

A Genome Scan for ESRD in Black Families Enriched for Nondiabetic Nephropathy

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Abstract. Nephropathy is a complex disorder, with predisposition influenced by the interplay of both genetic and environmental factors. As part of an effort to map genes that predispose to ESRD, a genome scan was performed in 264 black pedigrees that contained 296 ESRD-affected sibling pairs using multipoint nonparametric linkage analysis methods. The cause of ESRD in index cases was consistent with hypertension-associated ESRD. Nonparametric linkage (NPL) regression provided modest evidence of linkage to 9p21.3 near D9S1121 (logarithm of odds [LOD] = 2.03), 1q25.1 near D1S1589 (LOD = 1.62), and 13q33.3 near D13S796 (LOD = 1.02). Adjusting for the evidence of linkage at the other loci through the NPL regression analysis provided evidence for linkage to 1q25.1, 6p23, and 9p21.3. The NPL regression and ordered subset analyses suggest that the evidence for linkage signifi-

cantly increased with early onset of ESRD (2q32.1 LOD = 3.89, 13q13.1 LOD = 3.90), increased BMI (8p22 LOD = 3.37, 13q33.3 LOD = 5.20, 18p11.3 LOD = 2.38), early onset of hypertension (14q21.1 LOD = 3.19, 20q13.2 LOD = 2.32), and late onset of hypertension (4q13.1 LOD = 3.44, 5p15.33 LOD = 2.82). Multipoint single-locus linkage analysis provided modest evidence of linkage to nondiabetic ESRD on 9p21.3, 1q25.1 (in the region of the podocin gene), and 13q33.3. NPL regression and ordered subset analyses also identified loci on 13q13.1 and 13q33.3 as contributing to early-onset ESRD and ESRD in the presence of increased BMI, respectively. These regions should receive priority in the search for loci that contribute susceptibility to nondiabetic nephropathy.

Hypertensive ESRD (H-ESRD) is a serious public health concern in the United States, accounting for ~26% of all incident patients who require renal replacement therapy (1). In addition to the personal losses associated with H-ESRD morbidity and mortality, H-ESRD carries a significant health care cost (1). Minority populations are disproportionately affected by H-ESRD, as blacks have a fourfold increased risk of receiving a diagnosis of H-ESRD, relative to whites. This disparity increases to 20-fold in the southeast (2). Racial differences in the diagnosis of H-ESRD begin in the third decade of life, when the short duration of elevated BP is less likely to lead to advanced nephropathy (3). Thus, elevated BP, *per se*, may not fully account for the initiation of renal disease (4,5).

Patients with H-ESRD often present to nephrologists with advanced renal disease, making an accurate diagnosis difficult, they often lack preexisting essential hypertension, and physician bias in diagnosis is present (6,7). There may also be a relative lack of accuracy in this cause of ESRD, as listed in the U.S. Renal Data System registry (8). Two recent reports demonstrated the typical renal pathologic changes in putative hypertensive nephrosclerosis (arterionephrosclerosis, arteriolonephrosclerosis, interstitial fibrosis, and global glomerulosclerosis) (9,10). In both reports, the observed renal microvascular changes did not correlate with the degree of BP elevation (9,10).

H-ESRD is widely held to be a complex genetic trait with significant environmental and genetic causes. In addition to the diagnostic challenges noted above, abnormalities in multiple biologic pathways likely result in an increased risk for H-ESRD. Multiple genes likely control these pathways, and the influences of these genes are almost surely modified by interaction with other genes and environmental factors. There is extensive evidence that H-ESRD in the black population has a genetic component (11). Only a minority of hypertensive patients develop an elevated serum creatinine concentration (12,13), and strict BP control does not reliably slow the progression of nephropathy (14). However, blacks with a first-degree relative who has ESRD are at ninefold increased risk of

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developing future ESRD, and 40% of hypertensive blacks with a phenotype compatible with H-ESRD have close relatives on dialysis (15). When less rigid phenotypic criteria were applied, nearly 25% of blacks with a common cause of ESRD (H-ESRD, chronic glomerulonephritis, and diabetic ESRD) had first- or second-degree relatives on dialysis (16). Different causes of ESRD, including H-ESRD, often coexist within single black families (15).

The search for genes that predispose individuals to hypertensive and chronic glomerular disease-associated ESRD requires that we use methods that account for the characteristics of complex genetic traits (*e.g.*, genetic heterogeneity, gene-gene and gene-environment interactions). Here we report the results of the first genome scan in multiple black families with nondiabetic ESRD, with an emphasis on the potential influences of obesity and age of onset for hypertension and ESRD. On the basis of these analyses, we identify several priority regions for further study in an effort to map and clone genes that predispose to hypertensive and chronic glomerular disease-associated ESRD.

Materials and Methods

This study was approved by the Institutional Review Board at the Wake Forest University School of Medicine, and all participants provided written, informed consent. DNA samples were collected from self-described black families who have multiple siblings concordant for ESRD. Families were originally identified through a proband reported by his or her nephrologist as having H-ESRD on the HCFA 2728 form. Proband confirmed that they had hypertension preceding the development of ESRD, the absence of other risk factors for nephropathy, and (1) proteinuria <1.5 g/d (or ≤ 100 mg/dl on urine dipstick, or <1.5 g protein/g creatinine on spot sample), (2) electrocardiogram evidence of left ventricular hypertrophy, or (3) hypertensive retinopathy on medical record review. As previously reported, it is often difficult to confirm whether index cases actually have hypertensive nephrosclerosis or a chronic glomerular disease (7,8). Siblings of the H-ESRD index case often had disparate causes of ESRD, including definitive diabetic nephropathy, chronic glomerular disease (idiopathic focal and segmental glomerulosclerosis [FSGS]), systemic lupus erythematosus, human immunodeficiency virus (HIV)-associated nephropathy, and unknown causes. Families with any member who had ESRD attributed to polycystic kidney disease, Alport's syndrome, urologic disease, or surgical nephrectomy were excluded. Recruitment strategies and selection criteria have previously been described in detail (17–22).

Genotyping

DNA extraction was performed using the PureGene system (Gentra Systems, Minneapolis, MN). Through the National Institute of Diabetes and Digestive and Kidney Diseases-funded Family Investigation of Nephropathy and Diabetes, a genomewide scan was completed by the Center for Inherited Disease Research. The marker set used was based on Marshfield Panel 8, with $\sim 10\%$ of the markers changed from the previous Marshfield panel. The scan consisted of 385 di-, tri-, and tetranucleotide repeat markers, with an average spacing of 9.0 cM and a maximal intermarker gap of 20 cM.

Each pedigree was examined for consistency of familial relationships using the Pedigree Relationship Statistical Test (23). When the self-reported familial relationships were inconsistent with that deter-

mined from the observed genotypic data for that pedigree, (1) the pedigree was modified when the identity by descent statistics suggested a clear alternative or (2) a minimal set of genotypic data were converted to missing. A total of 52 (20%) pedigrees exhibited probable incorrect familial relationships and were modified as above, with 98% (51 of 52 families) of these changes being from a full-sibling to half-sibling relationship. Each genetic marker was also examined for Mendelian inconsistencies using PedCheck (24), and sporadic problem genotypes were converted to missing. Allele frequency estimates were computed using the maximum likelihood methods implemented in the software Recode (<http://watson.hgen.pitt.edu/register/docs/recode.html>). Map distances were based on the Marshfield genetic map (25).

Linkage Analyses

Multipoint linkage analyses were carried out, as described in detail by Sale *et al.* (26) and Bowden *et al.* (27). In brief, NPL regression analyses and the NPL_{pairs} statistics output from a modified version of Genehunter were used (28–32). The NPL regression approach is a conditional logistic regression in which the family-specific NPL statistic (*e.g.*, NPL_{pairs}) at one or more loci is the predictor variable.

Ordered Subsets Analysis

If a subset of pedigrees that are phenotypically more homogeneous can be identified, then it should be possible to improve the power of linkage analysis. Ordered subset analyses (OSA) (33) is a method developed to address this possibility. OSA were computed to investigate the influence of a pedigree's mean age at diagnosis of ESRD, mean age at diagnosis of hypertension, and BMI (similar to the NPL regression analysis above). See the appendix for full methods of NPL regression and OSA.

Results

The genome scan was conducted on 264 black pedigrees that contained 296 ESRD-affected sibling pairs (230 full-siblings and 66 half-siblings, total 558 affected individuals). A total of 250 families contained two affected siblings, 12 families contained three affected siblings, and two families contained four affected siblings. Family data consisted primarily of individuals from a single generation, with both parents available in no families and one ESRD-affected parent available in two families. On the basis of the phenotypic information received, a single investigator (B.I.F.) coded 290 participants with ESRD as likely having H-ESRD, 125 with glomerular disease-associated ESRD, and 143 with diabetic ESRD (diabetic cases were included in these analyses because their sibling had nondiabetic ESRD). Forty-four participants had renal biopsy information available for review.

The clinical and phenotypic characteristics for the genotyped individuals are summarized in Table 1. The genotyped population was 46% female, mean \pm SD (median) age at diagnosis of hypertension was 34.8 ± 13.2 (32.5) years, with a mean duration of hypertension of 11.1 ± 12.1 (7.0) years before the onset of ESRD. Mean BMI at enrollment was 27.8 ± 7.2 (26.3) kg/m^2 .

Linkage Analysis Results

Multipoint single-locus linkage analysis provided modest evidence of linkage to nondiabetic ESRD (Table 2; ASM

Table 1. Characteristics of nondiabetic ESRD in 264 black pedigrees

Phenotype	Mean \pm SD (Median)	Range
Age (yr)	52.4 \pm 13.5 (52.0)	19–86
Age at onset of ESRD (yr)	47.8 \pm 15.0 (47.0)	15–85
Age at onset of hypertension (yr)	34.8 \pm 13.2 (32.5)	8–79
Duration—hypertension to ESRD	11.1 \pm 12.1 (7.0)	21–59
BMI at entry into study (kg/m ²)	27.8 \pm 7.2 (26.3)	16–57.1

analysis). The strongest evidence for linkage was to 9p21.3 near D9S1121 (44.5 cM; logarithm of odds [LOD] = 2.03). Two other chromosomal regions exhibited a LOD >1, 1q25.1 near D1S1589 (191.9 cM; LOD = 1.62) and 13q33.3 near D13S796 (93.8 cM; LOD = 1.02).

As with all complex genetic traits, it is expected that multiple loci will likely contribute to susceptibility to nondiabetic ESRD. As such, analytical methods that account for genetic heterogeneity may improve the ability to detect linkage. The results of the multilocus nonparametric linkage analysis provided evidence that nondiabetic ESRD linked to three chromosomal regions (Table 2). Specifically considering these three loci in one model jointly and computing the corresponding 1 degree of freedom test, the evidence for linkage was greatest at 9p21.3 near D9S1112 (LOD = 2.16) and 1q25.1 near D1S1589 (LOD = 1.37). Evidence for linkage of the chromosome 13 loci disappeared and evidence for another locus at 6p23 near D6S2434/D6S1660 (LOD = 1.20) increased. After adjusting for the evidence for linkage at these three loci, no other region of the genome provided significant evidence for linkage. The LOD – 1 linkage support interval for each of the three positions tended to remain constant or be reduced when considering the evidence of linkage at the other two loci (Table 2). The evidence for linkage at any one of these loci (chromosomes 9, 1, and 6) did not seem to be influenced by the evidence for linkage at the remaining two loci. That is, there was no evidence of an epistatic relationship among these loci.

NPL Regression Analysis: Interaction with Phenotypic Traits

In addition to potential genetic heterogeneity, it is possible that other phenotypic characteristics of the individual influence the effect of a predisposing gene. For testing whether age of ESRD diagnosis, age at hypertension diagnosis, or BMI might influence the evidence for linkage, a series of locus by trait interaction analyses were computed using NPL regression analysis. The results of the NPL regression locus-specific interaction analyses are summarized in Table 3. Here the regions that show evidence of a statistical interaction with the specific trait are listed with the mean trait values for those families who link *versus* those families who do not link to that

region. The Pearson correlation coefficient summarizes the correlation between the evidence for linkage within a family and the mean trait value.

Age of Hypertension Diagnosis. The evidence for linkage tended to be greater in pedigrees with an earlier mean age of diagnosis of hypertension at 1q41, 14q13.1, 16q21, 19q13.31, and 20q13.32. This difference was greatest at 16q21 near D16S1385 ($P = 0.0084$) in which linked families mean age of onset tended to be nearly 5 yr earlier. In contrast, the evidence for linkage tended to be greater in pedigrees with later mean age of diagnosis of hypertension at 1p32.1, 2q22.3, 4q13.1, 5p15.33, 5q33.3, and 6q27. This difference was greatest at 4p13.1 near D4S3248 (p -value = 0.0007), with a mean difference nearly 6 yr in those families who link *versus* those who do not link.

Age at ESRD Diagnosis. The evidence for linkage tended to vary by the age of onset of ESRD at two primary loci (Table 3). The pedigrees that linked to 10q21.1 near D10S1221 tended to have an earlier age of ESRD onset than those that did not link to this locus ($P = 0.0062$). Similarly, the evidence for linkage at 13q13.1 near D13S1493 tended to be greater in those families with an earlier mean age of ESRD diagnosis ($P = 0.0098$). Nine other regions provided modest evidence of an interaction between age of ESRD diagnosis and the evidence for linkage ($P = 0.01$ to 0.05).

BMI. The results of the NPL regression interaction analysis suggest that the evidence for linkage varied with BMI at four primary loci (8p22, 13q33.3, 16p13.3, and 16q22.2).

OSA with Phenotypic Traits

OSA is an analytical approach designed to test for linkage by attempting to identify the subset of families that maximize the evidence for linkage. In contrast to the NPL regression interaction analysis that test for an interaction in the entire set of data, OSA test for differences in the evidence for linkage on the basis of the optimal subset of pedigrees. Thus, the two approaches attempt to test the same hypothesis but using fundamentally different but complementary approaches (*i.e.*, stratification for OSA and regression models for NPL regression). The OSA approach will be statistically more powerful when there are distinct subpopulations identified through the subsets of the covariate that are genetically more homogeneous, and the NPL regression approach should be more powerful when these *ad hoc* subsets are not genetically more homogeneous. Regions that display a significant change in the chromosome-specific P value for the change in the LOD score ($\Delta P < 0.05$) are shown in Table 4.

Age at Hypertension Diagnosis. The OSA analysis provided supporting evidence that the age at hypertension diagnosis tended to influence the evidence for linkage. Subsetting on the 12 and 14% of the pedigrees with the earliest mean age of hypertension onset significantly increased the evidence for linkage to 14q21.1 near D14S306 ($\Delta P = 0.0024$, LOD = 3.19) and 20q13.2 near D20S480 ($\Delta P = 0.0419$, LOD = 2.32), respectively. Conversely, subsetting on the 21 and 8% of the pedigrees with the latest age of hypertension diagnosis provided the greatest increase in the evidence for linkage at 4q13.1

Table 2. Results of the genomewide nonparametric linkage analyses of black families

Chromosome	Position (cM)	Nearest Marker	ASM		Single-Locus Model		NPL Regression	
			LOD	LOD-1 Interval	LOD	LOD-1 Interval	LOD	LOD-1 Interval
1	191.9	D1S1589	1.62	175.4–200.9	1.52	172.9–201.4	1.37	167.9–201.3
2	109.8	D2S1790/D2S2972	0.85	90.3–148.8	0.86	90.3–149.3		
3	118.5	D3S2459	0.32	104.5–134.0	0.29	104.5–134.0		
4	51.9	D4S2632	0.68	pter–97.9	0.71	pter–97.9		
5	69.5	D5S2500	0.36	61.0–78.5	0.32	61.0–78.5		
6	30.5	D6S2434/D6S1660	0.83	6.5–56.0	0.86	6.5–56.0	1.20	9.5–52.5
7	41.5	D7S1808	0.01	41.0–42.0	0.01	41.0–42.0		
8	4.2	D8S262	0.64	pter–16.2	0.63	pter–16.2		
9	44.5	D9S1121	2.03	36.5–53.0	1.87	35.5–54.0	2.16	37.0–53.0
10	46.6	D10S1423			0.16	38.5–56.5		
10	165.5	D10S1248	0.16	160.0–qter				
11	42.9	D11S1392	0.15	10.9–50.9	0.15	11.4–50.9		
12	6.4	D12S372	0.29	pter–12.9	0.31	pter–12.9		
13	93.8	D13S796	1.02	75.8–qter	1.11	78.8–qter		
14	12.5	D14S742	0.60	pter–28.0	0.63	pter–28.0		
15	6.1	D15S128	0.00		0.00			
16	64.0	D16S3396	0.15	57.5–74.0	0.16	57.5–74.0		
17	23.6	D17S1303	0.48	pter–83.6	0.47	pter–83.6		
18	71.3	D18S535/D18S851	0.54	50.8–95.3	0.51	50.8–94.8		
19	20.8	D19S1034	0.45	pter–49.8	0.45	pter–50.3		
20	89.6	D20S451	0.35	72.6–qter	0.33	72.6–qter		
21	23.5	D21S2052	0.43	3.5–33.5	0.46	3.5–33.5		
22	60.6	D22S1169	0.20	46.6–qter	0.21	46.6–qter		

Table 3. Results of NPL regression interaction analyses across the genome for age at diagnosis of ESRD, age at diagnosis of hypertension, or BMI in black families

Chromosomal Position (cM)	Nearest Marker	Interaction	Trait Mean ± SD		Pearson Correlation	
			Linked	Unlinked		
Interaction with age at diagnosis of hypertension						
1	90.85	D1S3728	0.0024	37.9 ± 11.9 (92)	33.4 ± 11.5 (127)	0.21
1	231.85	D1S2141	0.0161	33.9 ± 10.2 (86)	36.2 ± 12.7 (133)	−0.14
2	157.23	D2S1399	0.0388	37.0 ± 12.8 (95)	34.0 ± 10.9 (124)	0.13
4	72.43	D4S3248	0.0007	38.8 ± 12.4 (89)	32.9 ± 10.8 (130)	0.22
5	0.0	D5S2488	0.0222	37.5 ± 12.8 (95)	33.6 ± 10.8 (124)	0.15
5	159.5	D5S820	0.0020	38.2 ± 11.6 (70)	33.9 ± 11.7 (149)	0.20
6	181.5	D6S1027	0.0008	37.8 ± 11.7 (93)	33.4 ± 11.6 (126)	0.21
14	38.96	D14S599	0.0494	33.8 ± 12.2 (102)	36.6 ± 11.4 (117)	−0.13
16	80.03	C16S1385	0.0084	32.6 ± 10.6 (96)	37.4 ± 12.3 (123)	−0.17
19	67.84	D19S178	0.0281	33.1 ± 11.7 (78)	36.5 ± 11.8 (141)	−0.15
20	89.63	D20S451	0.0472	34.2 ± 12.0 (97)	36.1 ± 11.7 (122)	−0.14
Interaction with age at diagnosis of ESRD						
1	87.85	D1S3728	0.0234	49.9 ± 13.4 (119)	46.3 ± 13.6 (149)	0.14
1	230.85	D1S2141	0.0277	46.9 ± 12.5 (106)	48.6 ± 14.3 (162)	−0.13
4	73.43	D4S3248	0.0137	50.3 ± 13.7 (120)	46.0 ± 13.3 (148)	0.15
4	138.43	D4S51644	0.0348	49.7 ± 13.6 (121)	46.5 ± 13.5 (147)	0.13
6	102.5	D6S1056	0.0367	49.2 ± 14.4 (102)	47.1 ± 13.1 (166)	0.13
6	187.0	D6S1027	0.0268	49.5 ± 13.2 (99)	47.0 ± 13.8 (169)	0.13
8	53.73	D8S1771	0.0373	45.9 ± 13.3 (120)	49.5 ± 13.7 (148)	−0.13
8	94.23	D8S2324	0.0118	45.8 ± 12.3 (100)	49.2 ± 14.2 (168)	−0.14
10	55.0	D10S1426	0.0071	45.6 ± 12.8 (117)	49.7 ± 14.0 (151)	−0.16
10	72.0	D10S1221	0.0062	45.6 ± 13.0 (104)	49.4 ± 13.8 (164)	−0.15
13	21.79	D13S1493	0.0098	46.2 ± 13.8 (124)	49.4 ± 13.3 (144)	−0.14
14	43.96	D14S306	0.0400	45.5 ± 13.9 (109)	49.6 ± 13.2 (159)	−0.12
20	79.63	D20S480	0.0447	50.1 ± 13.3 (113)	46.3 ± 13.7 (155)	0.12
Interaction with BMI						
3	9.54	D3S2387	0.0107	28.2 ± 5.8 (110)	27.4 ± 5.8 (139)	0.16
4	36.43	D4S2639	0.0276	26.8 ± 4.7 (112)	28.5 ± 6.5 (137)	−0.14
4	207.93	D4S1652	0.0428	28.1 ± 5.7 (117)	27.5 ± 5.9 (132)	0.11
6	135.5	D6S1009	0.0348	28.6 ± 5.8 (106)	27.1 ± 5.8 (143)	0.14
7	33.5	D7S1802	0.0212	28.5 ± 5.9 (102)	27.3 ± 5.7 (147)	0.14
7	110.5	D7S2212	0.0398	26.8 ± 5.3 (96)	28.3 ± 6.1 (153)	−0.12
8	29.73	D8S1106	0.0058	28.7 ± 6.6 (102)	27.1 ± 5.1 (147)	0.18
8	72.73	D8S1110	0.0215	28.5 ± 6.4 (103)	27.2 ± 5.3 (146)	0.13
13	98.79	D13S1265	0.0014	29.1 ± 5.5 (96)	26.9 ± 5.9 (153)	0.20
16	0.03	D16S3401	0.0002	29.5 ± 6.1 (81)	26.9 ± 5.5 (168)	0.23
16	85.03	D16S2624	0.0074	28.8 ± 6.2 (104)	27.0 ± 5.5 (145)	0.16
18	40.84	D18S542	0.0282	28.7 ± 6.3 (90)	27.2 ± 5.5 (159)	0.14
19	99.34	D19S254	0.0418	27.0 ± 5.5 (101)	28.3 ± 6.0 (148)	−0.12
20	62.63	D20S481	0.0495	28.7 ± 6.2 (93)	27.2 ± 5.5 (156)	0.12

near D4S3248 ($\Delta P = 0.0095$, LOD = 3.44) and 5p15.33 near D5S2488 ($\Delta P = 0.0065$, LOD = 2.82), respectively.

Age at ESRD Diagnosis

Earlier age of ESRD diagnosis was associated with greater evidence of linkage at three loci. Subsetting for the earliest age at ESRD diagnosis increased the LOD score to 3.89 at 2q32.1

near D2S1391 ($\Delta P = 0.0035$) in 24% of the pedigrees, 3.90 at 13q13.1 ($\Delta P = 0.0069$) in 40% of the pedigrees, and 1.85 at 16p13.3 ($\Delta P = 0.0409$) in 7% of the pedigrees.

BMI. The subset of pedigrees with the highest mean BMI tended to provide the greatest evidence for linkage. Subsetting for the pedigrees with the largest BMI increased the LOD score to 3.37 at 8p22 near D8S1106 ($\Delta P = 0.0028$) in 12% of the

Table 4. Ordered subsets analysis across the genome for age at diagnosis (ESRD/HTN) or BMI in 264 black pedigrees

Chromosome	Position (cM)	Nearest Marker	Entire Sample LOD	Maximized LOD	Trait Mean \pm SD		Empirical <i>P</i> Value for Change	% Peds
					Optimal Subset	Remaining Families		
Subsetting by age at diagnosis of hypertension								
4 late onset	72.4	D4S3248	0.29	3.44	53.4 \pm 5.8	30.5 \pm 7.6	0.0095	21
5 late onset	0.0	D5S2488	0.05	2.82	59.2 \pm 4.1	33.2 \pm 9.7	0.0065	8
14 early onset	44.0	D14S306	0.16	3.19	18.7 \pm 2.9	37.6 \pm 10.7	0.0024	12
20 early onset	84.13	D20S480	0.30	2.32	19.0 \pm 3.0	37.9 \pm 10.6	0.0419	14
Subsetting by age at diagnosis of ESRD								
2 early onset	186.3	D2S1391	0.54	3.89	30.9 \pm 4.7	53.4 \pm 10.7	0.0035	24
13 early onset	25.79	D13S1493	0.96	3.90	34.8 \pm 6.2	56.5 \pm 9.7	0.0069	40
14 early onset	42.5	D14S599	0.18	2.20	36.4 \pm 6.7	58.3 \pm 9.1	0.0643	47
16 early onset	11.53	D16S2616	0.00	1.85	24.8 \pm 3.0	49.6 \pm 12.5	0.0409	7
Subsetting by BMI								
8 high BMI	29.2	D8S1106	0.00	3.37	38.8 \pm 3.4	26.2 \pm 4.2	0.0028	12
13 high BMI	94.79	D13S796	0.94	5.20	32.8 \pm 4.4	23.5 \pm 2.6	0.00020	46
18 high BMI	2.8	D18S1781	0.22	2.38	39.0 \pm 3.4	26.3 \pm 4.3	0.0760	11

pedigrees and 5.20 at 13q33.3 near D13S796 ($\Delta P = 0.0002$) in 46% of the pedigrees.

Discussion

This investigation represents the largest effort to map the chromosomal locations of genes that specifically contribute to nondiabetic ESRD in the black population. It is likely that blacks who were reported as having H-ESRD are actually a heterogeneous group of patients, particularly in the absence of renal biopsy material and quantification of proteinuria (7,8). This difficulty in classification has affected previous epidemiologic analyses (34). We postulated that susceptibility genes for the common, complex causes of ESRD exist and that a family-based genome scan could identify homogeneous patient subsets with similar genetic susceptibility. Therefore, in carrying out this study, we proceeded from the assumption that H-ESRD was genetically complex and had both genetic and environmental components. Consequently, the search for nondiabetic ESRD genes required consideration of both multigenic and phenotypic influences. This study incorporated relatively novel approaches for evaluating these types of interactions (*e.g.*, nonparametric linkage regression multilocus modeling and OSA).

Only limited evidence of linkage was evident at the first stage of analysis (Table 2) with three LOD scores ≥ 1.0 and only one > 2 . The highest LOD score was on chromosome 9p21.3 at 44.5 cM. In the multilocus analysis, incorporating an evaluation of heterogeneity, three chromosomal regions showed evidence of significant interaction in the multilocus models, with the strongest evidence (LOD = 2.16) on 9p21.3. When analytical approaches that incorporate phenotypic trait data were applied, as summarized in Tables 3 and 4, evidence for multiple chromosomal loci contributing to ESRD susceptibility was revealed. Using the OSA approach to subset fam-

ilies on the basis of BMI and age at ESRD onset, we identified subsets that in some cases showed dramatic increases in LOD scores, compared with the entire family set. The two chromosome 13 loci (26 cM for age at ESRD diagnosis and 95 cM for BMI) have been identified using two complementary analytical methods (NPL and OSA), providing strong evidence for linkage in a large proportion of pedigrees. Subsetting on the basis of age at ESRD onset was performed because there is a higher sibling risk ratio (λ_s) in dialysis patients with early onset of ESRD (35) and a stronger familial aggregation of early-onset ESRD (16,36). BMI was also evaluated as a result of associations between morbid obesity and secondary FSGS (37) and the relationship among BMI, birth weight, and risk for ESRD (38). Low birth weight has been demonstrated to be a risk factor for adult obesity, hypertension, diabetes, and renal failure.

The NPL regression interaction analysis is based on the entire collection of pedigrees, whereas the OSA attempts to find the subset of pedigrees that maximize the evidence for linkage. Thus, if the evidence for linkage is in a relatively small proportion of the pedigrees (*e.g.*, $< 15\%$) and correlates with a phenotype such as age at ESRD onset, then OSA likely will have more statistical power. However, if the evidence for linkage is not restricted to a small proportion of pedigrees, then the NPL regression interaction analyses will likely have more power. This tends to be the pattern in Tables 3 and 4. The results in these tables are dominated by their consistent findings. Specifically, eight of the 11 OSA results identified in Table 4 are identified in the NPL regression interaction analyses in Table 3. Those that were not are based on 24, 7, and 11% of the pedigrees. That the NPL regression analyses identify more loci is likely due to the increased statistical power when using the entire sample and a locus-specific *P* value; OSA reports a chromosome-wide *P* value. It should also be

stated that the failure of subsetting or interaction analyses to improve the evidence for linkage on chromosomes 1 and 9 is not an inconsistency. It simply reflects that the linkage evidence does not vary by these phenotypes. Alternatively stated, there is no evidence in this sample using these methods that these traits influence the penetrance of putative ESRD genes in the region.

Linkage has been reported in families with autosomal dominant forms of FSGS. Mutations in the α -actinin 4 gene on chromosome 19q13 have been identified as a cause of autosomal dominant FSGS (39), as have regions on chromosomes 1q25–31 (40,41) and 11q21–22 (42). Focal sclerosis may be misdiagnosed as H-ESRD in the absence of renal biopsy material, as it disproportionately affects blacks and can present in a nonnephrotic form with severe hypertension. The linkage peak that we observed on chromosome 1q25.1 at 191.9 cM (LOD 1.62) overlaps with the 1q25–31 region identified as contributing to familial FSGS (40,41). Our nondiabetic ESRD peak marker is proximal to the FSGS haplotype of Tsukaguchi *et al.* (40) (D1S254, 200 cM; D1S222, 210.5 cM), but the LOD-1 interval encompasses the steroid-resistant nephrotic syndrome locus (NPHS2/podocin gene at 195 cM) and overlaps with the Rana *et al.* (41) FSGS haplotype (D1S416, 192 cM; D1S466, 198 cM; D1S240, 200 cM). Future analyses will evaluate podocin and NPHS1 as candidate genes to determine whether they contribute to susceptibility to what is clinically labeled “H-ESRD” in blacks.

We have also performed linkage analyses in black families that contain multiple members who were concordant for type 2 diabetes–associated ESRD (27). These analyses demonstrated suggestive evidence for linkage between markers on chromosomes 3q, 18q, and 10q, broadly consistent with previous reports of linkage between diabetic nephropathy and these regions (22,43,44). We did not detect linkage in these regions for nondiabetic ESRD in blacks. This suggests that genetic susceptibility to the diabetic and nondiabetic forms of nephropathy are mediated by different loci, despite the observed clustering of disparate causes of ESRD in black families. The corresponding LOD scores seen in the black diabetes family set were comparable to those calculated in the nondiabetic families in this report.

It remains unclear why only a minority of essential hypertensive patients develop ESRD and why this illness demonstrates marked racial disparity. Familial clustering of H-ESRD is widely observed throughout the United States. It is clear that the phenotype referred to as “H-ESRD” in blacks includes cases that have multiple causes of renal failure. Some causes (arteriolar nephrosclerosis) are causally related to high BP, and others (global glomerulosclerosis and chronic glomerular diseases) seem to develop independently from hypertension (although hypertension speeds their progression to ESRD). We report the results of linkage analyses in a large number of black families with nondiabetic ESRD. Modest evidence for linkage was detected on chromosomes 9q21.3, 1q25.1, and 13q33.3 in the overall family set. Loci on chromosomes 13q13.1 and 13q33.3 demonstrated consistent evidence for linkage in families with early age at ESRD onset and high BMI, respectively.

These regions will receive priority in our search for the genes that predispose to nondiabetic ESRD in blacks. As with any linkage analysis of this type, the results will require replication in an independent population. Ultimate proof that an ESRD gene lies in these regions will require gene identification. Identification of the genes that cause H-ESRD may help to explain the excessive incidence rates of hypertension-associated kidney disease in blacks and lead to novel therapies to prevent progressive renal disease.

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Appendix: Methods for Linkage Analysis

NPL Regression Analysis

The NPL regression approach is a conditional logistic regression in which the family-specific NPL statistic (*e.g.*, NPL_{pairs}) at one or more loci is the predictor variable. Consider a sample of m independent pedigrees and a chromosomal region with one or more markers and a locus of interest. Let τ_i be the pedigree-specific contribution to the NPL statistic at the locus of interest. The likelihood function for a conditional logistic regression with τ_i as a predictor is

$$Lik(\beta; y_i, \tau) \propto \prod_{i=1}^m \left[\frac{\exp\{y_i \tau_i \beta\}}{1 + \exp\{\tau_i \beta\}} \right].$$

Here, $y_i = 1$ for all i , and β is the conditional logistic regression parameter. It can be shown that the score test from this likelihood is asymptotically equivalent to Whittemore and Halpern’s class of tests (32). Although unaffected individuals can be used to help estimate the possible inheritance vectors for that pedigree, an NPL regression analysis is an “affecteds only” analysis. The primary advantage of the NPL regression approach is that it allows us to evaluate simultaneously, either by joint or conditional tests, the effects of multiple loci (*i.e.*, heterogeneity) and test for interactions among sets of loci (*e.g.*, epistasis). For testing for an interaction between two loci, the two locations and their statistical interaction (*i.e.*, centered cross-product) were included in the model, and the 1 degree of freedom test of the interaction coefficient was computed. We also tested for interactions between the degree of sharing (identity by descent) at a location and (1) the mean age at diagnosis of ESRD, (2) mean age at onset of hypertension, and (3) the mean BMI.

OSA

OSA ranks each family by the family-level value of a covariate of interest and identifies the contiguous subset of

families that maximize the evidence for linkage. In the OSA with the mean age at ESRD diagnosis, each pedigree was ranked from lowest to highest for age at ESRD diagnosis. The family with the lowest mean age at ESRD diagnosis entered into the analysis, and the corresponding LOD score was computed on the target chromosome (e.g., chromosome 1) for that family. Next, a second linkage analysis on the target chromosome 1 was computed combining the two families with the two lowest mean ages at ESRD diagnosis values. The *i*th OSA analysis proceeds by computing a linkage analysis on the target chromosome using the subset of families with the *i*th lowest mean ages at ESRD diagnosis. This process is repeated until all families have been added to the linkage analysis. The subset of families that yield the largest LOD score on the target chromosome is taken as the LOD score of interest. The location that maximizes the LOD score on a chromosome will vary as the subset of families analyzed changes. The statistical significance of the change in the LOD score was evaluated by a permutation test under the null hypothesis that the ranking of the covariate is independent of the family's LOD score on the target chromosome. Thus, the families were permuted randomly with respect to the covariate ranking, and an analysis proceeded as above for each permutation of these data. The resulting empirical distribution of the change in the LOD scores yielded a chromosome-specific *P* value. In this example, the family-level means were ranked in ascending order. However, we also repeated the analysis ranking in descending order.

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