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Clinical review

Science, medicine, and the future
Genetics and cardiovascular risk

Ian N M Day, David I Wilson

Cardiovascular disease is the commonest cause of death in the Western world, and congenital heart disease affects almost 1% of liveborn infants. Both diseases have substantial genetic components. The identification of specific genetic variations that underpin cardiovascular disease is providing new opportunities for diagnostic testing, pharmacogenetics, and drug development. In this review we describe genes that are known to have an important impact on the development of cardiovascular disease and discuss how their identification may improve risk management.

Methods

This review is based on our research and clinical experience in human genetics, pathology, and medicine. Citations for the topics discussed derive from literature searches in PubMed, OMIM (Online McKusick Inheritance in Man), and online US and European patent databases (USPTO and Esp@cenet respectively).

Genetic influences on blood cholesterol concentrations

Variations in the genes for low density lipoprotein receptor, apolipoprotein B, and apolipoprotein E have a considerable effect on individuals' plasma cholesterol concentrations (fig 1).

Variations in the low density lipoprotein receptor gene

Familial hypercholesterolaemia is known to be caused by codominant mutations in the low density lipoprotein (LDL) receptor gene.[1] Affected individuals typically have cholesterol concentrations twice the population average for their age and sex. This disorder often results in heart attacks in middle age in the 1 in 500 heterozygotes in populations exposed to a Western diet and in childhood coronary disease in the 1 in a million homozygotes in all populations. The disorder illustrates four important principles.

Taking a good family history
Taking a family history is one of the oldest tools in clinical medicine and is frequently neglected. Case finding through probands, for identifying people at risk of having an autosomal dominant mutation in the LDL receptor gene, is still rarely carried out in routine practice despite the availability of effective preventive treatment with statins.[2] Statins inhibit cholesterol

Anticipated developments

Progress in unravelling the genetic components of coronary artery disease and congenital heart disease

Further identification of genetic variants which have predictive value and help direct preventive therapy and counselling

In the next decade gene testing is likely to become an established component of preventing coronary artery disease

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synthesis and secondarily lead to upregulation of the LDL receptor and thus greater clearance of LDL cholesterol to bile by the liver. They are of proved effectiveness in both primary and secondary prevention of atherosclerotic disease.[3]

Tailoring treatment to genetic variation
Individuals who are identified (from family based case finding) as being heterozygous for the LDL receptor gene should be advised about primary preventive
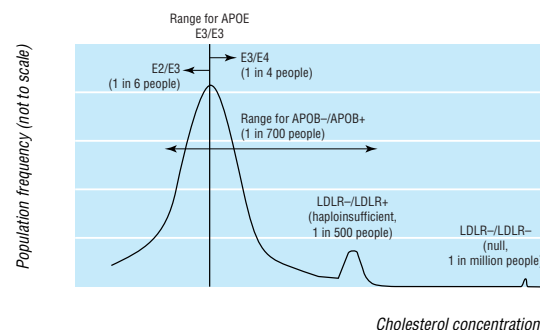

Fig 1 Effects of genetic variation on population distribution of plasma cholesterol concentration. Polymorphisms in the apolipoprotein E gene (APOE) cause an average 10% lowering (E2 allele) or raising (E4 allele) of cholesterol concentration compared with homozygosity for E3. A single defective allele of the apolipoprotein B gene (APOB) has a heterogeneous effect on cholesterol. A defect in the low density lipoprotein receptor gene (LDLR) leads to a rough doubling in cholesterol concentration for heterozygotes and greater increases for homozygotes

measures, including the critical importance of not smoking, attention to diet, and attention to other risk factors and treatment with statins. Families with a child homozygous for inactivation of the LDL receptor should be counselled about the risk of cardiovascular disease. The risk of recurrence (for homozygosity) in siblings is 25%, prenatal tests may be possible, and both parents will have the coronary risks of heterozygotes. Those who are identified as homozygotes should be followed up carefully from early childhood in specialist clinics that can offer LDL apheresis and related techniques. (Homozygotes have no functioning LDL receptor gene, so cholesterol clearance cannot occur.)

One new approach to treatment entails removing a patient's hepatocytes, inserting a functioning LDL receptor gene into the cells, and then returning the cells to the patient.[4] This is complex, but in time procedures may become more simple.

Changing the expression of a gene in endothelial or smooth muscle cells of the coronary vasculature may prove to be easier, without ex vivo steps.[5] This may be by nuclear gene insertion or by transitory block of a specific messenger RNA by antisense oligonucleotide to prevent translation of a protein. This approach is relevant to any form of coronary disease.

Genetic and environmental risk factors interact
Chinese people who are heterozygous for the LDL receptor gene present with coronary disease less frequently than do people in Western countries, presumably because the traditional Chinese diet does not contain the same excess of saturated fat even though smoking rates are high.[6] In contrast, individuals from Europe and North America with familial hypercholesterolaemia are at increased risk because of diet, and smoking increases their risk further.[7]

Genetic variation is extensive
In Britain many different mutations in the LDL receptor gene are recognised, and some severely hypercholesterolaemic families have mutations in other genes (see below).[8 9] This has made genetic diagnostic testing hard to establish because it is still difficult to provide reliable, high throughput, cost effective gene analysis for multiple mutations in large numbers of patients. However, technological progress means that this is unlikely to be limiting in the long term (fig 2).

In familial hypercholesterolaemia genetic status can often be inferred from the intermediate trait of plasma cholesterol concentration, but in other disorders pre-disease "intermediate traits" (biochemical or other features intermediate between the gene and the disease it can cause) may be harder to detect.

Variations in the apolipoprotein B gene
Some patients with a family history of hypercholesterolaemia have a defect in the gene for apolipoprotein B, the component of low density lipoprotein that binds the receptor.[10] This ligand defect is called familial defective apoB. One common mutation of the gene is R3500Q, which occurs in 1/700-1/1000 people in central and western Europe. The risk of coronary disease is seven times higher in people with this mutation than in the general population.[11] Cholesterol concentrations in affected individuals tend to vary more between family members and within individuals over time compared with concentrations in families with
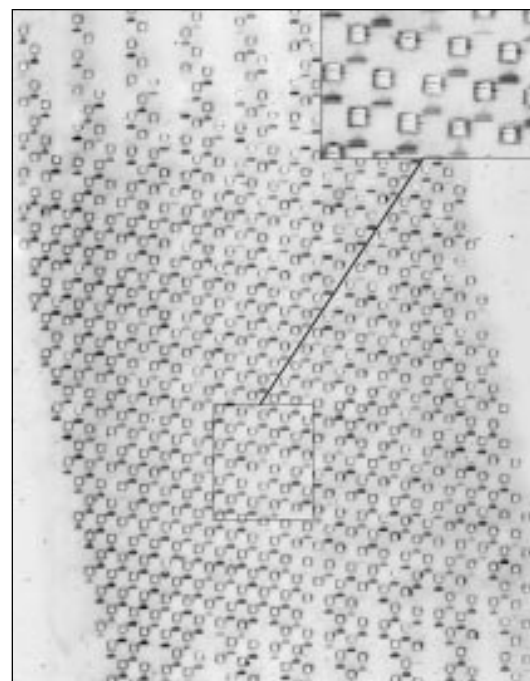

Fig 2 Analysis of a potential cardiovascular genetic risk marker in 768 subjects (one tenth of a worker's morning's work). One patient is represented by each square (a well into which a DNA sample is loaded). Possession of the marker is indicated by two "bands" in the electrophoresis lane derived from each well, while one band indicates lack of the marker. (Image courtesy of Himanshu Patel, Les Hinks, and Tom Gaunt, Division of Human Genetics, School of Medicine, Southampton University)

abnormalities in the LDL receptor gene, and some patients have normal cholesterol concentrations.[12]

A simple genetic test for the R3500Q mutation is available, and some family members of known probands ask to be tested for the gene. The clinical significance of a positive test may be hard to determine, however, especially if the individual concerned has a normal cholesterol level. At present, relatives may be better advised to forgo testing and just have their cholesterol concentrations regularly monitored. As public and professional knowledge about genetic predisposition to cardiovascular disease increases, however, clinical practice will doubtless change, not least to respond to new demands from patients.

Variations in the apolipoprotein E gene
Apolipoprotein E acts as a ligand for the LDL receptor and has an important role in clearing cholesterol-rich lipoproteins from plasma. The E4 allele of the apolipoprotein E gene occurs in about 30% of the general population (E3 being the most common allele).[13] Heterozygotes possessing alleles E3/E4 have cholesterol concentrations on average 10% higher than those of E3/E3 homozygotes because of the differential allelic effects on lipoprotein particle turnover. This difference extends to coronary events, although the risk associated with the E4 allele seems greater than is indicated from its effect on cholesterol levels. We give this example not because it is currently relevant to clinical practice, but as an example of the impact of a common genetic polymorphism on population cholesterol levels. If a hypolipidaemic drug were found to be effective in only one genetic subgroup the genotypes would be of more interest.

A third version of the gene, the E2 allele, occurs in its homozygous form in 1% of the UK population. When combined with a "second hit," such as diabetes or hypothyroidism, the E2/E2 genotype results in type III dysbetalipoproteinaemia, which is associated with a high risk of coronary and peripheral vascular disease and occurs in 0.01-0.02% of the population.

Genetics and smoking

The heritability of behavioural traits such as smoking is now recognised to be high. About 2% of the population have one inactive copy (haploinsufficiency) of the gene CYP2A6, which encodes a cytochrome P450 enzyme that inactivates nicotine to cotinine. Among this group smoking is less prevalent than in the general population.[14] The inference is that those who are slow to inactivate the addictive ingredient, nicotine, either do not become regular smokers or, if they do, consume fewer packs. Among established smokers, propensity to quit over a lifetime is almost twice as great in haploinsufficient people as in those with two active CYP2A6 genes.[15] In the future smoking cessation clinics may treat smokers who are CYP2A6 "poor metabolisers" differently from other smokers. Nicotine substitutes or substrates or inhibitors for the CYP2A6 enzyme (akin to Antabuse for alcoholics) might be more suitable treatment in some groups than others. Contrasting variations in addiction may involve polymorphism in central serotonergic and dopaminergic pathways.

Genetics and hypertension

Angiotensinogen, a peptide hormone, is a major component of the renin-angiotensin system, which is important in salt-water homoeostasis and maintenance of vascular tone and is thus an important determinant of blood pressure. Common molecular variants in angiotensinogen are associated with essential hypertension, pregnancy induced hypertension, angiotensin concentrations, and blood pressure response to low salt diet and some drugs. It seems that either a variation in the protein sequence of angiotensinogen or an associated polymorphism in the angiotensinogen gene promoter (on-off switch) cause these effects.[16] Myriad Genetics, a genomics company based in Salt Lake City, Utah offers a prognostic genotyping test for this angiotensinogen polymorphism.[17] The aim of the test to identify good responders to a low salt diet or to specific drugs.

Genetics and diabetes

Diabetes is a classical risk factor for late onset coronary disease. Researchers investigating type 2 diabetes have had to collect many thousands of nuclear families containing at least two affected siblings to try to determine what genomic regions are shared by affected pairs more frequently than chance would predict. One study has unearthed the "calpain 10" gene as one contributor to type 2 diabetes.[18] Previous knowledge of the condition would not have incriminated this otherwise obscure cysteine proteinase enzyme nor its genomic region. Although this approach (which is systematic rather than based on a "best guess" hypothesis) is expensive, it is currently popular because it can identify wholly new pathways of pathogenesis. New drug developments may depend as much on these approaches as existing drugs have on classic biochemical physiology.

Arrhythmias and pharmacogenetics

Investigations of families with the long QT syndrome, who are risk from sudden death from ventricular tachycardia and fibrillation, have revealed mutations within several genes that code for ion channel proteins (such as KVLQT1, HERG, SCN5A, minK, MiRP1, and RyR2).[19] Although such families are rare, their investigation has provided insight into the cellular mechanisms associated with acquired tachyarrhythmias, including drug associated long QT. Drug associated tachyarrhythmias and QT prolongation have also become a major issue in drug trialling and regulation because these side effects are quite common and are potentially fatal.

A classic gene in drug metabolism is CYP2D6, which encodes a liver cytochrome that hydroxylates many compounds. Common gene variants of CYP2D6 result in people being either "extensive" or "poor" metabolisers of a range of drugs. Those with the poor metaboliser phenotype are prone to diverse side effects from such drugs, but in the cardiovascular context QT prolongation is a particular risk. The phenotypes may be hard to identify until it is too late, and the genetic complexity is too great to permit cost effective and precise predictive testing, but there is intense effort to define the genetic complexity sufficiently to allow the science to be applied in routine clinical practice.

Genetics and congenital heart disease

Congenital heart disease is associated with significant morbidity and mortality in children and adults. The risk of disease recurring in a sibling of an affected child is in the order of 2-5%. This figure is influenced by the cardiac defect, and some studies have estimated sibling recurrence risk to be as high as 10%.[20] This, coupled with the risk of an adult with congenital heart disease having an affected child (offspring risk),[21] gives an indication of the proportion of cases of congenital heart disease that have a genetic aetiology. Recent research has identified specific genes that may predispose an embryo to cardiac maldevelopment—such as NKX2.5 causing atrial septal defects, ELN (elastin) causing supravalvar aortic stenosis, and mutations in TBX5 causing septal defects (fig 3).[22 23]

A major success in the investigation of causes of congenital heart disease comes from molecular cytogenetics and the use of chromosome fluorescent in situ hybridisation. This technique has dramatically improved the resolution with which chromosome microdeletions can be identified. Deletions within chromosome 22q11 are found in more than half of children born with interrupted aortic arch or truncus arteriosus,[24 25] and now many cytogenetic laboratories offer 22q11 deletion analysis as a routine investigation for any child or fetus with a substantial cardiac defect.

Future developments

As the genetic basis of cardiovascular disease is unravelled and more genetic prognostic and diagnostic tests become available, doctors will need clear guidance on how to use the new genetic knowledge to improve patient care. Over the next 10 years, the use of other

markers such as lipoprotein(a) and homocysteine, which have genetic determinants, will increase. In addition, direct genotype tests (applied to DNA extracted from blood or buccal wash) will be developed for markers such as angiotensinogen, which may have advantages over biochemical assays. Specific tests for risk factors for rare but severe cardiovascular conditions will be applied where therapeutic choices are available—such as in developmental genetics, arrhythmias, cardiomyopathies, and hypertension.

In the next 10-30 years a new battery of investigations is likely to improve diagnostic precision and inform prognosis. Algorithmic use of such additional risk factor data will require sophisticated software to aid clinicians to optimise therapeutic choices for patients.

In Britain direct marketing of genetic tests to the general public is permissible but subject to a (voluntary) code of practice and guidance established by the Advisory Committee on Genetic Testing, now subsumed by the Human Genetics Commission of the Department of Health.[26] If public knowledge and the availability and usefulness of tests all increase substantially, and costs diminish, tests by postal samples of DNA from buccal washes could abound. Some patients already present to their doctor with information gleaned from the internet, and a few avail themselves of high street kits for self testing of cholesterol concentration and blood pressure. The same could evolve for gene tests. Authoritarian control may be inappropriate, but public and professional education will be important.

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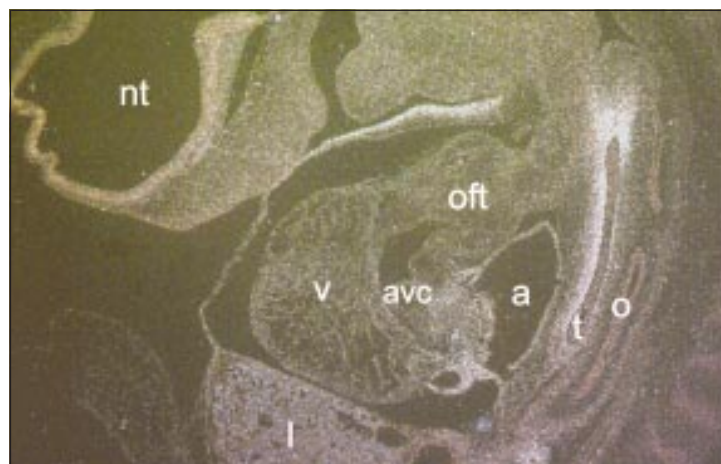26 Human Genetics Commission. www.hgc.gov.uk/ (accessed 12 Sep 2001).

Fig 3 Expression of the gene TBX5 in the developing heart. Mutations in TBX5 cause the autosomal dominant condition Holt-Oram syndrome, which includes cardiac septal defects. Gene expression (bright signal) is seen in the atrial wall, atrioventricular endocardial cushions, trachea, and upper anterior thoracic wall (a=atria, v=ventricle, avc=atrioventricular canal, oft=outflow tract, l=liver, o=oesophagus, t=trachea, nt=neural tube)