insulin-treated type 2 diabetic patients continue to have increased risk of cardiovascular events[3]. 'We intend to examine the effects of glucose-lowering drugs such as insulin, as well as other anti-diabetic drugs, on accelerated atherosclerosis. Unlike insulin, these drugs do not control blood-glucose levels, and could include anti-inflammatory drugs, inhibitors of second-messenger systems and factors mediating smooth-muscle proliferation and monocyte and macrophage function,' says Gerrity

In collaborative studies with diabetologist Jerry Nadler and cardiologist Ian Sarembock from the University of Virginia Health Sciences Center (Charlottesville, VA, USA), Gerrity and colleagues are performing angioplasty and stent procedures in these diabetic swine. This follows from observations that diabetic people who have undergone these procedures are more likely to restenose than non-diabetic controls[8]. The size and structure of the swine heart and arteries are such that the same angioplasty catheters and stents used in humans can be used in pigs. Preliminary studies by the group have shown that stents in diabetic swine do restenose more rapidly than in those in non-diabetic swine. The ultimate objective of the study is to see whether effective glucose control with insulin, or other anti-diabetic drugs, can be used to prevent the process.

'Our model provides an excellent system in which to carry out mechanistic studies aimed at furthering our understanding of diabetic atherosclerosis, and to test the effects of existing and experimental pharmaceutical interventions on both disease progression and stent restenosis, both of which are major concerns for diabetics,' says Gerrity. Hopefully, interventions that are successful in this humanoid model could rapidly be transferred into clinical trials in humans.

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New method links multiple genes to complex diseases

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One of the greatest challenges facing human geneticists is to identify the genes influencing complex, multifactorial diseases. Researchers at Vanderbilt University (Nashville, TN, USA) have developed a statistical technique that allows multi-locus genetic effects to be identified from studies involving relatively small patient samples[1].

Most common diseases have no clear pattern of inheritance. However, many are strongly suspected to have a genetic component, which probably involves subtle interactions between polymorphisms in several different genes. The interaction of genes with environmental factors could also have a role.

Data reduction

Traditional parametric statistical techniques are of limited use in detecting which of many possible genetic combinations make a person susceptible to disease. Contingency tables generated from polymorphism data usually contain many empty cells, that is, genetic combinations that were not observed in the study. Large sample sizes are needed to make such studies statistically valid, and it is often prohibitively expensive to obtain genetic information from enough subjects.

Data reduction methods have been used successfully to analyze quantitative genetic traits[2]. Inspired by this, the Vanderbilt group has developed a computer-based method called multifactor-dimensionality reduction (MDR)[1]. Designed to analyze discrete traits in case-control and discordant sibling-pair studies, it can detect and describe gene–gene and gene–environment interactions involving as many as ten or more different gene loci.

MDR involves constructing a series of contingency tables showing the incidence of all the possible pairs of polymorphisms in cases and controls[1]. Each cell in the table is designated as either high or low risk depending on whether the combination of genotypes occurs more commonly in cases (high risk) or controls (low risk). This reduces the number of dimensions involved to one,

1359-6446/01/$ – see front matter PII: S1359-6446(01)01955-9

that is, high or low risk. The one-dimensional variable is then subjected to recognized statistical techniques, known as cross-validation and permutation testing, to find the genetic combination that can best predict whether or not a person will be affected by the disease under analysis.

MDR was first evaluated with simulated data comprising four sets of 50 replicates of 200 cases and 200 controls. These were fed into four different models of multilocus epistasis (gene–gene interaction). The models were based on arbitrary definitions of disease-causing combinations of up to five polymorphisms. 'We were testing the MDR program to see if it could find what we knew was the answer,' says lead researcher Jason Moore, 'and it consistently found it.'

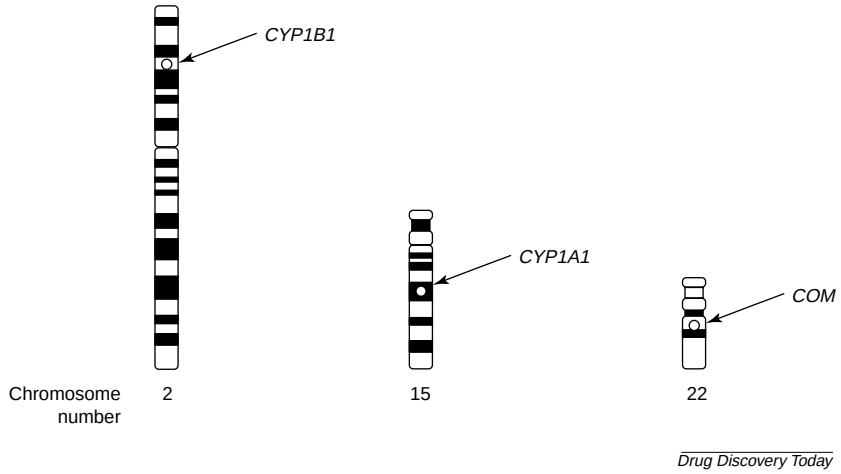

Figure 1. Locations of the genes involved in oestrogen metabolism that were jointly associated with increased risk for sporadic breast cancer. Adapted from a figure supplied by Marilyn Ritchie, Vanderbilt University.

Potential applications

The group then used MDR to look for combinations of single nucleotide polymorphisms (SNPs) that might increase susceptibility to sporadic breast cancer. Only ~10% of breast cancers are directly heritable, with the remainder classed as sporadic. Sporadic tumours are probably multifactorial, but there is substantial evidence of a causative role for oestrogen[3]. Therefore, five genes for enzymes involved in oestrogen metabolism were chosen for the analysis, each having two polymorphisms known to affect levels of carcinogenic oestrogen metabolites[4]. Data were obtained from stored tissue samples from 200 patients with sporadic breast cancer and 200 matched controls with no history of the disease.

They identified a combination of four polymorphisms, at three gene loci, which had a statistically significant ($p = 0.001$) association with increased breast cancer risk[1] (Fig. 1). None of the loci showed an independent main effect, indicating an epistatic process. 'To our knowledge, this is the first time that such a multiple-gene interaction involving four loci has been associated with a common multifactorial disease,' says Moore. He believes that machine learning algorithms and Vanderbilt's new supercomputer will allow them to search for interactions among up to 20 genes. These algorithms, currently under development, allow the computer to perform an 'intelligent' search by learning from previous results. Work is also under way to improve the predictive ability of MDR with greater numbers of variables. MDR is currently being modified to cope with studies with unequal numbers of cases and controls.

Another potential application for MDR is in pharmacogenetics: the Vanderbilt team will use it in a National Institutes of Health-funded study to look for gene combinations that are predictive of drug response. The group will also evaluate its applicability to microarray gene-expression data, where it will be used to identify combinations of expressed genes that are associated with a clinical endpoint. Moore is also confident that MDR will prove useful for identifying genes for development as drug targets.

'This novel method represents a useful extension of existing single-locus methods to test for associations between genes and disease,' says Eden Martin, a statistical geneticist in the Department of Medicine at Duke University (Durham, NC, USA). 'It will be useful in uncovering targets for drug action and could highlight genetic interactions that may suggest potent drug combinations. It could also give insight into the physiology of diseases.' Martin believes that the most challenging aspect of MDR will be interpreting the results: 'It may be difficult to sort out the nature of complex interactions. Additionally, the computational power required to assess higher-order interactions may make MDR of limited utility to research groups without extensive computer resources.'

The MDR software is available free to academic users, and to profit-making organizations for a fee (go to: http://phg.mc.vanderbilt.edu/Software/MDR).

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