Genetic Initiation of Hypertensive and Diabetic Nephropathy

The factors initiating the common etiologies of chronic renal failure remain elusive. This article reviews the evidence in support of a generalized genetic susceptibility to human end-stage renal disease, including kidney failure attributed to the systemic diseases of hypertension, diabetes mellitus, and glomerulonephritis. Molecular genetic techniques are powerful tools, assisting in the detection of the initiating factors in many complex diseases. In kidney disease, genetic methodologies complement the available anatomic, epidemiologic, and physiologic analyses. This article provides strategies to allow for the detection of human renal failure susceptibility genes. The identification of human renal failure genes would provide useful markers for disease susceptibility and speed the development of novel therapeutic strategies. Am J Hypertens 1998;11:251–257 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Diabetic nephropathy, hypertensive nephrosclerosis, blacks, genetic linkage analysis.

The factors that initiate renal failure are likely to differ from those that contribute to progression. These factors may be difficult to detect because renal disease is typically not apparent until microalbuminuria, overt proteinuria, or hypercreatininemia are present. In contrast, there has been great success in identifying the determinants that contribute to the progression of established nephropathy. Elevated blood pressure, glomerular hypertension, hypercholesterolemia, excessive dietary protein intake, and hyperparathyroidism all appear to hasten the loss of residual renal function to end-stage renal disease (ESRD) in many forms of nephropathy. This review focuses on the processes and phenotypes that are reported to initiate renal failure in susceptible hypertensive and diabetic patients. Initiators of renal disease, particularly inherited factors or “renal failure genes,” would be of great public health importance. The molecular genetic methodologies that have altered our concepts of the pathophysiology in essential hypertension and diabetes mellitus are now being applied to the diverse etiologies of renal failure. In the future, these genetic techniques are likely to provide useful markers for disease susceptibility and speed the development of novel treatment strategies by identifying genes whose protein products can serve as pharmacologic targets. These developments might have been overlooked by other investigative approaches.

FACTORS INITIATING HYPERTENSION-ASSOCIATED NEPHROPATHY

The clinical syndrome of “hypertension-associated ESRD” (H-ESRD) remains poorly defined. The majority of ESRD patients in the US who are labeled as having hypertension as the etiology of ESRD may have alternate etiologies and physician bias plays a role in this misclassification. Flisher et al recently demonstrated that inulin clearances were the same in healthy elderly individuals and hypertensive elderly...
patients. This suggests that high blood pressure, per se, did not reduce glomerular filtration rate.\(^\text{16}\) Strictly speaking, hypertensive nephrosclerosis refers to diseases with predominant pathologic changes occurring in the preglomerular microvasculature and secondarily involving the glomeruli and interstitium.\(^\text{13}\)

The pilot phase of the African American Study of Kidney Diseases (AASK) clinical trial attempted to define the renal structural abnormalities in black Americans with the clinical diagnosis of hypertensive nephrosclerosis.\(^\text{17}\) The renal biopsies in this trial were interpreted as demonstrating the renal vascular lesions consistent with hypertensive nephrosclerosis in nondiabetic, hypertensive black Americans with mild to moderate renal insufficiency and without marked proteinuria.\(^\text{17}\) This suggests that essential hypertension caused the renal histologic changes of nephrosclerosis and was the primary cause of the renal disease. In our opinion, this conclusion is difficult to reach. Interestingly, there was no correlation between higher blood pressure and the severity of the arteriolar and arterial sclerosis seen in this report.\(^\text{17}\) In addition to the arterial and arteriolar vascular changes, global glomerulosclerosis and marked interstitial fibrosis were observed.\(^\text{17}\) The extent of global glomerulosclerosis correlated with the degree of blood pressure elevation, interstitial fibrosis, and the reciprocal of the serum creatinine. These biopsy findings could have been interpreted as demonstrating that a primary glomerular disease (global glomerulosclerosis) or an interstitial lesion initiated the renal disease in the AASK population. Secondarily elevated blood pressure, not unexpected in this scenario, could have accounted for the observed arterial and arteriolar lesions. Alternatively, the vascular changes could represent a primary renal microvascular disease, with subsequent development of glomerular and interstitial changes and secondary hypertension. These scenarios are supported by a recent report from the investigators in Birmingham, Alabama. First-degree relatives of black Americans with clinically diagnosed H-ESRD had reduced effective renal plasma flow and reduced glomerular filtration rate despite normal serum creatinine and unremarkable urinalyses.\(^\text{18}\) This report supports the presence of a primary renal disease before the development of hypertension in close relatives of H-ESRD patients. The AASK authors mention this scenario in their discussion; however, their conclusion fails to focus on the importance of this point.\(^\text{17}\) Therefore, we feel that the nature of the lesion initiating renal failure in mild-to-moderate hypertensives remains unclear.

Salt sensitive hypertension and microalbuminuria\(^\text{19,20}\) have been touted as possible risk factors for renal disease susceptibility in essential hypertensives. In the case of salt sensitivity, Campese et al analyzed the alterations in blood pressure, glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and filtration fraction in black American and white hypertensives who were either salt sensitive or salt resistant.\(^\text{19}\) Despite a stable GFR, a fall in ERPF associated with an elevated filtration fraction and intraglomerular pressure was observed in the salt sensitive black Americans when fed a diet high in sodium. Nifedipine reversed these renal hemodynamic alterations in salt sensitive patients.\(^\text{19}\) Similarly, Weir et al demonstrated that increasing dietary sodium twofold resulted in a 15% increase in glomerular filtration rate without a change in ERPF.\(^\text{20}\) This was accompanied by a modest increase in urinary protein excretion. The observed “adverse renal hemodynamic profile” induced by high sodium intake in salt-sensitive patients appears to be reproducible. Unfortunately, it is neither a predictor nor a marker of susceptibility to future nephropathy in any individual hypertensive patient. This is based upon the frequent presence of salt sensitivity in the general hypertensive population and in normotensives.\(^\text{21}\) Approximately 50% of hypertensives, and a greater proportion of black American hypertensives, are salt sensitive.\(^\text{21}\) The southeast US has the highest incidence rate of ESRD in the country, yet fewer than 1 in 1500 black Americans and 1 in 6000 whites will develop ESRD from any cause in their lifetime.\(^\text{22}\) Therefore, salt sensitivity, despite the associated alterations in renal blood flow and filtration fraction, cannot play a major role in the initiation of nephropathy as it is so common in the hypertensive population. In a similar fashion, the modest increase in urinary protein excretion that has been detected in salt sensitive hypertensives fed a high sodium diet may be linked to salt sensitivity itself, but does not predict the rare hypertensive who is destined to develop future nephropathy. Microalbuminuria in essential hypertension has proved to be a risk factor for cardiovascular morbidity and mortality,\(^\text{23}\) but has not been linked to future nephropathy.

Finally, neither the prevalence\(^\text{24}\) nor the severity of mild-to-moderate essential hypertension\(^\text{25}\) has proved to be a reliable predictor of individuals at risk for subsequent nephropathy. This may result from the presence of different etiologies of renal disease in many patients classified as having H-ESRD. Additionally, controlling elevated blood pressure to the “usual” levels fails to protect hypertensive black Americans with renal insufficiency from losing additional renal function.\(^\text{26,27}\) Intensive blood pressure lowering has been shown to stabilize renal function in this population.\(^\text{28}\) The AASK trial will determine whether intensive blood pressure control and the use of either angiotensin converting enzyme inhibition (ACEI) or calcium channel blockade will have additional renoprotective effects.

Patients labeled as having H-ESRD (selected using entry criteria similar to the AASK trial) manifest a
striking tendency toward familial aggregation of ESRD, overt proteinuria, and hypercreatinemia. Forty percent of patients labeled as having H-ESRD from our center in Winston-Salem had close relatives with ESRD. Seventy percent of H-ESRD patients from the University of Alabama at Birmingham had first degree relatives with either proteinuria, elevated serum creatinine values, or ESRD. Similar results were reported from Los Angeles using less rigorous diagnostic criteria. We have also assessed the prevalence of relatives with ESRD from 4365 incident dialysis patients beginning renal replacement therapy in 1994 and residing in ESRD Network 6 (North Carolina, South Carolina, and Georgia). Nineteen percent of patients in this cohort (black and white) who were specified as having H-ESRD by their treating nephrologist were found to have either a first or second degree relative with ESRD. The somewhat lower frequency of a positive family history of ESRD in this cohort is likely accounted for by both the inclusion of white patients and black patients with causes of renal disease other than H-ESRD (because strict diagnostic criteria were not employed). Therefore, black Americans with a close relative having ESRD, long-standing hypertension, and < 1.5 g of proteinuria per day are at markedly increased risk for development of future nephropathy. This familial risk is of far greater predictive value for future nephropathy than is the presence of more severe hypertension, salt sensitivity, or microalbuminuria in hypertensive patients.

In summary, we feel that hypertensive black Americans with moderate renal insufficiency and low levels of proteinuria often have a familial syndrome. The renal disease may be caused by either: 1) a primary renal process (either in the microvasculature, glomeruli with global glomerulosclerosis, or interstitial fibrosis) with resultant secondary hypertension; or 2) arteriolar/arterial sclerosis may develop predominantly in genetically predisposed hypertensives who are uniquely susceptible to high blood pressure-induced vascular damage.

**FACTORS INITIATING DIABETIC RENAL DISEASE**

In contrast to hypertension, the nephropathy in diabetes mellitus appears to be a uniform syndrome with a well described histopathology. Renal biopsies in diabetic patients with overt proteinuria and proliferative retinopathy typically reveal mesangial matrix expansion, uniform thickening of the glomerular basement membranes, or nodular glomerulosclerosis. The renal pathology and cumulative risk of renal involvement over time appear similar in insulin-dependent (IDDM) and non–insulin-dependent (NIDDM) forms of diabetes mellitus. Microalbuminuria developing within the first decade of diabetes mellitus appears to predict susceptibility to progressive nephropathy and ESRD. Progression to ESRD may be reversible if the microalbuminuria subsides with intensive glycemic control or the use of ACEI early in the course of the illness. It is apparent that most, if not all, diabetics with ESRD progress through the initial stage of microalbuminuria.

In IDDM and NIDDM, studies have repeatedly demonstrated that the susceptibility of a diabetic to future renal failure is best predicted by the presence or absence of renal disease in their diabetic relatives. The familial clustering of diabetic nephropathy is of far greater predictive value than is the level of blood pressure or glycemic control. Familial aggregation of diabetic nephropathy has been reported in European whites with IDDM and Pima Indians and black Americans with NIDDM. In IDDM, the presence of hypertensive relatives increases the risk for development of diabetic nephropathy. A familial aggregation of elevated urinary albumin excretion rates has also been observed in patients with NIDDM. This familial clustering of diabetic renal disease susceptibility clearly suggests that in addition to the inherited and environmental factors that produce hyperglycemia, the predisposition to nephropathy is under independent genetic control (ie, diabetic nephropathy genes exist). Therefore, microalbuminuria will only be observed in diabetics who are genetically susceptible to renal disease in the presence of poorly controlled blood sugars. Individuals who are susceptible to renal disease may not manifest microalbuminuria or overt nephropathy if the environment is not conducive to its development (blood sugar is normalized).

It is apparent that 70% of nonintensively managed diabetics are not susceptible to renal disease. These patients will not manifest overt proteinuria even after many decades of severe hyperglycemia. Quinn et al demonstrated that the risk for nephropathy in IDDM increased from a low of 10% in well controlled diabetics up to 30% in poorly controlled diabetics. They felt that the 30% rate for nephropathy reflected the maximal genetic contribution, seen when the environmental contribution to nephropathy was greatest. These results have been confirmed by the Diabetes Control and Complications Trial (DCCT).

**FAMILIAL PREDISPOSITION TO RENAL DISEASE IN BLACK AMERICANS**

A significant proportion of black American families multiply affected with renal failure consist of individuals with disparate etiologies of ESRD. In our initial report, approximately 20% of multiplex families from North Carolina had members with ESRD attributed to either hypertension, IDDM, NIDDM, renal-limited chronic glomerular diseases, or human immunodeficiency virus (HIV) infection. A subsequent report
from our group demonstrated the familial aggregation of ESRD in relatives of black Americans with lupus nephritis (non-ESRD index cases).46 Eighty-eight percent of the ESRD relatives had neither lupus nor a collagen vascular disease as the etiology of their renal failure.

The clustering of disparate etiologies of ESRD in single black American families has been confirmed by Bergman et al.30 This study used H-ESRD index cases. Of the first degree relatives with ESRD from these carefully selected index cases, 50% had diabetic nephropathy.30 In a national study from 63 dialysis facilities, nearly 30% of black Americans with HIV nephropathy-associated ESRD were reported to have close relatives with ESRD.47 None of the relatives of these index cases had HIV as the cause of their nephropathy.

The demonstration that ESRD clusters independently from the systemic diseases of hypertension, diabetes mellitus, HIV infection, and systemic lupus erythematosus, coupled with the observation that disparate etiologies of ESRD exist within families from widely separated geographic regions of the US, strongly supports the contention that inherited factors play a major role in the development of the common etiologies of nephropathy. Lei et al reported that the familial clustering of renal disease in black Americans is in excess of that expected from the clustering of hypertension and diabetes mellitus in families and that genetic susceptibility increases the risk of developing ESRD.48

The presence of human renal failure genes is strongly supported by work in the fawn hooded rat, an animal model of hypertension and glomerulosclerosis.49 Two renal failure genes, Rf-1 and Rf-2, have been identified and are linked to nephrosclerosis and proteinuria in these rats. A separate locus, Bpfh-1, has been linked to hypertension. As in human renal disease, it appears that the inherited factors producing nephrosclerosis in rodents are distinct from those producing systemic hypertension.

STRATEGIES TO DETECT HUMAN RENAL FAILURE SUSCEPTIBILITY GENES

In any complex illness, the key elements required to detect disease susceptibility genes include the availability of large numbers of multiply affected families for genotyping, informative genetic markers and appropriate analytic methods to evaluate evidence for linkage. For diseases demonstrating late age-at-onset, such as kidney failure, family structures such as affected sibling pairs are commonly employed because few families have living parents, and offspring of affected individuals may be too young to demonstrate the disease phenotype. In studies of affected sibling pairs, the critical statistic determining the number of families (pairs) needed to have reasonable power to detect linkage is the ratio of disease in siblings to the risk in the general population (\(\lambda_s\)).50 Higher values of \(\lambda_s\) suggest significant clustering (genetic effects) and that fewer sibling pairs are needed to detect linkage. Our estimate of \(\lambda_s\) for renal failure in black Americans is nine for all-cause renal disease29 and 10 for NIDDM-associated nephropathy.42

Two strategies have been widely used in searches for genetic linkage. The first, a candidate gene approach, assesses coinheritance of a polymorphic DNA marker within or near a potentially causative gene and a disease in families. This approach is limited because only known genes can be tested. However, genes or chromosomal regions identified in inbred animals with hypertension or nephrosclerosis can serve as candidate loci if the homologous regions of the human genome are known.49 The second approach, a systematic genome-wide search, has the potential to locate previously unknown genes contributing to disease. This strategy employs 400 or more highly polymorphic microsatellite markers, which provide complete coverage of all the human chromosomes, to be tested for coinherence with the disease. This approach can presumably identify all genes making a detectable contribution to a phenotype. Recent advances in automation have accelerated the rate at which a total genome search can be completed. With both strategies, siblings sharing a disease are genotyped and scored for sharing 0, 1, or 2 alleles identical by descent (IBD) from parents. Linkage is assumed if the sharing ratio deviates significantly from the expected 1:2:1 ratio. This methodology can be applied to family structures other than siblings, using different frequencies of expected allele sharing. This method has limitations in adult onset diseases, because absence of parental data means that IBD statistics must be calculated rather than deduced by direct observation.

If linkage is observed using sibling pair analyses, the lod score method of linkage analysis can be used as a confirmatory test.51 Logarithm of Odds (LOD) score analyses require the specification of a mode of inheritance for the disease. In complex diseases such as renal failure, the mode of inheritance is unknown and data can be analyzed under several competing models. Likelihood-based methods offer the advantages of statistical efficiency when the model is known, and allow estimation of genetic parameters such as recombination fraction. Methods include pairwise linkage analysis for each candidate gene and the disease, and multipoint linkage analysis, given the candidate gene and multiple markers in the region of a putative locus.

We have detected evidence for linkage between polymorphic loci that map to the long arms of human chromosomes 20 and 12 (in the regions containing
Maturity Onset Diabetes of the Young 1 [MODY1] and MODY3 genes, respectively) with NIDDM in white families enriched for nephropathy.\textsuperscript{52} Nonparametric analysis of chromosome 20 inheritance data collected with the MODY1-linked marker D20S197 demonstrated evidence for linkage to NIDDM with a $P = .005$ in white sibling pairs using affected sibling pair analyses (ASP). Nonparametric analysis of chromosome 12 inheritance data collected with the MODY3-linked markers D12S349 and D12S86 demonstrated evidence for linkage to NIDDM with $P = .04$ and .006, respectively. Results of multipoint maximum LOD scores were consistent with the ASP results. A maximum LOD score of 1.48 was calculated for linkage to MODY1-linked markers and 1.45 to MODY3-linked loci in white sibling pairs. No evidence for linkage of MODY1 and MODY3 markers to NIDDM in black American sibling pairs was observed. It is unclear whether these markers are linked with NIDDM or with nephropathy. However, the results of candidate gene analyses in a complex, polygenic disorder suggest that genes contributing to NIDDM susceptibility in the general white population are located in the regions containing the MODY1 and MODY3 genes. We have also performed linkage analyses with ESRD in black Americans using candidate genes in the renin-angiotensin axis\textsuperscript{53} and growth factor loci.\textsuperscript{54} The preliminary results have been negative.

For most complex diseases, both genes and environmental factors are important. Thus, an individual may be susceptible or resistant to a disease based upon either genotype, environment, or both. An understanding of the impact of environmental factors on complex diseases would be improved by evaluating populations that have been stratified based on genetic risk.

In summary, molecular genetic approaches are likely to provide important clues to the causative or initiating factors in complex diseases. Their major advantage lies in the ability to detect a clearly defined, fixed alteration in DNA from patients exhibiting similar phenotypes. This methodology is a powerful complement to physiologic and epidemiologic analyses. In hypertensive and diabetic renal disease, physiologic studies using interrelated intermediate phenotypes cannot distinguish causative from compensatory responses. Epidemiologic approaches to detecting causes of hypertensive renal failure have been hampered by the lack of rigorous disease criteria. Although the identification of renal disease genes will not be easy, it is clear that they will be enumerated. Once identified, these genes can be applied to study individuals who are genetically susceptible, but as yet unaffected. Physiologic analyses can then proceed in more homogeneous subsets of patients and should greatly improve our ability to determine the initial events producing complex disease.

REFERENCES


