The genetic basis of X-linked nephrolithiasis: leaving no stone unturned.

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Editorial

In this issue of *The Journal of Clinical Investigation,* Scheinman et al. (1) report the recognition of genetic linkage of a unique form of nephrolithiasis with a polymorphic locus previously mapped to the short arm of the human X chromosome. This work represents a significant accomplishment in efforts to understand the fundamental etiology of this condition, and also serves to illustrate some of the current possibilities and problems which face those who are trying to clone disease related genes. There has been much recent interest in the process of characterizing the basic pathophysiology of a number of genetic disorders by positional cloning of the responsible genes (2, 3). The enthusiasm for conducting this type of work continues to grow as the successful progress of the Human Genome Project leads to more refined maps which enhance the feasibility of such research endeavors. Even though it comprises only about 5% of the total human genome, the map of the X chromosome currently has the most detailed cartography. This is because of the historic ease of assigning gene loci to the X chromosome due to the characteristic sex-linked pattern of inheritance typical of X-linked traits and because a great deal of attention has been devoted to this particular chromosome by scientists active in gene mapping. At present, some 237 defined genes and a total of 1,631 markers have been mapped with varying degrees of resolution to the human X chromosome (4).

The authors of the paper cited above have studied 102 members of a single family and are thus assured of investigating a homogeneous genetic condition, a problem of some considerable concern to scientists investigating other disorders who must of necessity pool data from a number of families to achieve statistically significant results. Many times, it has been discovered, often in the conduct of linkage mapping studies, that patients who at first appeared to have a single genetic entity, are in fact heterogeneous with respect to the specific locus at fault. The strength of the present data in this regard carries the counterbalancing problem that the results can only be applied with certainty to this unique kindred. Scheinman et al. have found that with a very high likelihood, the gene responsible for this progressive form of tubular dysfunction and renal failure family is located approximately 3.6 centimorgans from the anonymous DNA marker DXS255. Furthermore, multipoint linkage analysis makes it probable that the gene in question is situated between DXS255 and the dystrophin locus. This information could be of immediate utility for the genetic counseling of individuals within this pedigree. However, much additional information will be needed before the involved gene can be identified and its function elucidated.

There are still many hurdles which any investigator needs to negotiate in going from a preliminary linkage result to an identified genetic defect. Direct inspection of the data provided in this article indicate that while a recombination fraction of 3.6% with DXS255 is most likely, map distances of 10 centimorgans are almost equally possible. It is generally assumed that 1 centimorgan on the genetic recombination map is

roughly equivalent to 1 megabase of DNA in physical terms. However, there are substantial variations to this equivalency, and in particular, it seems that the pericentomeric regions of human chromosomes may have a particularly high ratio of physical to genetic distance. Thus, it is possible that the gene being searched for is located more than 10 megabases from this nearest available landmark. The steps required to cover this amount of distance by chromosome walking, to identify functional genes in the interval covered, and then to recognize the specific gene involved in this condition are formidable indeed. As an example of the effort and persistence which can be required to accomplish such ends, one need only examine the recent experience in cloning the gene responsible for Huntington's disease (5). It took 10 years, and the efforts of numerous collaborating groups working with many more patients than are available for X-linked nephrolithiasis to move from a linked marker some 4 centimorgans distant, to the actual gene itself. Using a more nephrologic example, the gene that causes adult type polycystic kidney disease was located on chromosome 16 in 1985 (6), and the identity of this locus is still elusive. More precise boundaries for the X-linked nephrolithiasis gene need to be established through further linkage studies, or through the recognition of discrete chromosomal rearrangements in additional families. Then, candidate genes will have to be found based on sequences that predict a function in tubular transport, or that are expressed in a tissue-specific fashion that might be predicted for a gene which causes this phenotype. Finally, a mutation will have to be recognized in the candidate gene in affected subjects, and the identified change demonstrated to have functional significance (i.e., not a benign polymorphism). While the overall tasks can seem daunting, numerous recent successes along with the steady development of new methodologies for genomic analysis, rapid sequencing, computer comparisons of sequence information, and means to functionally express and analyze new gene products make such undertakings more hopeful. Indeed, it should be appreciated that such efforts are often the best or only means available to try to garner information that will ultimately be helpful to patients and families affected with many genetic diseases.

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