Although meta-analyses of genetic studies are useful for providing the measure of association studies, they do not completely weight differences in phenotype heterogeneity, and in measuring environmental factors, which may influence the risk-disease in subjects with the same genetic susceptibility.

Genome-wide association studies

Recently, increased genome-wide panels of common polymorphisms (single nucleotide polymorphisms, SNPs) are available (20), so providing a powerful resource of genetic markers used to search for susceptibility to common diseases through genotyping platforms built with SNPs encompassing the whole genome or specific region of interest and permitting a systematic search for inherited components. Several genetic loci, individually and in aggregate, affect the risk of development of cardiovascular diseases (Table II). Data from the genome-wide association study for subclinical atherosclerosis in the community-based Framingham Heart Study (21), by evaluating over 100,000 SNPs genotyped in 1,345 subjects from 310 families, generated hypotheses regarding the association between several SNPs and subclinical atherosclerosis phenotypes in multiple arterial beds. Due to the role of obesity as risk factor for CVD, relevant data from population of Sardinia (22), and replicated in the GenNet study, showed three obesity-related quantitative traits associated with changes in BMI, hip circumference, and body weight, thus influencing the risk of obesity-related morbidity. A total of 1,087 Framingham Heart Study family members have been geno-

Table II - Genome-wide association studies.

Study	Population	SNPs evaluated (n)	Study sample (number of participants)	SNPs/chromosomal loci associated	Phenotype
O'Donnel CJ et al. (21)	American	100,000	1,345 subjects from 310 families	-rs1376877(2q33)	IMT
O'Donnel CJ et al. (21)	American	100,000	1,345 subjects from 310 families	-rs4814615(20p12)	IMT
O'Donnel CJ et al. (21)	American	100,000	1,345 subjects from 310 families	-9p21	CHD
Scuteri A et al. (22)	American, European	362,129	4,741 subjects from Sardinia; 3,467 subjects from the GenNet Study	-16q12 -rs6602024 (10p15)	Obesity-related traits
Florez CJ et al. (23)	American	66,543	1,087 Framingham Heart Study Family members	-rs2863389 (3q26) -rs7935082 (11q12) -rs952635 (1p31)	Diabetes
Larson MG et al. (24)	American	70,987	1,345 Framingham Heart Study participants from 310 families	-9p21	CVD
Samani NJ et al. (25)	European Whites	500,000	1,988 CHD patients and 3,004 from the WTCCC Study/875 CHD patients and 1644 controls from the German MI Family Study	-9p21.3 -6q25.1 -3q26.3	CAD

typed with 66,543 SNPs for association with incident diabetes and six diabetes-related quantitative traits demonstrating promising associations of 25 SNPs (23). Interestingly, a community-based genome-wide association study of major CVD outcomes (myocardial infarction, stroke, CHD death, heart failure and atrial fibrillation) performed on 1,345 Framingham Heart Study participants from 310 pedigrees, demonstrated no significant association, but suggested an intriguing finding for the chromosome 9p21 and major CVD (24).

Sequential and combined analysis from the Wellcome Trust Case Control Consortium (WTCCC) study (data from 1,000 cases of seven common diseases and 3,000 shared controls) looked for replication in the German Myocardial Infarction Family Study (875 myocardial infarction people and 1644 controls) and obtained by genotyping approximately 500,000 genetic variants, showed strong associations between several loci and coronary artery disease (25). However, some loci from the WTCCC study attempted to replicate, did not show association in the German Myocardial Infarction Family Study, thus underscoring the need to weight genome-wide association studies with caution, despite their statistical strength, until they have been replicated in appropriate validation group. Furthermore, in order to avoid bias and population stratification, cases and controls should be drawn from the same geographical area and matched for age, gender and race.

An accurate assessing and measurement of environmental factors relevant for CVD are needed for a better phenotyping. Multiple risk factors and their interactions may affect the pathophysiology of CVD, through influencing plaque stability and inflammation, platelet function, and the coagulation cascade, so predisposing to the development of different phenotypes of CVD (26).

Pharmacogenetics

Current areas of interest and investigation in the genetics of CVD include gene-environment interaction, pharmacogenetics and genetic counselling.

Complex diseases are influenced by multiple genes interacting with each other and with the environment. Interventional studies, in which an environmental exposure is normalized across subjects, allow to identify gene-environmental interactions and provide evidence for translation of findings into clinical practice. To date, blood pressure response to a low- sodium diet has been shown to vary according to polymorphisms in the renin angiotensin system, as suggested by findings from the DASH (Dietary approaches to Stop Hypertension) study, which has demonstrated that the *AGT*-6AA genotype was associated with a significant decrease in blood pressure (27). Findings from studies evaluating the response to dietary fat intake (28) and physical activity (29) have demonstrated variable interactions according to genotypes. The gene-environment interaction may mask the relevance of the genetic association, but may provide tools for correcting modifiable factors.

Progress has been made in pharmacogenetics, in which the response to a drug is a phenotype influenced by both genetic and non-genetic components. Many common polymorphisms have influence on drug efficacy and toxicity, and in turn the clinical practice, by providing an alternative treatment that through testing for the genotype might tailor the treatment strategy more clinically effective or cost effective than a merely treating everyone. Recent advances have identified genetic variants able to influence drug action, such as two SNPs in gene encoding for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), the target enzyme that is inhibited by pravastatin (30). Subjects heterozygous for these genetic variants may experience significantly smaller reductions in cholesterol when treated with pravastatin. Moreover, carriers of the gly460trp polymorphism in the gene encoding for adducing 1 (*ADD1*) and taking diuretic drugs were found to be at lower risk of MI or stroke, than subjects using other antihypertensive therapies (31). As hypertension is considered, other studies have reported interactions between diuretics and polymorphisms in gene encoding for endothelial nitric oxide synthase (eNOS); in particular, the *eNOS* Glu298Asp polymorphism made a statistically significant contribution to predicting blood pressure response to diuretics (32). Interventional studies reported a role for genes encoding components of the renin angiotensin system (*ACE* and *AT1R*) in influencing blood pressure variations, and provided a possible epistatic interaction between these two loci (33). Finally, a recent study demonstrates a role for the CYP2C19*2 polymorphism in modulating platelet aggregability and residual platelet reactivity in high-risk vascular patients on dual anti-platelet treat-

ment, thus providing a relevant information on the future design of pharmacogenetic antiaggregant strategies (34).

Genetic testing

The search for and characterization of genes predicting the susceptibility to CVD, thus improving prevention, treatment and quality of care, represent objectives of interest, and advances in molecular genetics are adding genetic tests to the diagnostic and predictive tools available for the management of CVD. A useful model of applied genetic testing for common diseases into practice is the identification of common polymorphisms associated with venous thromboembolism. Although there is not universal consensus about the use of genetic tests, the American College of Medical Genetics and the College of the American Pathologists have proposed guidelines about the utility of genetic testing for factor V Leiden and other thrombophilias (35, 36). Actually, genetic testing is not part of current CVD risk stratification algorithms, however it is likely that in the future genotyping will become part of clinical practice in order to assess CVD risk stratification.

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