Evidence of Gene–Gene Interactions in the Genetic Susceptibility to Renal Impairment after Unilateral Nephrectomy

MASAHIDE SHIOZAWA,* ABRAHAM P. PROVOOST,†
RICHARD P.E. VAN DOKKUM,† REBECCA R. MAJEWSKI,* and
HOWARD J. JACOB*

**Laboratory for Genetics Research, Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin; and †Department of Pediatric Surgery, Erasmus University, Rotterdam, The Netherlands.*

Abstract. The number of patients with hypertension-associated end-stage renal failure (ESRF) continues to increase despite improved antihypertensive management and early detection programs. Variation for the development of renal complications in hypertension may reflect independent genetic susceptibility to ESRF. The genetically hypertensive fawn-hooded rat is characterized by the early presence of systolic hypertension, glomerular hypertension, progressive proteinuria (UPV), and focal glomerulosclerosis (FGS), resulting in premature death as a result of renal failure. In the present study, the genetic basis of hypertension-associated ESRF in an F2 intercross consisting of 337 animals, in which systolic BP, UPV, albuminuria, and FGS, were studied at 8 wk after a unilateral nephrectomy performed at 5 to 6 wk of age. A total genome scan, consisting of 418 markers, was used to identify regions that contribute to the pathogenesis of UPV and FGS. Linkage analysis revealed five loci involved in the development of renal impairment. Of these five, two (*Rf-1*, *Rf-2*) had been identified previously. There seems to be strong interactive effects between the various loci and their impact on UPV and the other parameters of renal impairment, as well as an interaction with BP. In particular, *Rf-1* seems to play a major role in determining the severity of the disease. This study is the first to report the interaction of more than two loci to produce progressive renal failure, suggesting that the genetic dissection of renal failure in humans will require understanding of how multiple genes interact with each other and BP to produce ESRF.

Hypertension and diabetic nephropathy are considered to be major causes of end-stage renal failure (ESRF) in humans, accounting for approximately 50% of the patients treated in the United States (1). However, not all patients with hypertension or diabetes uniformly develop ESRF, which suggests that other factors must contribute to the development of the renal disease. Various epidemiologic studies demonstrate familial clustering of patients with ESRF (2–4). Ethnic differences and familial clustering are used as evidence that ESRF has a genetic component. However, familial clustering of ESRF may result from a shared environment, genetic components, or both. Freedman *et al.* (3,4) examined the relative risks of race and socioeconomic factors and concluded that a genetic predisposition to ESRF may exist in African Americans.

Direct evidence for the involvement of genetic factors in renal impairment has been obtained in various animal models. The Milan normotensive strain rat is more susceptible to renal damage than Milan hypertensive strain rats (5). The Buffalo (BUF) rat develops spontaneous proteinuria and structural renal lesions, and the authors predicted that two autosomal recessive genes were responsible for these traits (6). However, the study that used the fawn-hooded hypertensive (FHH/Eur) rat first demonstrated that susceptibility loci, that are genetically independent of BP, are responsible for hypertension-associated ESRF (7). This strain develops a moderately elevated level of systolic BP (SBP), progressive proteinuria (UPV) mainly consisting of albuminuria (UAV), and focal glomerulosclerosis (FGS) at a relatively young age, leading to premature death as a result of ESRF (8–19). Using a backcross design (FHH/Eur × ACI/NCrEur) F1 × FHH rats, we identified two quantitative trait loci (QTL)—*Rf-1*, which was independent of BP, and *Rf-2*—that were responsible for UPV and structural renal damage (7). In addition, we localized a QTL, *Bpfh-1*, that was partly responsible for the increased SBP level (7). *Bpfh-1* maps close to *Rf-2* and the S_A region, also known to contain an important gene in determining SBP in other crosses of genetic hypertensive rat strains (20,21). However, there was not sufficient genetic power to separate *Rf-2* from *Bpfh-1* with this backcross design.

More recently, other experimental models and genetic studies have been used to implicate susceptibility genes. A QTL named *Pur-1*, on rat chromosome 13, was identified in a backcross of BUF and (BUF × Wister Kyoto [WKY])F1 rats

Received December 15, 1999. Accepted March 29, 2000.
Correspondence to Dr. Howard J. Jacob, Human and Molecular Genetics Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. Phone: 414-456-4887; Fax: 414-456-6516; E-mail: Jacob@mcw.edu

1046-6673/1111-2068
Journal of the American Society of Nephrology

(22). Studies in mice indicated that genetic factors were more important than nephron reduction in determining renal damage (23). Additional studies seem to indicate that 8 to 10 interacting genes make up the renal susceptibility for glomerulosclerosis (24).

Taken together, these experimental studies indicate that a substantial number of genetic loci may be involved in determining the susceptibility of the kidney to renal impairment. In the present study, we wanted to expand our knowledge of the susceptibility genes that are responsible for renal failure in the FHH/Eur rat and the complex interactions with BP. To accomplish this goal, we studied 337 male rats from an F2 intercross derived from the FHH/Eur and ACI/NCrEur grandparent strains. A secondary goal was to determine whether there was a genetic basis to the accelerated progressive ESRF after unilateral nephrectomy (16,25), as we had noticed that ACI rats do not develop ESRF after the same procedure.

Here, we replicate the study of *Rf-1* and *Rf-2* in determining not only the susceptibility to renal damage but also the localization of three additional genes, *Rf-3*, *Rf-4*, and *Rf-5*. In total, these five genes are responsible for approximately 63% of the genetic variation. We also confirmed *Bpfh-1* and genetically mapped a second BP locus, *Bpfh-2*; together, they are responsible for approximately 13% of the total variation in BP. One of the most important discoveries was the complex interaction between the different genes. The data presented here suggest that various combinations of susceptibility genes interact to influence the rate of renal failure progression.

Materials and Methods

Animals

FHH/Eur and ACI/NCrEur rats were obtained from the breeding colonies maintained at the Animal Research Center of the Erasmus University, Rotterdam, The Netherlands. Inbreeding procedures for FHH have been described in detail previously (12). Animals were housed in standard rat cages with lights on from 8:00 a.m. to 8:00 p.m. Food, consisting of standard commercial rat chow containing 56% carbohydrates, 26% digestible protein, 7% fat, 4% fiber, and 5% minerals (AM II; Hope Farms, Woerden, The Netherlands), and drinking fluid (tap water, acidified to pH 3) were provided *ad libitum*.

We studied 337 male F2 rats from an intercross derived from the FHH/Eur and ACI/NCrEur grandparent strains. Female F2 rats were not studied because there was a very different time course for the development of renal damage (26). Breeding and phenotyping of the F2 intercross was completed in Rotterdam. A reciprocal cross design was used, because in preliminary studies, the renal phenotypes showed no correlation with grandparent gender.

Unilateral Nephrectomy

At 5 to 6 wk of age, rats were anesthetized with ethyl ether and the right kidney was removed after exposure by a mid-line laparotomy and careful separation from the adrenal gland and associated connective tissue.

Phenotypes

At 8 wk after unilateral nephrectomy (UNX), we determined the levels of UPV, UAV, residual proteinuria (rUPV = UPV − UAV), urinary kallikrein levels, urinary osmolality, SBP, and the incidence of FGS in all 337 male F2 progeny. To collect urine, animals were kept in metabolic cages (Tecniplast Gazzada, Buguggiate, Italy) and allowed to adapt to the new situation over the weekend. Urine was then collected during two consecutive 24-h periods. UPV and UAV were determined colorimetrically using pyrogallol red/molybdate complex (27) and bromocresol-green (28), respectively. Both assays were carried out using an automatic analyzer (ELAN, Eppendorf/Merck, Darmstadt, Germany).

Because of the platelet storage pool bleeding disorder in the FHH/Eur, SBP was measured by the indirect tail-cuff plethysmography in awake but restrained animals using a semiautomatic system (Model 1279, IITC Life Science, Woodland Hills, CA). Animals were trained by exposing them to the restraint during the week before taking the measurements. For BP determinations, at least three measurements were taken on each of three consecutive days. The mean of these three values was used for analysis.

After the urine collections and the BP measurements were completed, the rats were killed and the kidney and spleen were removed. The spleen was used for extraction of genomic DNA. The kidney was weighed, fixed, and embedded in paraffin. Sections (3 μm thick) were stained with hematoxylin and eosin and with periodic acid-Schiff reagent. The incidence of FGS was scored in at least 50 glomeruli and expressed as the percentage of glomeruli with sclerotic lesions, as described earlier (13,29).

Genotyping and Linkage Analysis

Genomic DNA was extracted from the spleens of the intercross male progeny by standard methods (30) and was diluted to 4 ng/μl stocks in sterile, distilled water. Genotyping with simple-sequence length polymorphism was performed, essentially, as described previously (31). On the basis of existing maps, we selected 418 markers to provide a genetic map with an expected average intermarker distance of approximately 4 cM and covering the majority of the genome.

A subgroup of 46 animals, the number of samples able to be run on a single polyacrylamide gel, were chosen for the initial genome-wide scan (all 418 genetic markers). These 46 animals were selected on the basis of calculated expected logarithm of odds (LOD) scores to provide a representative sample covering the extremes for both SBP and UPV (unpublished algorithm). This targeting approach allowed us to build a genetic linkage map for this cross and to provide preliminary QTL for the trait of SBP and UPV with greater efficiency than scanning all markers in all F2 rats (32). When putative QTL were found to exceed predicted LOD score, more rats were genotyped; when the LOD score continued above threshold, all animals were genotyped for the markers flanking the final QTL.

Genetic markers were mapped relative to each other by using the MAPMAKER/QTL computer package (33), using an error detection procedure (34). After the map was constructed, QTL that affected phenotypes were mapped relative to genotypes using the MAPMAKER/QTL software package (32,33). Briefly, the program calculates the most likely phenotypic effect that has genotypes FHH/FHH, FHH/ACI, or ACI/ACI at a putative QTL and then calculates a LOD score that reflects the strength of evidence for the existence of the QTL and the proportion of the total phenotypic variance explained. A LOD score of more than 4.3 indicated significant linkage and a LOD score of between 2.6 and 4.3 was suggestive of linkage (35). To assess the impact of multiple QTL on a particular trait, a second analysis was conducted with the MAPMAKER/QTL software. Briefly, identified QTL are "fixed," removing that portion of the variance that is explained by that locus from the subsequent analysis. The genome is then rescanned to identify additional QTL. In the same manner,

phenotypes can be fixed and the genome rescanned to test the effects of one phenotypic trait on another. In addition, residual analyses were performed to test the interaction between traits and the results analyzed as a new trait using MAPMAKER/QTL (33).

Comparative Mapping

To determine the syntenic regions among rat, human, and mouse, we began by identifying genes in evolutionarily conserved genomic regions among mammalian species (human, mouse, and rat) that were mapped in rat and mouse and were listed in at least one database that contained rat genomic data (http://ratmap.gen.gu.se; http://www.well.ox.ac.uk; http://rgd.mcw.edu). Conserved regions and evolutionary break points between rat and mouse genomes were identified using the Mouse Genome Database; the mapped genes served as anchoring points within the published genetic maps for both species (http://www.informatics.jax.org). This information was used to define regions of conserved gene order and evolutionary break points with the human genome, using mapping information of homologous genes in the human genome available in the Mouse Genome Database, The Genome Database (http://gdbwww.gdb.org), and the UniGene set at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The cytogenetic locations were established on the basis of mapped genes in the Human Gene Map at NCBI (http://www.ncbi.nlm.nih.gov/genemap98).

Statistical Analyses

Data are presented as mean ± SEM unless stated otherwise. Comparison of the various phenotypes between different genotype groups was performed by using ANOVA followed by the Student-Newman-Keuls test to identify the groups that were different from each other. $P < 0.05$ was used as an indication of statistical significance.

Results

Effects of UNX on Renal Impairment in Parental Strains and F1 Rats

At 8 wk after UNX (performed at 5 to 6 wk of age), male FHH rats developed severe UPV, UAV, and FGS. In contrast, none of ACI rats or (FHH × ACI) progeny (F1) developed marked signs of renal damage (Table 1). The F1 rats are heterozygous at all genes and, therefore, provide the best estimate of environmental noise. All renal parameters in F1 rats are much closer to the values obtained in ACI rats. Therefore, we can conclude that after UNX, renal failure has a mainly recessive mode of inheritance, as reported earlier for FHH with both kidneys present (7), suggesting that UNX accelerates a similar type of chronic renal failure seen previously in the backcross.

Comparisons of SBP values indicated that the values for F1 were intermediate to those of the hypertensive FHH and the normotensive ACI rat, suggesting an additive mode of inheritance of this phenotype. However, given that the variance in SBP within the F2 was the same as in the F1, we were not sure whether this trait would segregate.

Confirmation of Rf-1, Rf-2, and Bpfh-1

Linkage analysis of the F2 cross revealed evidence of an important gene locus on chromosome 1, previously designated *Rf-1* (Figure 1). The *Rf-1* locus showed a maximum LOD score of 16.7 for UPV, 16.9 for UAV, and 8.6 for FGS, occurring within the 95% confidence interval (D1Rat119 and D1Mit8). The *Rf-1* locus explains 19.6%, 20.5%, and 14.2% of the genetic variance in UPV, UAV, and FGS, respectively, in the F2 cross. As expected, all three traits had a recessive mode of inheritance at this locus (Table 2). The LOD score scans for UPV and UAV implicate that *Rf-1* seems to have two peaks—the interval between D1Rat119 and D1Mgh12 and the interval between D1Mgh12 and D1Mit8. Maximum LOD scores are 15.9 and 16.7 for UPV and 16.7 and 16.9 for UAV, respectively. However, from the analysis the fix command of MAPMAKER, we could not conclude that these peaks were independent. Peak LOD scores for UPV, UAV, and FGS all occur in essentially the same location of rat chromosome 1.

Linkage analysis also validated that *Rf-2* (Figure 1) cosegregates with UPV, UAV, and FGS with maximum LOD scores of 5.4, 6.5, and 2.5, respectively, within the interval between D1Wox7 and D1Mgh26. The inheritance pattern is consistent; all three traits are inherited with a recessive mode of inheritance (Table 2).

Figure 1 also shows the LOD plot for SBP, which illustrates suggestive linkage (2.7) at *Rf-1* and a suggestive LOD score at *Bpfh-1* (LOD score 3.8) between D1Wox6 and D1Mit28. It is

Table 1. Phenotypes of male FHH and ACI parental strains (FHH × ACI), F1, and F2 progeny obtained at 8 wk after unilateral nephrectomy at 5 to 6 wk of age[a]

	FHH (26)	ACI (21)	F1 (330)	F2 (337)
BW (gram)	310 ± 15	242 ± 14[b]	282 ± 17[c]	279 ± 31[c]
SBP (mmHg)	151 ± 10	113 ± 9[b]	131 ± 11[c]	133 ± 31[c]
UPV (mg/d per 100 g BW)	77.2 ± 20.4	5.9 ± 2.7[b]	10.1 ± 2.9[b]	16.3 ± 15.2[d]
UAV (mg/d per 100 g BW)	57.6 ± 17.8	3.2 ± 2.3[b]	4.6 ± 2.3[b]	9.6 ± 12.8[b]
FGS (% glomeruli)	52.1 ± 11.7	3.2 ± 1.8[b]	5.2 ± 4.0[b]	10.8 ± 11.9[d]

All data are mean ± SD for (n) rats.

[a] FHH, fawn-hooded hypertensive; ACI, A × C 9935 Irish; BW, body weight; SBP, tail-cuff systolic blood pressure; UPV, proteinuria; UAV, albuminuria; FGS, incidence of focal glomerulosclerosis.

[b] $P < 0.05$ *versus* FHH.

[c] $P < 0.05$ *versus* FHH and ACI.

[d] $P < 0.05$ *versus* FHH, ACI, and F1 (ANOVA followed by Student-Newman-Keuls test).

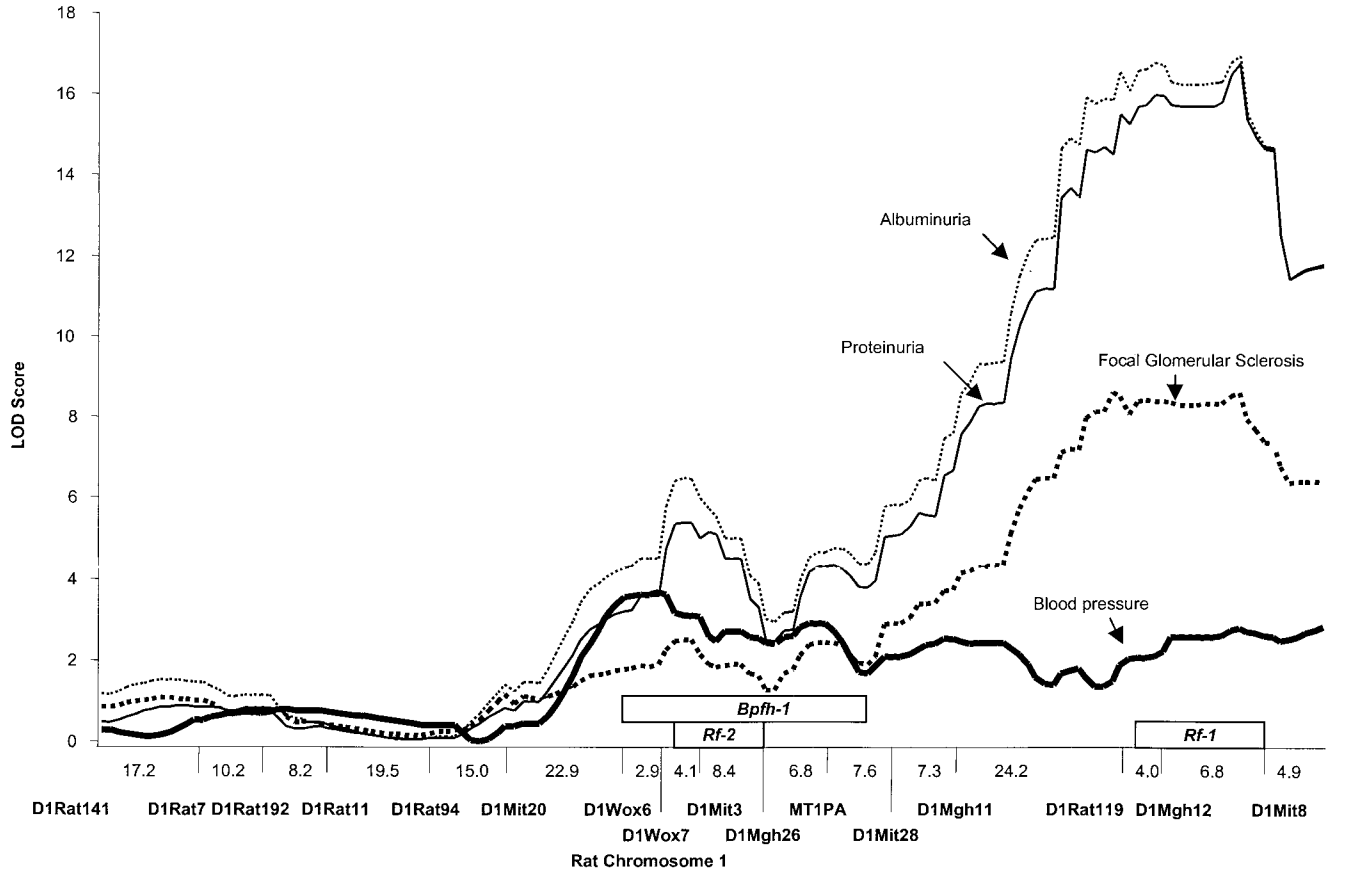


Figure 1. Logarithm of odds (LOD) plot of proteinuria (solid), albuminuria (small dotted line), focal glomerulosclerosis (large dotted line), and systolic BP (thick dashed line) for rat chromosome 1 after unilateral nephrectomy (UNX). A horizontal line indicates the threshold for significance of the LOD scores (4.3). Maximum likelihood positions and LOD 1-support intervals for each quantitative trait locus QTL are indicated by the boxes. *Rf-1* is localized between D1Rat119 and D1Mit8, *Rf-2* between D1Wox7 and D1Mgh26, and *Bpfh-1* between D1Wox6 and D1Mit28 using a LOD 1 confidence interval. centimorgans (cM) determined using the Kosambi map function. Map distances are presented in cM.

apparent from the LOD plot that there is substantial overlap between *Rf-2* and *Bpfh-1*. This degree of overlap was surprising, as we expected to be able to separate these two traits using an intercross design. Furthermore, the suggestive effect of *Rf-1* on SBP was not expected, because the backcross data had not shown a LOD peak for SBP greater than 1.0. One explanation of the results is that UNX is having an impact. Comparison of the ACI animals with and without UNX revealed a trend toward an increase in SBP, as well as higher UPV (data not shown), suggesting that UNX increases the interaction between SBP and UPV, obscuring our ability to separate these traits in this cross.

Additional Loci Rf-3, Rf-4, Rf-5, and Bpfh-2

In addition to the confirmation of the already known loci, we found evidence for three additional QTL that affect UPV, UAV, and FGS. First was a locus on chromosome 3, D3Mit4 (*Rf-3*), with LOD scores indicative of significant linkage for UPV (6.1), UAV (6.5), and FGS (4.0). However, as shown in Table 2, SBP was not even suggestive of linkage, despite an apparent significant difference ($P < 0.05$) by ANOVA. This discrepant result is easily explained by the need to increase the level of significance to account for the large number of markers tested in the genome scan (35). *Rf-4*, on chromosome 14 located around D14Mgh7, showed LOD scores of 4.1 for UPV, 3.7 for UAV, and 2.6 for FGS. Although none of these traits formally exceeds the 4.3 threshold, the UPV and UAV traits are very different from the histologically based FGS and suggest that these traits are not coincidentally mapped to the same locus. *Rf-5*, on chromosome 17 located around D17Mit12, showed a LOD score of 3.0 for UPV, 2.9 for UAV, and 2.1 for FGS. Taken together, the five *Rf* loci are responsible for approximately 63% of the genetic variation in UPV.

At none of these three additional *Rf* loci was there a LOD score indicative or suggestive for statistically significant linkage with SBP. However, a QTL on chromosome 17, located near D17Rat54 or *Bpfh-2*, the peak of which is 30 cM (approximately 60 million bp) from the peak of *Rf-5*, showed a LOD score of 2.6 for SBP, suggestive for linkage. Together, the two *Bpfh* loci are responsible for approximately 14% of the total variation in SBP. In contrast to *Bpfh-1*, none of the LOD scores of the renal phenotypes were even suggestive for link-

Table 2. Maximal LOD scores at the various identified QTL for proteinuria and SBP in the male F2 progeny, and the mean values for the various phenotypes after segregation according to the inheritance of the paternal alleles[a]

Locus	LOD Score	Genotype			ANOVA (*P* Value)
		FHH/FHH	FHH/ACI	ACI/ACI	
Proteinuria QTL					
D1Mgh12 (Rf-1)		(87)	(160)	(90)	
SBP	***2.70***	136 ± 11**	132 ± 12	131 ± 11	0.008
UPV	**16.74**	26.7 ± 23.2**	13.9 ± 9.6	10.5 ± 5.9	< 0.001
UAV	**16.88**	18.5 ± 19.8**	7.5 ± 7.8	4.8 ± 4.5	<0.001
FGS	**8.61**	18.3 ± 14.1**	9.4 ± 10.7*	5.9 ± 7.6	< 0.001
		(66)	(117)	(69)	
D1Mit3 (Rf-2)		(93)	(151)	(93)	
SBP	***3.41***	136 ± 12**	132 ± 11	130 ± 10	< 0.001
UPV	**5.39**	21.0 ± 16.4**	15.4 ± 16.4	13.0 ± 10.2	< 0.001
UAV	**6.50**	13.7 ± 14.0**	8.7 ± 13.7	7.0 ± 6.3	<0.001
FGS	2.50	15.0 ± 13.7**	9.3 ± 10.8	8.9 ± 10.9	0.002
		(69)	(115)	(68)	
D3Mit4 (Rf-3)		(86)	(166)	(85)	
SBP	1.87	135 ± 12**	133 ± 11	130 ± 11	0.014
UPV	**6.06**	22.2 ± 18.6**	15.1 ± 13.1	12.7 ± 13.5	< 0.001
UAV	**6.51**	14.6 ± 16.1**	8.5 ± 10.7	6.8 ± 11.3	< 0.001
FGS	***4.04***	15.9 ± 14.1**	10.1 ± 11.5	6.9 ± 7.9	< 0.001
		(64)	(125)	(63)	
D14Mgh7 (Rf-4)		(88)	(181)	(68)	
SBP	0.63	134 ± 13	132 ± 11	132 ± 11	0.379
UPV	***4.07***	19.8 ± 16.6*	16.4 ± 16.2*	11.3 ± 7.4	0.002
UAV	**3.75**	12.7 ± 14.3*	9.6 ± 13.5*	5.6 ± 6.1	0.003
FGS	2.57	14.1 ± 14.4*	10.9 ± 11.5*	6.3 ± 7.4	0.002
		(65)	(134)	(53)	
D17Mit12 (Rf-5)		(86)	(173)	(78)	
SBP	1.70	135 ± 12**	132 ± 12	130 ± 9	0.018
UPV	***2.99***	21.2 ± 21.1**	15.8 ± 13.8*	11.8 ± 6.6	< 0.001
UAV	**2.90**	14.0 ± 18.0**	9.2 ± 11.4	5.8 ± 5.2	< 0.001
FGS	2.06	15.1 ± 15.6**	9.9 ± 10.2	7.8 ± 9.0	0.001
		(67)	(125)	(60)	
Systolic Blood Pressure QTL					
Chr. 1 Mtlpa (Bpfh-1)		(88)	(169)	(80)	
SBP	**3.03**	136 ± 12**	132 ± 11*	129 ± 10	< 0.001
UPV	4.31	20.5 ± 18.7**	15.7 ± 14.8	12.8 ± 13.8	0.006
UAV	**4.80**	13.0 ± 15.7**	9.2 ± 12.6	6.9 ± 12.1	0.011
FGS	2.42	14.1 ± 13.1	9.7 ± 11.5	9.6 ± 10.9	0.036
		(64)	(130)	(58)	
D17Rat54 (Bpfh-2)		(83)	(170)	(84)	
SBP	**2.61**	136 ± 13**	133 ± 10*	130 ± 11	0.002
UPV	0.57	18.8 ± 19.2	16.1 ± 15.2	14.1 ± 9.5	0.133
UAV	1.01	11.8 ± 16.2	9.5 ± 12.7	7.7 ± 8.0	0.114
FGS	0.46	12.2 ± 12.4	10.1 ± 12.2	10.7 ± 10.9	0.513
		(66)	(125)	(61)	

[a] All phenotype data are mean ± SD for (n) rats. LOD scores reported are at the marker indicated. In many instances, the LOD score between markers is higher. LOD, logarithm of odds; QTL, quantitative trait loci.

FGS was scored in 252 rats; the numbers per group are given below the FGS values. ***bold italic*** indicates a LOD score suggestive for linkage (2.6 to 4.3); **bold** indicates a LOD score with significant linkage (> 4.3). *, $P < 0.05$ with ACI/ACI; **, $P < 0.05$ with FHH/ACI and ACI/ACI as assessed by ANOVA and Student-Newman-Keuls test.

age at the *Bpfh-2* locus. There was no interaction between the two loci responsible for BP (data not shown).

Interaction between Rf Loci

In Figure 2A, we plotted against mean UPV values the number of *Rf* loci that were homozygous for FHH. It is obvious that the higher the number of homozygous *Rf* loci, the higher the level of UPV. The difference in UPV between those that possessed none or four to five homozygous *Rf* loci that were homozygous for FHH is approximately 35 mg/d per 100 g body wt (approximately 105 mg/d). These data suggest that ESRF in the FHH required more than three loci, a threshold effect. This was not anticipated, as *Rf-1* has previously been shown to be the major gene responsible for ESRF (7). We reanalyzed the impact of *Rf-1* by plotting the number of *Rf* loci that were homozygous for FHH *versus* UPV, while separating combinations with or without *Rf-1* that were homozygous for FHH. The result is depicted in Figure 2B. Clearly, having *Rf-1* has a major impact on the development of UPV; however, these data suggest that there may be an interaction among the various loci.

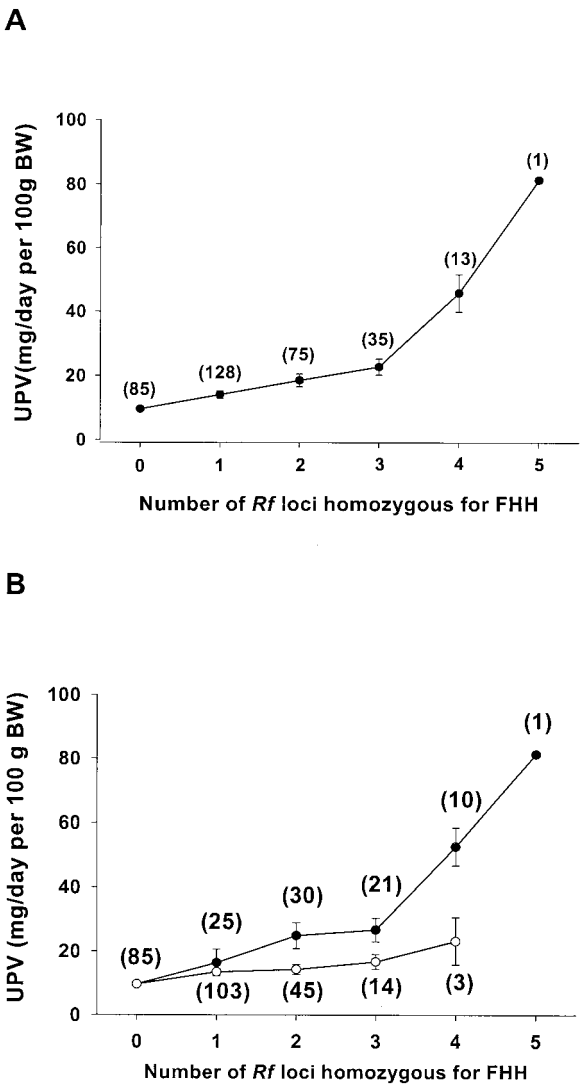


Figure 2. (A) Urinary protein excretion (UPV) at 8 wk after UNX in F2 rats grouped according to the number of *Rf* loci that are homozygous for fawn-hooded hypertensive (FHH). The data represented are not continuous. Data are mean ± SEM for (n) rats. (B) UPV at 8 wk after UNX in F2 rats grouped according to the number of *Rf* loci that are homozygous for FHH. For each number of *Rf* loci that are homozygous for FHH, groups of rats with *Rf-1* included (●) differ significantly ($P < 0.05$) from groups that do not include *Rf-1* (○). With *Rf-1*, number 1 means *Rf-1* only, 2 means *Rf-1* plus one of the other four loci, 3 means *Rf-1* plus two other loci, etc. Without *Rf-1*, number 1 means one *Rf* locus homozygous for FHH with *Rf-1* being excluded, 2 means two *Rf* loci homozygous FHH again excluding *Rf-1*, etc. The highest possible number without *Rf-1* is 4, because five homozygous *Rf* loci will automatically include *Rf-1*. The data represented are not continuous. Data are mean ± SEM for (n) rats.

Figure 3 shows the interactions between two loci. If there were no interaction between the loci, then the slopes of the lines would be parallel and any increase would be due to the additive effect of the two loci. Figure 3, A through D, illustrates the effect of *Rf-1* plus one of the other *Rf* loci. In all combinations with *Rf-1*, there seems to be an interaction with *Rf-1* resulting in a marked increase in the level of UPV as *Rf-1* goes from homozygous for ACI to homozygous for FHH. Furthermore, these interactions seem to be additive. In contrast, *Rf-2* × *Rf-3* (Figure 3E) shows an interaction only when both loci are homozygous for FHH. With the exception of *Rf-2* × *Rf-5*, there seems to be two locus interactions, albeit at a lower level than with *Rf-1* (Figure 3, E through J). Collectively, these data demonstrate complex interactions between the loci that will require additional investigation.

Synteny between Rat Rf *Loci and Human Chromosomal Regions*

Localization of the chromosomal regions that contain susceptibility genes is only the beginning of a long road that leads to the identification of the individual genes. Gene identification in animal models may be helpful in the search for susceptibility genes in human ESRD (36). Genetic homology between rats and humans provides a powerful tool to translate the experimental findings. The comparative mapping of genes in different species has made considerable progress on the basis of the delineation of contiguous conserved syntenic regions between different species. Although still insufficiently detailed, the location of the human regions that are homologous to the various rat chromosomal regions that carry the *Rf* genes can already be outlined (37,38). In Table 3, we present the locations of these homologous regions, comparing rat, mouse, and human.

Discussion

Using an FHH × ACI F2 intercross, apart from confirming the presence of two previously reported QTL for UPV, *Rf-1* and *Rf-2* on rat chromosome 1, we identified three new genetic loci, designated *Rf-3*, *Rf-4*, and *Rf-5*, that influence the development of renal damage after UNX. These three additional loci cosegregated with UPV, UAV, and FGS and located on rat chromosomes 3, 14, and 17, respectively. Previously, using

128 backcross animals, we demonstrated that *Rf-1* had a LOD score peak of 8.9 for UPV (7). In the present study, using 337 F2 animals, we could detect a LOD score peak of 16.7 for UPV at the *Rf-1* locus. This high LOD score indicates that *Rf-1* must contain one or more genes that play an important role in defining the high susceptibility to renal failure in the FHH rat. However, the presence of four additional QTL that influence UPV and UAV underscores the complexity of the genetics of renal failure.

In addition, the large F2 intercross increased our chances of detecting genetic loci that make minor contributions to the hypertension phenotype. Thus, we could confirm the presence of a suggestive QTL for SBP at the previously reported *Bpfh-1* locus on rat chromosome 1 and another suggestive QTL, *Bpfh-2*, on chromosome 17. As in the backcross, the relationship between *Bpfh-1* and *Rf-2* is still unclear. The most likely positions for the two loci are separated by approximately 15.6 cM, which is not far enough to conclude that they segregate independent of each other. Furthermore, the use of UNX may have obscured our ability to separate *Bpfh-1* and *Rf-2*. Generally, it is desirable to have the linkage of a chromosomal location to the phenotypic trait supported by a LOD score that is 4.3 or higher (35). The LOD score for *Bpfh-1*, itself, demonstrates that the evidence for linkage is only suggestive. However, it has been replicated, and the proximity of *Bpfh-1* to a region that cosegregates with BP in other crosses of genetic hypertensive rat strains (20,21) allows us to be confident of the presence of a BP gene on *Bpfh-1*. Similarly, the LOD score for *Bpfh-2*, at the locus of D17rat54, was suggestive only for linkage. At the *Hith* locus on rat chromosome17, Gu *et al.* (39) demonstrated a suggestive QTL for BP in a LEW × SHR cross on an 8% NaCl diet. We are, as yet, unable to confirm that *Bpfh-2* reflects the same QTL.

Susceptibility for the development of ESRD in rat models reported to date has a recessive mode of inheritance. Here, we demonstrated that even after UNX, F1 rats do not develop overt UPV, UAV, or FGS (Table 1). A recessive mode of inheritance is also indicated in the first-generation hybrids of the Milan rat strains (40). In a backcross of BUF × F1(BUF × ACI) and BUF × F1(BUF × WKY) rats, the inheritance pattern of albuminuria could be explained by the presence of at least two recessive genes (6), a QTL for one of which has been recently localized on rat chromosome 13 in the BUF × F1(BUF × WKY) backcross (22). The same inheritance pattern holds true for mice. It has been reported that spontaneous development of glomerulosclerosis is inherited in a recessive fashion, involving at least 8 to 10 loci (24). Taken together, these studies in experimental models indicate that the genetics of progressive renal damage is extremely complex, involving a substantial number of genes.

Localization of the chromosomal regions that contain susceptibility genes is only the beginning of a long road that leads to the identification of the individual genes. Gene identification is essential for further understanding of the function of genes and the pathway by which the mutated genes may result in an increased susceptibility to renal damage. A strategy to reach these goals has been outlined (41). The first step requires the generation of several strains of congenic rats, each carrying one of the *Rf* loci of the FHH strain on the ACI background. These strains are essential for the positional cloning of the various *Rf* genes but will also be used to determine whether and how each of the *Rf* loci influences the development of renal damage. In each of the strains that carry a single locus, it can be determined whether a single *Rf* gene is sufficient to increase renal susceptibility to UNX or to increase systemic BP. Simultaneously, studying the renal physiology of the congenic strains can unravel potential pathways that may explain an enhanced susceptibility and may, thus, be helpful in the gene identification studies.

In addition, complex gene–gene interactions should be revealed, as well as the interactions of the genes with environmental factors known to influence the progression of renal failure, such as hypertension, diabetes, renal mass reduction, protein intake, and so forth. Analyses of the F2 cross already indicate that different combinations of *Rf* loci enhance renal susceptibility. However, detailed studies can be obtained only by using congenics that carry two, three, four, or all five *Rf* loci concurrently. Ideally, the *Rf* loci should be transferred from FHH to a renal-resistant strain that is either normotensive, such as ACI, or hypertensive, such as SHR; as well, the *Rf* loci should be serially replaced on the FHH background. In the case of congenic normotensive rats on the ACI background, susceptibility to hypertension can be studied by raising BP with NG-nitro-L-arginine methyl ester. We previously reported that this is a very attractive and efficient way to uncover increased renal susceptibility in rats (29,42).

Gene identification in animal models may be helpful in the search for susceptibility genes in human ESRD (36). Genetic homology between rats and humans provides a powerful tool for translation of experimental findings. The comparative mapping of genes in different species has made considerable progress. Although still being developed, the location of the human regions that are syntenic to the various rat chromosomal regions that carry the *Rf* genes can be roughly outlined (37,38) (Table 3). It has been suggested that the genes of human chromosome 19q13 may be homologous with the *Rf-1* gene (22). This, however, is not in accordance with our present knowledge of the rat-human genetic homology maps. As indicated in Table 3, the rat region that carries *Rf-1* corresponds with mouse chromosome 19 and human chromosome 10q.

Recently, Yu *et al.* (43) examined the linkage between ESRD and markers on human chromosome 10q, the area homologous to the rat *Rf-1* region. They reported that no evidence for linkage was found in African-American sibling pairs concordant for non–insulin-dependent diabetes mellitus–associated nephropathy or with nondiabetic causes of ESRD; however, they did not have enough power to exclude the region either. Although a total of 129 sibling pairs were studied, the number is still relatively small, because it actually consisted of 58 diabetic and 71 nondiabetic sibling pairs. Using an even smaller number of sibling pairs, the same group previously reported negative results for linkage with loci of the renin-

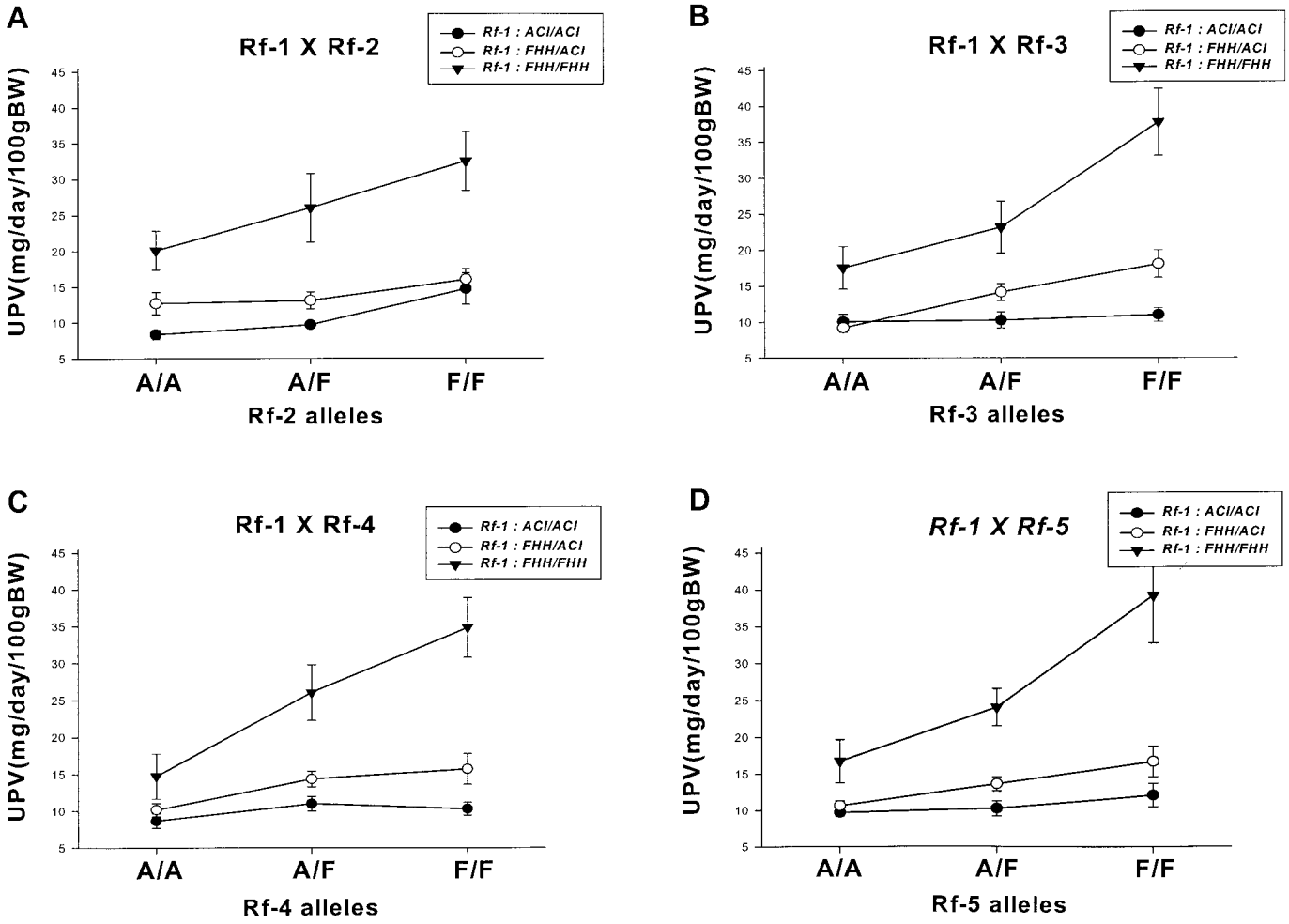
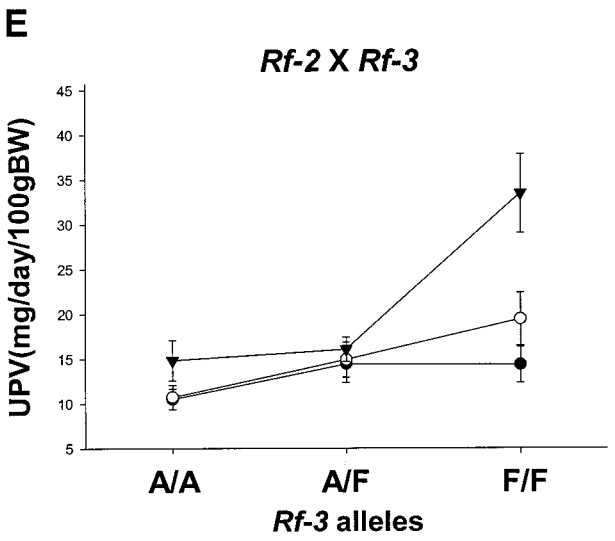


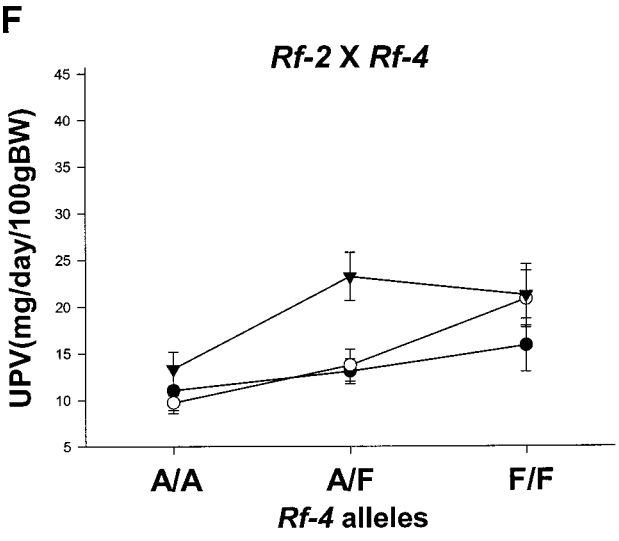
Figure 3. Effect on UPV of genotype combinations for two *Rf* loci changing from homozygous ACI (A/A) to heterozygous (F/A) and homozygous FHH (F/F). (A) combines *Rf-1* and *Rf-2*; (B) combines *Rf-1* and *Rf-3*; (C) combines *Rf-1* and *Rf-4*; (D) combines *Rf-1* and *Rf-5*; (E) combines *Rf-2* and *Rf-3*; (F) combines *Rf-2* and *Rf-4*; (G) combines *Rf-2* and *Rf-5*; (H) combines *Rf-3* and *Rf-4*; (I) combines *Rf-3* and *Rf-5*; (J) combines *Rf-4* and *Rf-5*. Data are mean ± SEM. The number of rats per group varies from 14 to 84. ●, ACI/ACI; ○, FHH/ACI; ▲, FHH/FHH.

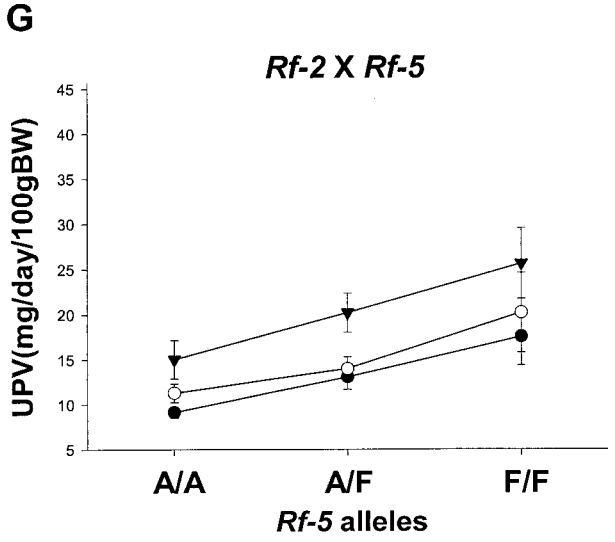
angiotensin axis, *i.e.*, AGTR1, AGT, REN, and ACE, and with the glandular kallikrein gene (44), as well as genes for growth factors and cytokines (45).

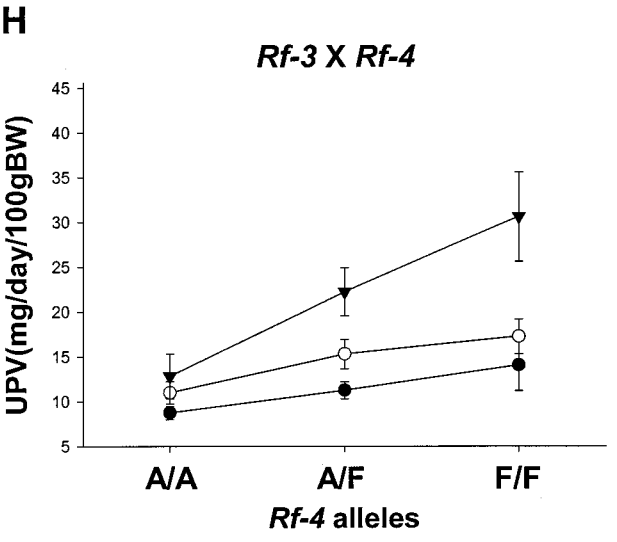
The number of sibling pairs required to detect linkage depends substantially on the relative risk determined by the ratio of the prevalence of the disease in sibling pairs divided by the prevalence of the disease in the general population. Thus, for polygenic disease with a moderate relative risk like ESRD, a larger number of sibling pairs are needed (46). The number of sibling pairs studied in the various reports of Freedman and colleagues (43–46) may have been too small to detect genetic linkage. An additional problem is associated with genetic heterogeneity when several genes influence the trait. Thus, the gene effect is not constant, detectable, or even present in all individuals (46–48). For the *Rf-1* region, in advance of identifying the gene in the rat, we would predict that a more efficient strategy would be to use a case-control study and single nucleotide polymorphisms (SNP) to test the entire region of *Rf-1* (36). On the basis of the rat data, these studies should be carried out in patients with hypertension-associated ESRD and these cases contrasted to hypertensive patients without renal disease while controlling for population stratification.

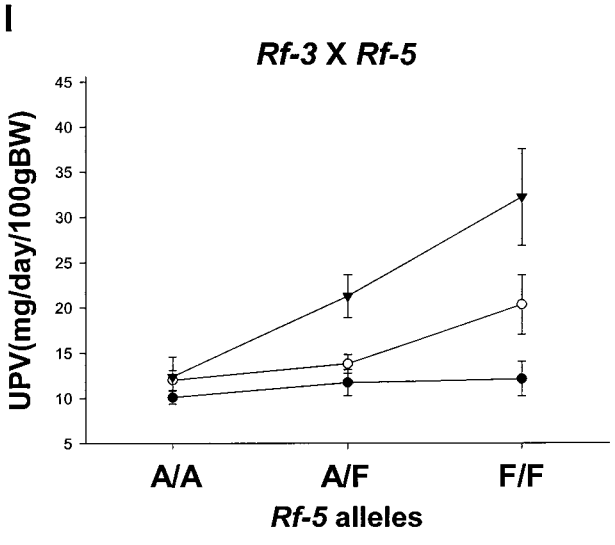
A final comment on the possibilities of successfully identifying genes that influence renal susceptibility relates to the relative lack of understanding of the pathways involved in progressive renal damage. This is in sharp contrast to hypertension, for which numerous BP regulating systems have been identified at the molecular level. Consequently, in the genetic analysis of hypertension, a substantial number of candidate genes can be easily generated. However, even in hypertension, with the exception of rare monogenic forms, little is known about the genetics of more complex human essential hypertension or genetic hypertension in rats. Causative gene mutations have not yet been identified. Because knowledge of the molecular basis of progressive renal damage is virtually absent, molecular genetic strategies offer the prospects of helping to unravel the disease process.


E
Rf-2 X Rf-3
UPV(mg/day/100gBW)
45
40
35
30
25
20
15
10
5
A/A
A/F
F/F
Rf-3 alleles


F
Rf-2 X Rf-4
UPV(mg/day/100gBW)
45
40
35
30
25
20
15
10
5
A/A
A/F
F/F
Rf-4 alleles


G
Rf-2 X Rf-5
UPV(mg/day/100gBW)
45
40
35
30
25
20
15
10
5
A/A
A/F
F/F
Rf-5 alleles


H
Rf-3 X Rf-4
UPV(mg/day/100gBW)
45
40
35
30
25
20
15
10
5
A/A
A/F
F/F
Rf-4 alleles


I
Rf-3 X Rf-5
UPV(mg/day/100gBW)
45
40
35
30
25
20
15
10
5
A/A
A/F
F/F
Rf-5 alleles

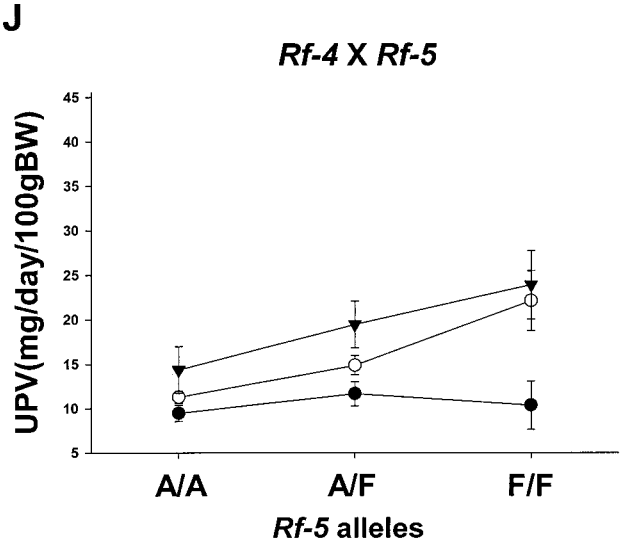

J
Rf-4 X Rf-5
UPV(mg/day/100gBW)
45
40
35
30
25
20
15
10
5
A/A
A/F
F/F
Rf-5 alleles

Table 3. Genetic synteny among the *Rf* regions in the rat (RNO), mouse (MMU), and human (HSA) chromosomal locations[a]

	RNO chr	MMU chr	HSA chr
Rf-1	1q3	19	10q21-q25
Rf-2	1q5	7	11q14-q21
Rf-3	3q1-q2	2	20p13, 2q, and 15q15-q21
Rf-4	14p1-q1	5	4q11-q23
Rf-5[b]	17p1-q1	13	5q or 6 (37)
		18	10p11-p15 (38)

[a] Based on references 37 and 38 and own data (Kwitek-Black, Jacob, *et al*., unpublished data).
[b] Published data markedly differ with regard to the homologous regions of *Rf-5*.

In conclusion, by studying the genetics of BP and renal damage in the FHH rat, we confirmed the roles of *Rf-1*, *Rf-2*, and *Bpfh-1*; in addition, *Rf-3*, *Rf-4*, *Rf-5*, and *Bpfh-2* were identified. *Rf-1* acts through a mechanism that is independent of BP and has a prominent additive effect on other *Rf* loci. The findings may be relevant for the differences in susceptibility for the development of ESRF in humans, but the translation to human and its interpretation are still very complex and speculative and require additional studies. Our results demonstrate that searching for gene–gene interactions may be required for unraveling the genetic basis of ESRF.

Acknowledgments

Studies were performed with financial support from the National Institutes of Health (RO1-HL56284 to HJJ and APP) and the Dutch Kidney Foundation (C 95.1436 to APP) and is part of EURHYPGEN II Concerted Action within the Biomed 2 program of the European Union.

The authors thank C. Luhrman-Schlomski, J. Mahabier, I.M. Hekking-Weyma, M. van Aken, A.P. Boijmans, and C. Peekstok at EUR and Dr. S. Twigger and K. Choate at MCW for their excellent assistance.

Parts of the studies were presented at the 31st Annual Meeting of the American Society of Nephrology, Philadelphia (October 25 to 28, 1998) and at the 59th Scientific Meeting of the Dutch Society of Nephrology, Leiden, The Netherlands (January 17, 1998) and have been printed in abstract form (*J Am Soc Nephrol* 9: 394A, 1998, and *Kidney Int* 55: 1167, 1999).

References

1. United States Renal Data System 1999 Annual Data Report: II. Incidence and prevalence of ESRD. *Am J Kidney Dis* 34[2 Suppl 1]: S40–S50, 1999
2. Ferguson R, Grim CE, Opgenorth TJ: A familial risk of chronic renal failure among blacks on dialysis? *J Clin Epidemiol* 41: 1189–1196, 1988
3. Freedman BI, Spray BJ, Tuttle AB, Buckalew VM: The familial risk of end-stage renal disease in African Americans. *Am J Kidney Dis* 21: 387–393, 1993
4. Freedman BI, Iskandar SS, Appel RG: The link between hypertension and nephrosclerosis. *Am J Kidney Dis* 25: 207–221, 1995
5. Brandis A, Bianchi G, Reale E, Helmchen U, Kuhn K: Age-dependent glomerulosclerosis and proteinuria occurring in rats of the Milan normotensive strain and not in rats of the Milan hypertensive strain. *Lab Invest* 55: 234–243, 1988
6. Matsuyama M, Ogiu T, Kontani K, Yamada C, Kawai M, Hiai H, Nakamura T, Shimizu F, Toyokawa T, Kinoshita Y: Genetic regulation of the development of sclerotic lesions in the BUF/Mna rat. *Nephron* 54: 334–337, 1990
7. Brown DM, Provoost AP, Daly MJ, Lander ES, Jacob HJ: Renal disease susceptibility and hypertension are under independent genetic control in the fawn-hooded rat. *Nat Genet* 12: 44–51, 1996
8. Kreisberg JI, Karnovsky MJ: Focal glomerular sclerosis in the fawn-hooded rat. *Am J Path* 92: 637–652, 1978
9. Kuijpers MH, de Jong W: Relationship between blood pressure level, renal histological lesions and plasma renin activity in fawn-hooded rats. *Br J Exp Pathol* 68: 179–187, 1987
10. Kuijpers MH, Provoost AP, Molenaar JC: Proteinuria is an early marker in the development of progressive renal failure in hypertensive fawn-hooded rats. *J Hypertens* 7: 525–528, 1989
11. De Keijzer MH, Provoost AP, Molenaar JC: Glomerular hyperfiltration in hypertensive fawn-hooded rats. *Renal Physiol Biochem* 11: 103–108, 1988
12. Provoost AP, De Keijzer MH: The fawn-hooded rat: A model for chronic renal failure. In: *Experimental and Genetic Rat Models of Chronic Renal Failure*, edited by Gretz N, Strauch M, Basel, Karger, 1993, pp 100–114
13. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM: Pathogenesis of glomerular injury in the fawn-hooded rat: Early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol* 3: 1775–1782, 1993
14. Simons JL, Provoost AP, De Keijzer MH, Anderson S, Rennke HG, Brenner BM: Pathogenesis of glomerular injury in the fawn-hooded rat: Effect of unilateral nephrectomy. *J Am Soc Nephrol* 4: 1362–1370, 1993
15. Oliver JD III, Simons JL, Troy JL, Provoost AP, Brenner BM, Deen WM: Proteinuria and impaired glomerular permselectivity in uninephrectomized fawn-hooded rats. *Am J Physiol* 267: F917–F925, 1994
16. Simons JL, Provoost AP, Anderson S, Rennke HG, Troy JL, Brenner BM: Glomerular hypertension and not hypertrophy defines susceptibility to progressive glomerular injury in uninephrectomized fawn hooded rats. *Kidney Int* 46: 396–404, 1994
17. Provoost AP: Spontaneous glomerulosclerosis: Insights from the fawn-hooded rat. *Kidney Int* 45[Suppl 45]: S2–S5, 1994
18. Kriz W, Hosser H, Hähnel B, Simons JL, Provoost AP: Development of vascular pole-associated glomerulosclerosis in the Fawn-hooded rat. *J Am Soc Nephrol* 9: 381–396, 1998
19. Kriz W, Hosser H, Hähnel B, Gretz N, Provoost AP: From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: A histopathological study in rat models and human glomerulopathies. *Nephrol Dial Transplant* 13: 2781–2798, 1998
20. Samani NJ, Lodwick D, Vincent M, Dubay C, Kaiser MA, Kelly MP, Lo M, Haris J, Sassard J, Lathrop M, Swales JD: A gene differentially expressed in the kidney of the spontaneously hypertensive rat co-segregates with increased blood pressure. *J Clin Invest* 92: 1099–1103, 1993

21. Lindpaintner K, Hilbert P, Ganten D, Nadal-Ginard B, Inagami T, Iwai N: Molecular genetics of the SA-gene: Co-segregation with hypertension and mapping to rat chromosome 1. *J Hypertens* 11: 19–23, 1993
22. Murayama S, Yagyu S, Higo K, Ye C, Mizuno T, Oyabu A, Ito M, Morita H, Maeda K, Serikawa T, Matsuyama M: A genetic locus susceptible to the overt proteinuria in BUF/Mna rat. *Mamm Genome* 9: 886–888, 1998
23. He C, Esposito C, Phillips C, Zalups RK, Henderson DA, Striker GE, Striker LJ: Dissociation of glomerular hypertrophy, cell proliferation, and glomerulosclerosis in mouse strains heterozygous for a mutation (Os) which induces a 50% reduction in nephron number. *J Clin Invest* 97: 1242–1249, 1996
24. Lenz O, Zheng F, Vilar J, Doublier S, Lupia E, Schwedler S, Striker LJ, Striker GE: The inheritance of glomerulosclerosis in mice is controlled by multiple quantitative trait loci. *Nephrol Dial Transplant* 13: 3074–3078, 1998
25. Provoost AP, De Keijzer MH, Molenaar JC: Effect of protein intake on lifelong changes in renal function of rats unilaterally nephrectomized at young age. *J Lab Clin Med* 114: 19–26, 1989
26. Brown DM, Van Dokkum RPE, Korte MR, McLaughlin MG, Shiozawa M, Jacob HJ, Provoost AP: Genetic control of susceptibility for renal damage in hypertensive fawn-hooded rats. *Renal Failure* 20: 407–411, 1998
27. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K: Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. *Clin Chem* 32: 1551–1554, 1986
28. Doumas BT, Watson WA, Biggs HG: Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 31: 87–96, 1971
29. Van Dokkum RPE, Jacob HJ, Provoost AP: Differences in susceptibility of developing renal damage in normotensive Fawn-Hooded (FHL) and August x Copenhagen Irish (ACI) rats after N^{w}-Nitro-L-arginine methyl ester induced hypertension. *Am J Hypertens* 10: 1109–1116, 1997
30. Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A: Simplified mammalian DNA isolation procedure. *Nucleic Acids Res* 19: 4293, 1991
31. Jacob HJ, Brown DM, Bunker RK, Daly MJ, Dzau VJ, Goodman A, Koike G, Kren V, Kurtz T, Lernmark A, Levan G, Mao YP, Petterson A, Pravenec M, Simon JS, Szpirer C, Troillet MR, Weiner ES, Lander ES: Genetic linkage map of the laboratory rat *Rattus norvegicus*. *Nat Genet* 9: 63–69, 1995
32. Lander ES, Botstein D: Mapping mendelian factors underlying quantitative traits using RFLP analysis. *Genetics* 121: 185–189, 1989
33. Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln S, Newberg L: MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174–181, 1987
34. Lincoln SE, Lander ES: Systematic detection of errors in genetic linkage data. *Genomics* 14: 604–610, 1992
35. Lander ES, Kruglyak L: Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nat Genet* 11: 241–247, 1995
36. Broeckel U, Shiozawa M, Kissebah AH, Provoost AP, Jacob HJ: Susceptibility genes for end-organ damage: New strategies to understand diabetic and hypertensive nephropathy. *Nephrol Dial Transplant* 13: 840–842, 1998
37. Grützner F, Himmelbauer H, Paulsen M, Ropers HH, Haaf T: Comparative mapping of mouse and rat chromosomes by fluorescence in situ hybridization. *Genomics* 55: 306–313, 1999
38. Watanabe TK, Bihoreau MT, McCarthy LC, Kiguwa SL, Hishigaki H, Tsuji A, Browne J, Yamasaki Y, Mizoguchi-Miyakita A, Oga K, Ono T, Okuno S, Kanemoto N, Takahashi E, Tomita K, Hayashi H, Adachi M, Webber C, Davis M, Kiel S, Knights C, Smith A, Critcher R, Miller J, James MR, et al.: A radiation hybrid map of the rat genome containing 5,255 markers. *Nat Genet* 22: 27–36, 1999
39. Gu L, Dene H, Deng AY, Hoebee B, Bihoreau MT, James M, Rapp JP: Genetic mapping of the two blood pressure quantitative trait loci on rat chromosome 1. *J Clin Invest* 3: 777–788, 1996
40. Stella P, Cusi D, Duzzi L, Bianchi G: Relations between hypertension and glomerulosclerosis in first generation hybrids of the Milan strains. *Child Nephrol Urol* 11: 6–9, 1991
41. Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES: Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* 67: 213–224, 1991
42. Van Dokkum RPE, Jacob HJ, Provoost AP: Genetic difference defines severity of renal damage after L-NAME-induced hypertension in rats. *J Am Soc Nephrol* 9: 363–371, 1998
43. Yu H, Sale M, Rich SS, Spray BJ, Roh BH, Bowden DW, Freedman BI: Evaluation of markers on human chromosome 10, including the homologue of the rodent *Rf-1* gene, for linkage to ESRD in black patients. *Am J Kidney Dis* 33: 294–300, 1999
44. Yu H, Bowden DW, Spray BJ, Rich SS, Freedman BI: Linkage analysis between loci in the renin-angiotensin axis and end-stage renal disease in African Americans. *J Am Soc Nephrol* 7: 2559–2564, 1996
45. Freedman BI, Yu H, Spray BJ, Rich SS, Rothschild CB, Bowden DW: Genetic linkage analysis of growth factor loci and end-stage renal disease in African Americans. *Kidney Int* 51: 819–825, 1997
46. Risch N, Merikangas K: The future of genetic studies of complex human diseases. *Science* 273: 1516–1517, 1996
47. Schork NJ: Genetically complex cardiovascular traits: Origins, problems, and potential solutions. *Hypertension* 29: 145–149, 1997
48. Lander ES, Schork NJ: Genetic dissection of complex traits. *Science* 265: 2037–2048, 1994