Editorial Comment

Will Gene Markers Predict Hypertension?

Roger R. Williams

Clinicians hearing about gene markers envision a simple blood test that will tell whether a particular patient carries a specific disease gene that runs in that patient's family. Investigators expect gene markers to elucidate pathophysiological mechanisms so we can tailor disease prevention and treatment to specific genes and contributing environmental cofactors.

Practical Help From Gene Markers

It may be unrealistic to expect a pure monogenic (single gene) explanation for most patients with a disorder as common and heterogeneous as essential hypertension. However, gene marker technology can be combined with risk factor epidemiology to try to quantitate the risk of hypertension and sort out heterogeneity. We can hope to define several discrete gene loci and associated environmental cofactors to explain the multifactorial pathophysiology of essential hypertension. In certain individuals one or two discrete gene loci may play a predominant role (e.g., altered cellular electrolyte metabolism and sodium retention in some, familial dyslipidemia and hyperinsulinemia in others). In persons with a predominant genetic susceptibility, we might be able to specify the most influential environmental cofactors for prevention or treatment of that person's hypertension (e.g., sodium restriction in some, exercise and weight loss in others). Choice of antihypertensive medication might also be tailored to the predominant causal factors (e.g., diuretics for some, converting enzyme inhibitors for others).

Genetic Linkage Studies

Clinically useful gene markers are usually identified from linkage studies within families. Genetic linkage studies test the assumption that a particular measurable gene marker (such as human leukocyte antigen [HLA] phenotype) sits on the same chromosome as the gene causing the disease of interest so that the disease and the gene marker cosegregate. If a person has a dominantly inherited disease gene, we would expect about one half of the children and about one fourth of the grandchildren to inherit the same disease. A hypothetical pedigree in Figure 1 illustrates cosegregation of HLA type with disease. If the location or locus of the marker gene is on the same chromosome "quite close" to the disease gene, we would expect all of the children and grandchildren who received the disease gene to also have the same polymorphism for the marker gene (HLA B12 in the hypothetical example). For a disease with onset in adults, a blood test for the marker gene could be used to find out which of the children and grandchildren had received the inherited disease tendency. "Tight genetic linkage" is defined as expecting 99% or more of all family members with the disease to also show the specific marker type that cosegregates with the disease in that family. In a different family the same disease could cosegregate with the same marker locus (HLA) but with a different polymorphism (e.g., B15 or B18).

If the disease gene locus and marker gene locus are farther apart on the same chromosome, they may occasionally be separated from each other when crossing over of chromosomes occurs between the two locations during meiosis, the process during which chromosomes are prepared for germ cells before reproduction. During meiosis, matched pairs of chromosomes repeatedly "cross over" and exchange segments of equal length producing recombination. If crossing over occurs between the location of the gene marker and the disease gene, recombination separates these two gene loci. After meiosis, offspring with the disease gene will no longer have the characteristic marker gene polymorphism found in their parent with the disease. How often crossing over and recombination separate a particular gene marker from its disease gene depends on how far apart they are on the chromosome. As long as the gene marker and disease gene are close enough together for recombination to occur less than 50% of the time, it should be theoretically possible to detect "statistically significant genetic linkage" by observing cosegregation between the marker and the disease more often than would be expected by chance among descendants of persons with both the disease gene and specific marker phenotype. As in other statistical tests, a striking finding (tight linkage) can be detected with a small number of subjects, whereas a weak finding (linkage with 30% recombination) requires a large sample size for reliable detection.

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In the past, traits used as gene markers included ABO blood groups, HLA histocompatibility antigens, and electrophoretic variations of proteins and enzymes in serum, saliva, urine, and cells. Biological specimens to measure these genetic markers included serum, red blood cells, white blood cells, saliva, and urine. To be most informative, a marker should have many different variations or polymorphisms. A good example is the HLA system, which is very polymorphic with over 90 different antigenic variations at this locus (e.g., A28, B17, DR7). In contrast, many of the old marker systems such as Rh (two types, positive or negative) and ABO blood groups (four types, A, B, O, and AB) had few polymorphisms. If a large percentage of the population had only one of the variations, little power was present for detection of significant genetic linkage with these markers even if the disease were close enough for linkage to occur.

New DNA technology has dramatically improved the opportunities for using genetic linkage to detect and study disease genes. New methods detect the variation in genetic coding contained in the DNA sequence. This has several advantages. First, only one biological sample is needed: white blood cells. Second, because the code being studied is DNA itself, a disease gene could be located on any chromosome and still have a good chance of being close to some measurable DNA marker that could show genetic linkage. Third, theoretically there should be thousands of possible DNA markers with sufficient degree of variation or polymorphism to detect genetic linkage. Tight linkage (less than 1% recombination) occurs between gene loci and markers located within a chromosomal region spanning about 1 million base pairs. With an average of one polymorphism for every 500 base pairs, there would be about 2,000 possible polymorphisms in that region. At least some of these polymorphisms should be detectable and useful for genetic linkage. For "statistically useful linkage" (5% recombination), there should be as many as 10,000 DNA polymorphisms available.

The possibility of finding half a dozen DNA markers close enough to a disease gene to provide a high probability for detection of genetic linkage seems very likely under these circumstances. This is one of the major motivations for a current worldwide effort to map the human genome. Molecular biologists, coordinated through a computer network, are rapidly expanding a catalog of specific gene markers spread over the whole human genome. The goal is to produce a library of sufficient markers to enable linkage studies for most genetic diseases. The next goal will be pinpointing the exact locations of the disease genes and characterizing the DNA coding variations at the disease locus. It is estimated that about 200 markers evenly spaced throughout the human genome would provide an opportunity for statistically useful linkage with disease genes anywhere on the human genome. About 1,000 evenly spaced markers would provide the tool necessary to find tight linkage with almost any disease gene. Progress is moving rapidly in this field, and in one laboratory alone nearly 600 DNA markers have been developed.

**Association Versus Linkage of Gene Markers With Disease**

The same gene markers used to test for genetic linkage can also be used to test for an association of the disease with a specific polymorphism of a gene marker. For example, ankylosing spondylitis is strongly associated with HLA-B27 in nearly every population that has been studied. In most general populations, this HLA polymorphism occurs in only 5–10% of the general population, whereas 90% or more patients with ankylosing spondylitis have...
the B27 phenotype. Although the basis for this association has not been established, it suggests that the specific B27 antigen may be related to the pathogenic mechanism leading to the disease. In other words, the marker gene does not just sit next to the disease gene, it actually is a gene involved in the pathogenic process leading to the disease. While linkage studies require disease and marker information on individuals within families, association studies look at data from unrelated individuals in the general population, preferably from several different populations. In linkage studies, the same gene marker polymorphism is consistently observed among members of a family with the disease. In other families with the same disease, a different polymorphism at the same locus would be observed. This is consistent with the hypothesis that the two genes tend to be inherited together; however, the specific marker that is cosegregating in that family is not necessarily involved in the disease process. In contrast, gene marker association with disease such as HLA-B27 with ankylosing spondylitis, shows exactly the same gene marker polymorphism in diseased persons in many different families.

Obtaining the data from family members for linkage studies is often more difficult than obtaining marker and disease status on many persons sampled from the general population (e.g., blood donors). On the other hand, with currently available marker technology, there is a much better chance of identifying significant linkage than significant association. The investigator has to be lucky enough to select the actual marker involved in the disease process to achieve a significant association. In contrast, significant linkage could be obtained by studying polymorphisms of any of approximately 2,000 theoretically available marker polymorphisms within the region of tight linkage or as many as 10,000 marker polymorphisms that would link with up to 5% recombination. In most situations, the odds of success are higher for finding linkage than associations. Thus, although the population data required to test linkage are harder to obtain, the probability of success is higher. In the past, association studies have been easier to do because of available population data and therefore, seem to be much more common than linkage studies. As a case in point, Gerbase-deLima et al reported that other than their study, only one other known study tested for linkage of HLA with hypertension. However, their review of the literature produced 20 association studies of HLA versus hypertension.

Most investigators doing both types of studies believe that linkage studies are more reliable than association studies. Because there are so many different gene markers available, hundreds of tests for association have been performed in general populations finding spurious "chance associations" simply because one of 20 tests will be significant at p<0.05 based on chance alone. A study wherein an investigator goes to the trouble of assembling families of persons with a particular disease is usually more focused and chance associations become less likely.

**Applications of Linkage to Hypertension**

Traditional linkage studies assume that imperfect cosegregation is caused by crossing over and recombination. The frequency of recombination is used in statistical calculations to infer genetic distances from the disease gene to the marker locus. Hypertension does not fit this classical model. It is likely heterogeneous and caused by multifactorial characteristics including several definable single gene contributions, polygenic blending, shared family environment, and individual environmental effects. If some gene marker is linked to a monogenic determinant of "essential hypertension", imperfect cosegregation will be expected based just on heterogeneity alone. In other words, at least some cases of hypertension will be caused by the influence of factors other than the single gene being tested, and other cases will exist where individuals inherit the disease gene but are not affected. Thus, one is less likely to expect tight linkage, but one should be willing to accept statistically significant linkage.

With the growing availability of DNA markers, it is tempting to jump straight to a genetic linkage study of hypertension with a given polymorphic marker. Such an impatient attempt to test for linkage could be considered a "long shot" or even a "shot in the dark" as described in Table 1. Results would often be negative as they were for a linkage study of the polymorphisms of the structural gene for renin versus hypertension in a single large pedigree. On the other hand, the multifactorial nature of hypertension implies multiple single gene traits provide several targets, improving the chances of a "hit" even for long shot linkage studies. The
reported linkage of HLA phenotype to hypertension\textsuperscript{4} may represent a successful long shot.

Where possible, informative subsets of the general population should be indexed for study with the best available phenotypes to improve the chances of success. Consider the analogous situation of testing for linkage to a major gene affecting blood cholesterol levels. Testing for genetic linkage of unselected polymorphic markers versus the phenotype "total cholesterol" with a random sample of families from the general population would be very unlikely to succeed in finding a single locus affecting cholesterol. However, by taking advantage of prior knowledge of dominant transmission of very high low density lipoprotein (LDL) cholesterol levels and using a candidate gene marker (DNA probe for LDL receptor locus), genetic linkage was found in the first family tested with a LOD score of 7.52 and 0\% recombination.\textsuperscript{5} Candidate phenotypes for hypertension with segregation data suggesting some major gene effects include sodium-lithium countertransport and urinary kallikrein excretion.\textsuperscript{1} Candidate DNA markers exist for each of these (sodium-hydrogen transporter gene and structural gene for renal kallikrein). Families showing clear segregation of the phenotype would be most informative for a linkage study.

Quantitative Predisposition

None of the currently identified risk factors for hypertension (e.g., obesity, family history, age, urinary kallikrein) acts as an all or none phenomenon. Most indicate a quantitative predisposition to hypertension just as cholesterol, HDL, blood pressure, and body mass index are quantitative risk factors for coronary artery disease.

As an example in hypertension research, high values of sodium-lithium countertransport are strongly associated with hypertension. However, in one study only 26\% of subjects with essential hypertension had values considered “high” by most other published reports.\textsuperscript{6} This overlap of quantitative countertransport level was also seen in normotensive persons, with 11\% having levels in the range commonly associated with hypertension.

Perhaps a battery of quantitative risk factors for essential hypertension will be useful in predicting the future risk of this disorder in a manner similar to the use of a battery of coronary risk factors for the detection and management of coronary risk. Judicious use of gene markers for linkage studies and innovative integration with epidemiological methods should bring us closer to this goal and closer to understanding the genetic and environmental determinants of hypertension.

References


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