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Genetics of kidney disease

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Genetics of kidney disease. Multiple lines of evidence suggest that susceptibility to develop end-stage renal disease (ESRD) has a significant genetic component. These studies include familial aggregation studies, comparisons of incidence rates between different racial or ethnic populations, and segregation analysis. Multiple approaches have been employed in an effort to identify genes that contribute to this genetic susceptibility. Many studies have now been carried out assessing the contribution of specific "candidate genes," that is, genes with functions consistent with involvement in renal pathogenesis. Independent evaluations of specific candidate genes have frequently provided contradictory results. This may be due, in part, to the modest contribution to genetic susceptibility that these genes impart. In contrast to the focused analysis of candidate genes, the genome scan approach employs a comprehensive evaluation of inheritance throughout the genome. The great potential advantage of the genome scan is the ability to identify chromosomal regions harboring novel, previously unrecognized, genes that contribute to renal disease. Results from whole genome scans of family collections are now beginning to appear and give the promise that multiple comprehensive genetic evaluations of end-stage renal disease will soon be available for evaluation.

The purpose of this article is to present an overview of current thinking and ongoing research in genetic studies of renal disease. Emphasis has been placed on current efforts to identify the genetic components of common forms of renal disease. It is now widely accepted that the genes an individual inherits from their parents contribute to susceptibility to inherit many forms of renal disease. All of us are familiar with the clearly genetic forms of kidney disease such as polycystic kidney disease, Alport syndrome, some forms of focal segmental glomerular sclerosis and IgA nephropathy. From the pattern of inheritance it is clear that such medical conditions are caused by single genes, and in several cases these genes have now been identified. Although these disorders have devastating impact on affected individuals and their families, the monogenic forms of kidney disease comprise a relatively small percentage of the total number of people with ESRD.

Key words: inherited kidney disease, heritable renal disease, genome screening, familial genetic evaluation, candidate genes.

At this time we can presume with some confidence that individual risk of developing ESRD is a function of not only inheritance, that is, genetic risk, but also the environment in which a person lives and the lifestyle that a person leads. We envision that ESRD susceptibility genes are not the "yes" or "no" genes of monogenic disorders, but genes whose risk is modified by elements such as diet, exercise, occupation, and, especially in renal disease the co-existence of diabetes or hypertension. In the United States the most common co-existing medical conditions are Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), and hypertension. Thus, lifestyle and environment can contribute to increased or decreased risk for renal disease.

EVIDENCE FOR A GENETIC COMPONENT TO RENAL DISEASE

There is now extensive evidence from a wide variety of approaches that suggests renal disease in the general population has a genetic component. The most numerous studies take the form of evaluating familial aggregation. In their simplest form, familial aggregation studies evaluate the incidence of renal disease in families with index cases with renal disease compared to the incidence of renal disease in families with index cases without renal disease. Seaquist et al published the first description of familial aggregation in renal disease, primarily studying Caucasian Type 1 diabetes families (T1DM) [1]. Families containing individuals who had undergone kidney transplantation were compared to families who contained diabetes-affected individuals without substantial renal disease. While 17% of the diabetic siblings of the probands without nephropathy had renal disease as judged by creatinine clearance and a urinary albumin excretion rate, 83% of diabetic siblings of probands receiving kidney transplants had renal involvement ($P < 0.001$). An interesting observation is that the two groups did not differ in their glycemic control. Similar results were reported by Borch-Johnsen et al in a European study of T1DM families [2] and American T1DM families [3]: nephropathy clustered in families, but there was no significant

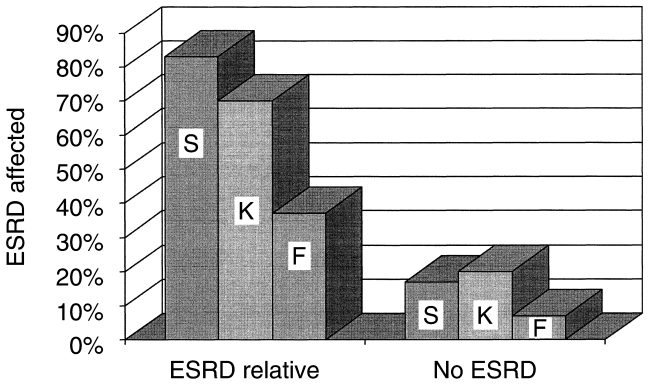

Fig. 1. Familial clustering of diabetes associated kidney disease. Comparison of frequency of renal disease in siblings or relative of diabetes-affected individuals with ESRD (bars on the left) compared to the frequency of renal disease in siblings or relative of diabetes-affected individuals without renal involvement (bars on the right). Bars represent data from analysis of: Caucasian T1DM subjects, Seaquist et al [1] labeled "S"; Caucasian type 1 diabetes mellitus (T1DM) subjects, Quinn et al [3] labeled "K", and African American T2DM subjects, Freedman et al [6] labeled "F".

difference between measures of glycemic control between cases and controls. Familial clustering of nephropathy in T1DM also has been observed in the Diabetes Control and Complications Trial (DCCT) [4].

Familial clustering of renal disease has been described in T2DM-affected Pima Indians [5], multiple studies of T2DM-affected African Americans [6], and T2DM-affected Caucasians [7, 8]. While not as extensively documented, similar evidence exists that non-diabetic ESRD also shows familial aggregation. For example, in the studies of African Americans, family history of ESRD was evaluated with the conclusion that the presence of a close relative with ESRD gave an African American an eight-fold increased risk of developing ESRD [9]. In Caucasians the increased risk was 2.7-fold. Subsequently, familial clustering of renal disease has been described in T2DM families from populations as diverse as south India [10] and Brazil [11]. Results of three of these familial aggregation studies are summarized in Figure 1. More recently, Fioretto et al have re-addressed familial clustering in T1DM families with a study incorporating the results of kidney biopsies in addition to more common measures of renal function such as urinary albumin excretion rate [12]. Mesangial fractional volume clustered in T1DM-affected siblings ($P < 0.01$). Patterns of glomerular lesions such as glomerular basement membrane thickening compared to mesangial matrix expansion were observed also.

Results of familial clustering studies have frequently included or been complemented by numerous studies that have evaluated incidence of complications in different racial or ethnic groups [6–8]. The assumption in these studies is that different racial and ethnic groups have lived and evolved in different environments with different lifestyles and diets that would contribute to differential selection of genetic alleles in the different populations. This different genetic heritage should be reflected, in the case of renal disease, in different rates of renal disease in different populations. One alternative explanation put forth to explain these disparities is that racial or ethnic minorities have lower economic status and consequently more limited access to medical care. While this may explain some of the differences, several studies have controlled for socioeconomic status and still observed significant differences between rates of renal complications when comparing American Caucasians and African American patients [6, 7, 9, 13]. As summarized earlier, Freedman and colleagues have introduced a new perspective on studies of racial/ethnic differences by incorporating a familial aggregation analysis into their comparisons of racial groups [6, 7, 9], observing that family clustering was more pronounced in African Americans than in Caucasians.

Finally, one of the most challenging approaches, segregation analysis, has been used by two groups to evaluate genetic components of diabetes complications [14, 15]. Fogarty et al studied 96 large, primarily Caucasian, multigeneration families with multiple T2DM-affected individuals encompassing a total of 1269 subjects including 630 with T2DM and 639 subjects without diabetes who had renal function measured as the urinary albumin-to-creatinine ratio (ACR) [14]. ACR was mathematically modeled as a continuous trait with the inclusion of age, sex, and duration of diabetes as covariates. The pattern of ACR inheritance was most consistent with a Mendelian model with multifactorial inheritance, that is, ACR is determined by a mixture of genes, variable effects, and other lifestyle/environment factors such as diabetes and hypertension. ACR heritability (h^2) was estimated to be 0.27, that is, 27% of ACR is determined genetically. Imperatore et al used a comparable measure of renal function, protein-to-creatinine ratio, from 2107 Pima Indians from 715 nuclear families [15]. They similarly concluded that a major genetic component contributes to nephropathy in Pima Indians with diabetes.

THE SEARCH FOR RENAL DISEASE GENES

With the wide acceptance that inheritance contributes to renal disease susceptibility, a broad range of efforts has been initiated to identify the genes contributing to susceptibility. Investigators have followed two main approaches with a variety of study designs in their effort to identify ESRD genes.

Candidate gene studies

In the candidate gene approach that is familiar to many investigators, specific genes are selected for analysis based on a function that could be closely related to

Table 1. Genes that have shown evidence of association to renal disease in candidate gene studies

Gene	Population
ACE	DM1, DM2 nephropathy
Aldose reductase	DM1 nephropathy
Angiotensinogen	DM1 nephropathy
APOE	DM1 nephropathy
Bradykinin receptors	ESRD
IL1RN	DM1, DM2 nephropathy
KLKB1	non-DM renal disease
KLK1 promoter	non-DM renal disease
NHE5	non-DM renal disease
eNOS	DM1 nephropathy
iNOS	non-DM renal disease

renal disease or through direct empirical evidence that the gene product, that is, protein, is associated with renal pathology. Examples of such genes are cytokines, growth factors, and nitric oxide synthases. Most frequently these types of studies are carried out in case-control populations, comparing allele frequencies in renal disease patients with allele frequencies in individuals without renal disease.

At this time the candidate gene approach has been used most widely in genetic studies, with over 100 articles appearing since 1999 with an analysis of candidate genes and renal disease. Table 1 lists some of the genes that have been tested and found to have evidence for association with renal disease. The great challenge of candidate gene studies is to assess the relative importance of the many reports. The candidate gene analysis method is very sensitive, but also limited by a number of features. Results from such studies have been notoriously difficult to independently replicate. If such studies are indeed reflecting true associations, one can envision that minor genetic components may differ significantly in their frequency from one population to another and even within populations from different locales. In addition, populations of both cases and controls in different studies are frequently ascertained by different criteria, and the results of genotyping are often interpreted using different statistical analysis approaches. These possibilities, in combination with the fact that many published candidate gene association analyses are probably underpowered, increases the likelihood that type 1 error will continually surface as evidence for association.

At Wake Forest we have taken a number of approaches for evaluating the genetics of renal disease including studies in families and case:control populations, and with genome screens and focused analysis of candidate genes. One area on which we have focused is the evaluation of kallikrein genes. Kallikreins are a class of serine proteases with high substrate specificity. The key feature of kallikreins relative to renal disease is that these enzymes cleave kininogen to release bradykinin, a powerful vasodilator. One can envision that an alteration in kallikrein activity, either by mutation or altered levels of expression could lead to local constriction of blood vessels and potentially contribute to ESRD. The two genes that we have evaluated in the greatest detail are the plasma and tissue kallikrein genes. Studies with the plasma kallikrein gene, *KLKB1*, illustrate a number of issues faced in candidate gene analysis.

These analyses were initiated with studies evaluating *KLKB1* in ESRD in genetic studies. Evidence of association of *KLKB1* with ESRD was observed in African American families with multiple cases of non-diabetic ESRD [16]. These studies were then extended to a detailed analysis of the *KLKB1* gene searching for DNA sequence variants or alleles of *KLKB1* that might contribute to ESRD [17]. Twelve allelic variants were identified in the 5′ proximal promoter and seven exons. Of note was a common polymorphism (30% of the population), at position 521 of *KLKB1* cDNA that leads to the replacement of asparagine with a serine, a seemingly a dramatic change, at position 124 in the substrate binding domain A2 (one of four substrate binding domains) of the protein. In addition, an A716C polymorphism in exon 7 resulting in the amino acid change H189P in the substrate binding domain A3 was observed in five patients belonging to three ESRD families. Another polymorphism in the coding sequence was a C699A shift that caused an amino acid change H183Q. This allele was observed in eight cases from six ESRD families, but was not found in any control DNAs from control subjects. The pedigree of a particularly interesting family is shown in Figure 2. In this family there are four children with diabetes (three with Type 2 diabetes, DM2, and one with Type 1 diabetes, DM1) and two children with chronic renal failure (CRF) or ESRD. Two different uncommon SNPs are segregating in this family: H183Q and K229E. Interestingly, the H183Q allele is found in both of the renal disease affected siblings and the K229E allele in the two diabetes affected, but renal disease unaffected individuals.

Individually or combined, the allelic variants observed are not statistically associated with ESRD, but in several cases, for example, H183Q, the small number of people in the population carrying these alleles limits our ability to statistically test for significant association with ESRD. As a consequence, to determine the impact of these various coding changes, the individual genetic variants will need to be expressed and the proteins assayed for functional activity. Importantly though, we may have identified genetic alleles that have a substantial impact on renal disease susceptibility, albeit in a small number of people.

Genome screen analysis

The genome screen approach is more difficult, time consuming, and expensive than candidate gene analysis,

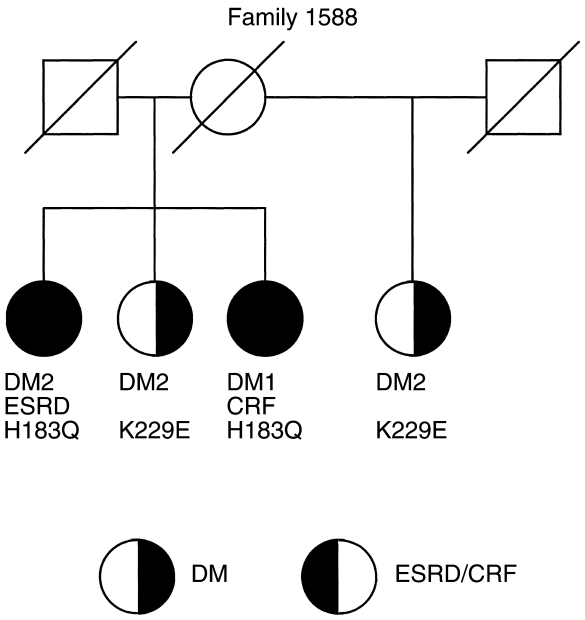

Fig. 2. Inheritance of *KLKB1* allelic variants in an African American family with multiple of cases of diabetes and renal disease. Abbreviations are: DM2, Type 2 diabetes mellitus; DM1, Type 1 diabetes mellitus; CRF, chronic renal failure; ESRD, end-stage renal disease.

but has the advantage of being able to locate new, as yet undiscovered, genes. Importantly, the genome screen approach is not limited to our current knowledge of renal disease. The genome screen methodology gives a comprehensive map of inheritance of all parts of the human genome in families expressing a specific trait, for example, of ESRD.

In a genome screen approximately 400 genetic markers that identify evenly spaced locations along each of the human chromosomes are genotyped in families with multiple cases of ESRD or other forms of renal disease. What this results in is a dataset that follows the inheritance of every part of every chromosome in each individual in the study. The idea then is to try to determine by statistical analysis which parts of which chromosome are being inherited with renal disease. This is performed by statistical calculations called linkage analysis.

The first genome screen looking for nephropathy genes was published by the Phoenix group working with the Pima Indians, where they followed Type 2 diabetes associated nephropathy and also another microvascular disease, retinopathy [18]. They did this study in 98 sibling pairs in which each sibling had T2DM and renal failure. After analyzing their data they saw strongest evidence for linkage on the long arm of chromosome 7. Results of these kinds of analyses are expressed statistically as LOD scores. LOD stands for log of the odds, in this case to the base 10. Thus, the LOD score is an expression of the probability that this part of the genome or chromosome is being inherited with renal disease. In this case the LOD score they saw on chromosome 7 was 2.7 or odds of approximately 500:1 that this part of the genome is linked, that is, co-inherited, with nephropathy in these Pima Indian families. If this part of chromosome 7 is inherited more commonly than expected in families with renal disease, this suggests that a renal disease gene is located in this part of the genome. They found less strong evidence for linkage in several other places on the genome: chromosomes 3, 9, and 20.

At the recent World Congress of Nephrology ASN/ISN meeting, Vardarli et al reported the results of a genome scan of 18 Turkish kindreds with multiple diabetic nephropathy-affected individuals (abstract; *J Am Soc Nephrol* 12:160A, 2001). These unusual families have multiple cousin-cousin marriages and consequently a significant inbreeding component. Using an autosomal dominant model, a strong linkage peak was observed on chromosome 18 with a LOD score of 6.6 between genetic markers D18S43 and D18S50, providing strong evidence for the existence of a novel renal disease gene. Evaluation of the loci in the Pima Indian dataset also showed evidence of confirmation ($P = 0.013$ to 0.006), although this region was not linked in the original Pima genome screen.

We are currently analyzing the results of a genome screen carried out in our laboratories at Wake Forest University School of Medicine. While not complete, there are some interesting results to date. Our strongest evidence for linkage is a LOD score of 3.4 (2500:1) on human chromosome 10q in our complete set of ESRD families that include families with both diabetes associated ESRD and ESRD families without diabetes (abstract; Freedman et al, *J Am Soc Nephrol* 12:71A, 2001). There is a lesser peak at another location on chromosome 10p. There is another peak of 2.35 (220:1 odds of linkage) on chromosome 7p that is only seen in diabetes families, but is at a different location than the peak observed by Imperatore et al [18]. We have looked at the chromosome 10 data in greater detail by evaluating linkage in diabetes and non-diabetic families alone, and then by analyzing the data by evaluating linkage based on age of ESRD onset. Interestingly, the 10q peak is contributed to equally by diabetic and non-diabetic families and early and late onset families. In contrast, the 10p peak is due solely to evidence of linkage in early onset, non-diabetic families, and the 7p peak is seen only with the diabetes affected families.

Our results, and those of our colleagues, suggest that the genetic origins of renal disease are heterogeneous: different genes on different chromosomes may contribute to susceptibility in different populations and may be specific for certain disease etiologies or general to all origins of renal disease. It is important to emphasize that researchers are just in the early stages of these types of comprehensive genetic analyses. Results so far are similar in content to studies of other complex diseases such

as Type 2 diabetes where different studies in different populations have suggested that different regions of the genome are important for further analysis.

SUMMARY

Evidence from multiple lines of inquiry suggests that the susceptibility to develop renal disease has a significant genetic component. Studies of familial aggregation, different incidence rates in different racial and ethnic groups, and segregation analysis are all consistent in pointing to a genetic contribution to renal disease. Efforts to identify genes that contribute renal disease risk are in their early stages. Many efforts have been made to link specific genes or small numbers of related genes to various forms of renal disease in the general population. These types of studies are now being complemented by genome scans that give a comprehensive evaluation of inheritance in renal disease families. The genome scan studies have the greatest potential for identifying the important genetic contributors to renal disease, but are limited in number at this time. Lessons from similar genetic studies of other complex diseases are that multiple genome scans in different populations will be very helpful or even critical to the identification of genomic regions that contain renal disease genes. It is sobering to note that the subsequent effort to identify renal disease predisposing genes—and even their function—is still a daunting technological undertaking that may require many years of effort. We are just beginning to see significant returns from the hard work that many geneticists have put into the study of renal diseases. The next few years should provide new insights into the origins of renal disease.

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