Associations Between Hypertension and Genes in the Renin-Angiotensin System

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Abstract—The genes of the renin-angiotensin system have been subjected to intense molecular scrutiny in cardiovascular disease studies, but their contribution to risk is still uncertain. In this study, we sampled 192 African American and 153 European American families (602 and 608 individuals, respectively) to evaluate the contribution of variations in genes that encode renin-angiotensin system components of susceptibility to hypertension. We genotyped 25 single-nucleotide polymorphisms in the renin-angiotensin system genes *ACE*, *AGT*, *AGTR1*, and *REN*. The family-based transmission/disequilibrium test was performed with each single-nucleotide polymorphism and with the multilocus haplotypes. Two individual single-nucleotide polymorphisms were significantly associated with hypertension among African Americans, and this result persisted when both groups were combined. The associations were confirmed in haplotype analysis for *REN*, *AGTR1*, and *ACE* in African Americans. Consistent but less significant evidence was found in European Americans. We also randomly sampled unrelated individuals across families to obtain 84 cases and 108 controls among the African Americans and 41 cases and 113 controls in the European Americans. Single-nucleotide polymorphism and haplotype analyses again showed consistent, albeit weaker, results. Thus, in this biracial population sample, we find evidence that interindividual variation in the renin-angiotensin system genes contributes to hypertension risk. **(*Hypertension*. 2003;41:1027-1034.)**

Key Words: hypertension, genetic ■ angiotensin-converting enzyme ■ haplotypes ■ angiotensin ■ renin-angiotensin system ■ case-control studies

The renin-angiotensin system (RAS) plays a key role in the regulation of blood pressure (BP). Genes that encode components of the RAS are in turn thought to play a role in determining genetic susceptibility to hypertension and have been intensively scrutinized. Up to the present at least, consistent associations have been difficult to demonstrate. We sought to reexamine this question in a large, biracial population–based sample of families. We focused on the 4 primary genes of the RAS: angiotensinogen (*AGT*), renin (*REN*), angiotensin I–converting enzyme (*ACE*), and the angiotensin II receptor, subtype 1 (*AGTR1*). The *AGT* gene encodes the precursor protein angiotensinogen. An *AGT* variant (C4072T) has been associated with hypertension in several European and Japanese studies but not in African Americans.[1–4] Renin cleaves angiotensinogen to form angiotensin I in the rate-limiting step in the tightly regulated cascade that produces angiotensin II. Evidence for a contribution of *REN* variants to hypertension is limited.[5–7] Statistically significant associations between *REN* alleles and essential hypertension have been reported in 2 independent studies: one examined an ethnically homogeneous population in the United Arab Emirates and selected individuals without a history of cigarette smoking or alcohol consumption, whereas the second[8] was based on a sample of US whites that was also studied for hypercholesterolemia. Angiotensin I–converting enzyme, ACE, cleaves angiotensin I to angiotensin II, a peptide hormone that stimulates aldosterone secretion and through its effects on the heart, kidneys, and vessels elevates arterial BP. The level of plasma and intracellular ACE is modulated by common polymorphisms of the gene.[9,10] Associations between an insertion/deletion polymorphism in the *ACE* gene and BP have been reported,[11–13] although conflicting evidence exists.[14–16] Two large, population-based studies found marginally significant linkage and association between BP and the insertion/deletion polymorphism that was restricted to males.[17,18] A genome scan for BP from the Framingham Heart Study found strong evidence for a quantitative trait locus on chromosome 17, close to the *ACE* gene.[19] AGTR1 mediates the effect of angiotensin II by increasing intracellular calcium concentration and protein

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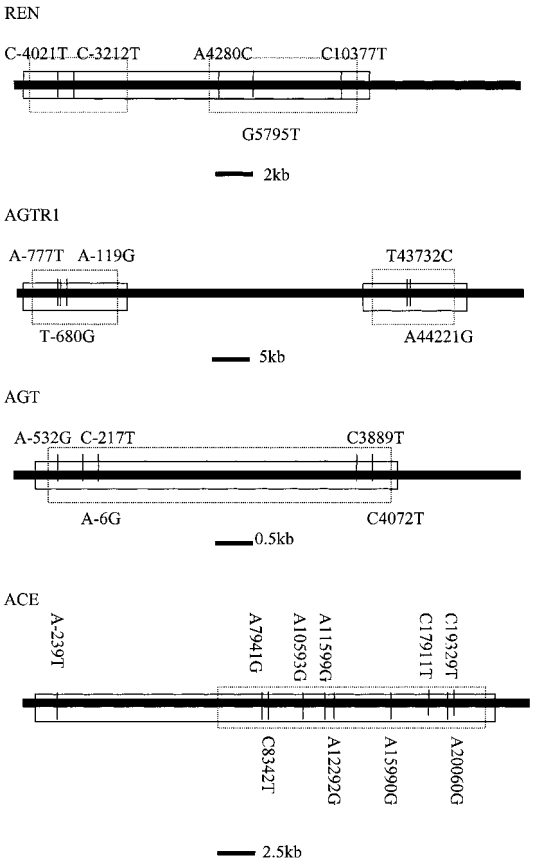
phosphorylation in target cells. *AGTR1* variants are associated with hypertension in a Finnish study,[20] but no association was observed in African Americans.[21,22]

The inconsistencies observed in RAS gene-association studies demonstrate the challenges of dissecting complex multifactorial traits like hypertension by single-locus analysis. Inconsistencies might arise from the inadequate power of small sample sizes, population structure, varying effects of several disease-predisposing variants, gene-environment interactions, or poor study design. Each of these factors can hinder the detection of a modest contribution of an individual locus to a trait such as hypertension. Overall, the role of RAS gene variants in hypertension remains unclear, and a more thorough understanding of the patterns of linkage disequilibrium within RAS candidate genes is necessary. Haplotype-based analysis, which considers all of the variants segregating at these loci, can provide additional precision in studies of complex disease. However, accommodating all genetic and nongenetic information in the analytic process is still a formidable challenge.

Methods

Participants in this study were enrolled in the GenNet component of the National Heart, Lung, and Blood Institute–sponsored Family Blood Pressure Program;[23] the design and sampling procedures have been described previously.[24] In brief, sibships including persons between the ages of 25 to 40 years were enrolled if their systolic (SBP) or diastolic (DBP) BPs were in the upper 25th and 15th percentiles for African Americans and European Americans, respectively. Controls were defined as those sibs without treatment and with an SBP $<$125 mm Hg and a DBP $<$75 mm Hg. African Americans were recruited from Maywood, Ill, whereas European Americans were enrolled in Tecumseh, Mich. The protocols were reviewed and approved by the review boards of the respective institutions. Single-nucleotide polymorphism (SNP) genotypes were determined for 602 African American individuals (192 families) and 606 white individuals (153 families) by using the TaqMan assay (ABI) as previously described.[25] The locations of the SNPs in and around *REN*, *AGT*, *AGTR1*, and *ACE* genes used in this study are shown in the Figure.



Polymorphic markers genotyped in *REN*, *AGTR1*, *AGT*, and *ACE*, corresponding to the genomic region, covers 14, 45, 4.5, and 21 kb, respectively. The vertical line indicates the position of a genotyped single-nucleotide polymorphism. Solid and dashed boxes indicate the haplotype blocks for European Americans and African Americans, respectively.[25]

Statistical Analyses

Descriptive statistics were obtained with SAS software (SAS Institute). Because antihypertensive medications are sometime prescribed to nonhypertensive individuals, hypertension was thus defined as treated individuals with an SBP$>$120 mm Hg or a DBP $>$70 mm Hg or persons not under treatment but with both SBP $>$140 mm Hg and DBP $>$90 mm Hg. Cases and controls were randomly selected from siblings within a family, with 1 sib sampled from each family. Because hypertensive individuals are, on average, older than normotensive individuals in this study, the oldest unaffected sib in a family was selected as the control. Hardy-Weinberg equilibrium (HWE) at each SNP was assessed by the χ^2 test with one degree of freedom[26] in cases and controls separately. Single-locus tests of association between an SNP and hypertension were performed with a standard contingency χ^2 test for a case-control design. We estimated haplotype frequencies by the maximum-likelihood method from genotype data through the use of the expectation-maximization (E-M) algorithm under the assumption of HWE.[27–30] A likelihood ratio (LR) statistic was computed by testing equality of the allele or haplotype frequency for cases and controls treated separately versus combined. The null distribution of the LR was calculated by randomizing case and control status in our samples.[31] We also compared individual haplotype frequencies between the cases and controls by χ^2 statistics from a series of simple 2$\times$2 contingency tables by combining all other haplotypes and assessed the probability value with the permutation test.

Our data are drawn from nuclear families in which many parents have missing genotypes and multiple affected or unaffected siblings are genotyped. By selecting 1 sib per family in our case-control analysis, substantial power is sacrificed in this sampling scheme. Because case-control analysis is subject to population stratification, we used the transmission/disequilibrium test (TDT) for further analysis. The TDT statistic proposed by Clayton[32] and used by the computer program TRANSMIT is therefore suitable. This method decomposes the full likelihood of the trio of parent and siblings ascertained by the affected offspring into 2 parts: one that depends on population stratification and one that does not. The test of no linkage or association is based on a partial score function that omits the terms most influenced by hidden population stratification. This approach handles missing parental genotype information by using the genotypes from unaffected sibs to infer the genotypes of missing parental genotypes, and it allows for multiple affected sibs. The significance level, indicated by *P* values, is assessed by a bootstrap procedure based on 10 000 replicates. For multiple polymorphisms, TRANSMIT also reconstructs haplotypes with an E-M algorithm and tests individual haplotype as well as the overall transmission distortion. Cervino and Hill[33] used simulations to show that TRANSMIT is robust against population stratification and is more powerful than other TDT approaches, such as STDT[34] and RCTDT.[35] To further verify the robustness of TRANSMIT to population stratification in our samples, we tested linkage and association in 100 microsatellite markers with an average intermarker distance of 35 cM extracted from a previous genome scan. The number of significant tests can be

TABLE 1. Demographic Features of the Samples

Features	African Americans	European Americans
Gender, M/F	242/360	287/321
Age, y	40.5±11.7	45±14
Body mass index, kg/m^2	30.2±8.3	29.2±6.1
Antihypertensive medication		
SBP, mm Hg	142.7±23.6 (141)	137.7±17.2 (151)
DBP, mm Hg	86.3±14.8 (141)	75.3±10.8 (151)
Not medicated		
SBP, mm Hg	122.2±17.1 (453)	117.9±16.1 (455)
DBP, mm Hg	75.6±12.8 (453)	68.6±10 (455)
Hypertension, %*	27.4	24.7
No. of pedigrees	192	153
No. of hypertensive offspring	110	56

*Hypertension is defined as individuals on antihypertensive medication and either SBP>120 or DBP >70, or nonmedicated individuals with both SBP>140 and DBP >90.

considered a guide to the probability that TRANSMIT protects us against population stratification. We also performed TRANSMIT by pooling African Americans and European Americans together. To eliminate the possible inflation of a type I error due to the effect of pooling the 2 different samples, we calculated the *P* value in this way. We retained the pedigree structure and the individual's affected status and simulated the genotypes according to the actual allele frequencies in African American and European American samples separately. We then merged the 2 samples to calculate the test statistic. The *P* values for the pooled sample are calculated on the basis of 2000 replications.

Analysis with haplotypes of multiple loci can be more powerful than associations based on a single locus. Recent studies that examined the structure of the human genome have documented that linkage disequilibrium is nonrandom and that most of the genome lies in blocks where 3 to 5 haplotypes account for >80% of the segregating variation.[36–38] In our recent study of linkage disequilibrium of RAS genes, we identified similar patterns.[25] Accordingly, we designed a test of the hypothesis that common variants found in the RAS genes contribute to susceptibility to hypertension and relied on the information provided by these haplotype blocks.

Results

Table 1 describes the demographic characteristics of the participants. The average SBPs and DBPs of individuals treated with antihypertensive medication were significantly higher than those of subjects under no treatment ($P<0.0001$). Applying our definition of a hypertensive case yielded 110 and 56 hypertensive sibs among African Americans and European Americans, respectively.

Case-Control Analysis

We selected cases and controls as follows. When there were hypertensive sibs in a family, we randomly selected 1 sib per family as a case, and this family was then excluded from control selection. We then selected 1 sib as a control per family from the remaining families. Because hypertensives were generally older than normotensives, we selected the oldest qualified sib in a control family as a control. Table 2 presents the characteristics of our selected cases and controls. The distributions of body mass index, SBP, and DBP did not change as a result of selecting another sib as a case from families with >2 hypertensive sibs (data not shown). We then carried out HWE tests for all loci among cases and controls separately. After multiple corrections, only *AGTR1* A44221G significantly deviated from HWE ($P=0.0075$ after correction) among the African American hypertensives. We next compared the genotype frequencies between cases and controls for each locus. This analysis indicated that *AGTR1* C43732T and A44221G in African Americans and *ACE* A-239T and *AGT* C4072T in European American were associated with hypertension at a nominal level of significance ($P<0.05$).

Single-Locus TDT Analysis

Table 3 summarizes the results of testing for associations for the 25 SNPs among African Americans and European Americans separately and in the combined sample. The Obs# column represents the observed number of transmissions from both homozygous and heterozygous parents to the affected offspring, and the Exp# column represents the number of transmissions that would be expected under the null hypothesis of no association. Under the null hypothesis, the score test statistic follows a χ^2 distribution with one degree of freedom, but we report a simulated probability value based on 10 000 replications.

We observed that allele *T* at REN C4021T ($P=0.0006$) and *G* at A44221G ($P=0.0006$) for *AGTR1* were transmitted to the affected offspring significantly more often than expected (Table 3) in the African Americans. The other loci with nominal transmission distortion were *REN* C-3212T, *AGTR1* C43732T, and *ACE* A-239T in African Americans ($P<0.05$). In European Americans, *ACE* C8342T, A10593G, A11599G, G12292G, and C19329T were observed with nominal trans-

TABLE 2. Demographic Characteristics for Subjects Randomly Selected From Each Pedigree*

Phenotype	Male	Female	Age, y	BMI, kg/m^2	SBP, mm Hg	DBP, mm Hg
African Americans						
Hypertensive	36	48	41.4±8.0	33.1±9.5	148.8±21.7	93.9±14.9
Normotensive	27	59	34.8±6.0	28.3±7.4	109±9.7	65.8±6.7
European Americans						
Hypertensive	22	19	39.8±6.4	33.1±6.3	135.7±15.9	79.6±8.8
Normotensive	39	68	36.4±7.4	26.8±6.1	107.8±8.4	63.6±6.6

*Only one offspring is selected per pedigree.

TABLE 3. Association Between Hypertension and RAS SNPs: Summary of TDT Results for Single SNP

			African Americans					European Americans					Pooled				
SNP	Allele	Transmitted Allele	Freq	Obs#	Exp#	TDT	*P**	Freq	Obs#	Exp#	TDT	*P**	Freq	Obs#	Exp#	TDT	*P***
REN																	
−4021	C/T	T	0.272	71	59.3	11.31	**0.0006**	0.130	10	13.2	1.98	0.163	0.213	81	69.3	7.58	**0.019**
−3212	C/T	T	0.142	37	31.2	4.02	**0.032**	0.086	5	6.7	0.98	0.439	0.119	42	36.7	2.37	0.115
4280	A/C	A	0.697	151	150.7	0.01	0.949	0.876	100	98.8	0.27	0.560	0.771	251	253.5	0.32	0.699
5795	G/T	T	0.716	164	161.1	0.74	0.429	0.740	80	77.6	0.75	0.302	0.726	244	239.1	1.21	0.281
10377	C/T	T	0.164	40	38.7	0.17	0.693	0.128	10	11.3	0.40	0.446	0.149	50	49.2	0.04	0.831
AGTR1																	
−777	A/T	T	0.719	141	140.4	0.04	0.840	0.838	60	59.9	0.00	0.956	0.758	201	201.8	0.04	0.862
−680	G/T	G	0.259	61	60	0.07	0.765	0.167	16	17	0.13	0.721	0.221	77	75.2	0.14	0.733
−119	A/G	G	0.265	64	62.1	0.24	0.560	0.180	17	18.1	0.16	0.684	0.230	81	78.5	0.27	0.625
43732	C/T	C	0.782	187	181.1	3.76	**0.049**	0.525	62	62.8	0.06	0.821	0.675	249	239.1	4.95	0.107
44221	A/G	G	0.304	86	74.8	8.20	**0.0006**	0.055	2	2.3	0.14	0.657	0.199	88	71	16.0	**0.004**
AGT																	
−532	C/T	T	0.132	30	28.4	0.31	0.466	0.129	13	13.7	0.10	0.731	0.131	43	42.1	0.06	0.804
−217	A/G	G	0.726	162	161.5	0.02	0.893	0.869	99	96.8	0.84	0.344	0.785	261	261.3	0.01	0.940
−6	A/G	G	0.139	32	28.7	1.60	0.231	0.542	61	62.4	0.14	0.685	0.302	93	98.1	1.22	0.685
3889	C/T	C	0.918	205	201.2	2.71	0.120	0.884	97	96.1	0.17	0.630	0.904	302	296.7	2.67	0.118
4072	C/T	C	0.842	84	83.8	0.01	0.906	0.490	36	36.2	0.01	0.934	0.647	120	116.9	0.73	0.715
ACE																	
−239	A/T	A	0.672	157	148.4	4.93	**0.017**	0.596	54	60.2	3.47	0.056	0.641	211	207.2	0.56	0.484
7941	A/G	G	0.217	51	48.3	0.54	0.380	0.040	5	4.7	0.22	0.383	0.143	56	49.6	2.75	0.283
8342	C/T	T	0.606	146	140.2	2.35	0.131	0.548	71	64.6	3.98	**0.028**	0.582	217	203.7	7.09	**0.008**
10593	A/G	A	0.750	173	170.5	0.67	0.382	0.459	41	47.4	3.97	**0.027**	0.630	214	212.3	0.14	0.828
11599	A/G	A	0.753	176	172.4	1.33	0.221	0.459	39	45.8	4.59	**0.016**	0.635	215	212.8	0.25	0.809
12292	A/G	G	0.624	148	144.3	0.96	0.352	0.547	71	64.6	4.00	**0.025**	0.592	219	207.4	5.46	**0.027**
15990	A/G	A	0.631	152	146.5	2.11	0.138	0.541	70	64.4	3.13	0.088	0.594	222	209.2	6.74	**0.018**
17911	C/T	C	0.579	126	125.7	0.01	0.943	0.452	42	47.6	3.07	0.085	0.473	168	165.2	0.32	0.601
19329	C/T	T	0.453	103	102.3	0.03	0.858	0.536	69	62.8	3.86	**0.050**	0.487	172	166.8	1.12	0.326
20060	A/G	A	0.569	123	118.3	1.75	0.155	0.455	42	47.7	3.18	0.085	0.521	165	163.6	0.08	0.793

TDT indicates transmission/disequilibrium test statistic; Obs#, observed number of transmissions from both homozygous and heterozygous parents to the affected offspring; Exp#, number of transmissions that would be expected under the null hypothesis of no association; freq, frequency. Bold numbers indicate statistical significance at 0.05.

*Based on 10 000 bootstrap samples.

**Based on 2000 replications as described in Methods.

mission distortions ($P<0.05$). Interestingly, some polymorphisms have different transmission distortion in the African Americans and European Americans. For example, the *A* allele of *ACE* A-239T is more often transmitted to affected offspring in the African American families but not among the European Americans. In the combined sample, *REN* C4021T, *AGTR1* A44221G, and *ACE* C8342T, G12292G, and A15990G showed evidence of transmission distortion ($P<0.05$).

Haplotype Analysis

In a previous study,[25] we determined the haplotype structure of the 4 RAS genes. To summarize those findings, SNPs that demonstrate significant linkage disequilibrium with each other are grouped to construct haplotypes, and these haplotype blocks, or linkage disequilibrium domains, are boxed and illustrated in the Figure. A single functional mutation existing in a candidate gene would fall within 1 of these blocks. Thus, haplotypes in the block could explain most of the variation attributable to functional alleles.

Case-Control Haplotype Analysis

Haplotype frequencies for the selected SNPs in a block were estimated with an E-M algorithm for cases and controls separately. Haplotype frequencies within a block were compared between cases and controls by the χ^2 square test. The overall haplotype distributions between cases and controls were also compared by the LR test. Significance levels (*P* values) were obtained by permuting cases and controls. Only the block consisting of C43732T and A44221G in

AGTR1 was significantly associated with hypertension ($P=0.005$).

Haplotype TDT Analysis

We next applied the program TRANSMIT to reconstruct haplotypes and test the association between a haplotype block and hypertension from family data. Table 4 summarizes the results of testing linkage and association between a haplotype block and hypertension. We first report the results for African Americans. In the block consisting of C-4021T and C-3212T in *REN*, haplotype *CC* is transmitted to the affected offspring significantly less often than expected ($P=0.0004$), whereas *TC* and *TT* are transmitted more often ($P<0.03$). A global test also suggests that this block is associated with hypertension ($P=0.0025$). For the block consisting of C43732G and A44221G in *AGTR1*, haplotype *CG* is overtransmitted to the hypertensive offspring. The permutation test shows that this discrepancy is highly significant ($P=0.0002$), and a global test adjusting for the multiple haplotypes is confirmatory ($P=0.0038$). For *ACE*, the haplotype consisting of A11599G, A15990G, and A20060G represents the major haplotypes in this block, after excluding A-239T;[25] these 3 SNPs also individually show a significant result in the TDT analysis. Considering the haplotypes consisting of these 3 SNPs, only haplotype *AAA* was transmitted more often than expected under the null hypothesis ($P=0.006$); the global test was not significant. When we added A-239T, however, the significance level was improved ($P=0.0002$), and a significant global test was also observed ($P=0.047$).

In our smaller sample of European Americans, similar TDT analysis was performed with the haplotypes. Significant transmission distortion was found in *REN* and *ACE*. In *REN*, haplotype *CCTC* was overtransmitted to hypertensives ($P=0.01$), whereas for *ACE*, haplotype *GGG* consisting of A10593G, A11599G, and A12292G is overtransmitted. We also observed that the *P* value for haplotype *TCTC* is very small, but the corresponding haplotype frequency is too small to consider this *P* value statistically significant. Global tests show that both genes are associated with hypertension. We did not find any significant association between haplotype and hypertension status in either *AGT* or *AGTR1*.

We then performed TDT analyses with TRANSMIT by using 100 microsatellite markers randomly selected from our previous genome-wide scan.[24] Only 4 of the 100 markers were in transmission distortion ($P<0.05$) in African Americans and none in European Americans. Therefore, the type I error of the TDT analysis with TRANSMIT is reasonably well controlled when analyses are performed in African Americans and European Americans separately.

Finally, we performed the TDT analysis in the combined samples from both samples, but we obtained the probability values by simulating the genotypes separately in both samples and retaining the pedigree structures and phenotypes. The haplotype blocks in African Americans were shorter and consisted of a subset of the SNPs in European Americans; thus, we performed analyses based on the haplotype structure found in African Americans. Again, the results show significant evidence of linkage and association between hypertension and *REN* and *AGTR1* (global $P<0.05$) and marginal evidence between hypertension and *ACE* (global *P* value$=0.067$).

Discussion

Studies of associations between DNA variants and disease have been widely used to identify genomic regions or candidate genes that contribute to disease. A major limitation of association studies is the potential for false-positive findings, given the multiple tests required by the large number of comparisons.[39] Careful selection, or use of family-based controls, reduces the risk of a type I error. When the haplotype structure of a targeted region is known, comparisons of the frequency of major haplotypes can identify specific haplotypes that play important roles in disease. This approach is based on the hypothesis that common variants are responsible for susceptibility to common diseases.[40,41] Consistent results across different populations or in independent studies further strengthen the evidence found in individual association studies.

On the basis of this reasoning, we performed case-control and family-based TDT analyses between *RAS* genes and hypertension in African Americans and European Americans. In the single-locus analysis with unrelated individuals as controls, we identified only A44221G of *AGTR1* as being associated with hypertension in African Americans, with the *G* allele increasing the risk of hypertension. The odds ratio associated with *G* was 2.41 (95% confidence interval, 1.26–4.63). In the single-locus family-based TDT analysis, A44221G in *AGTR1* remained strongly significant, with the *G* allele being transmitted to hypertensive offspring more often than expected in African Americans. This result is consistent with the analysis in unrelated case-controls, but the controls in TDT are different from the unrelated controls. A44221G also significantly deviated from HWE in cases. A deviation from HWE among affected individuals implies disease heterogeneity and marker-disease linkage disequilibrium.[42,43] Therefore, A44221G, located on exon 5 of the gene, could be an important polymorphism associated with hypertension. Although the A44221G SNP does not alter protein structure (proline→proline), it might alter the function of a nearby regulatory element or be in linkage equilibrium with another causative variant that is directly involved in hypertension susceptibility. We had very little power to detect linkage and association for this marker in European Americans. The *T* allele of C-4021T in *REN* was also transmitted more often than expected in African Americans but not in European Americans, and this might also have been due to the small sample size of European Americans. The combined European American and African American results demonstrate significant linkage and association between hypertension and *REN* C-4021T, *AGTR1* A44221G, and *ACE* C8342T, A12292G, and A15990G.

We next performed haplotype analysis based on blocks previously defined by Zhu et al.[25] Because of limited haplotype diversity in each haplotype block, we were able to restrict tests to the major haplotypes, which reduced the number of comparisons. We first compared the haplotype frequencies between cases and controls. The permutation test showed that the haplotype frequencies in cases were signifi-

TABLE 4. Association Between Hypertension and RAS SNPs: Summary of Significant TDT Results for Haplotypes Within RAS Genes

SNPs	Haplotype	Freq of Haplotype	O(T)	E(T)	χ^2	*P**	Global Test *P**
African Americans							
Renin							
−4021−−3212	CC	0.729	151	163	11.33	**0.0004**	
	TC	0.129	34	27.7	5.6	**0.021**	
	TT	0.142	37	31.2	3.9	**0.03**	**0.0025**
AGTR1							
43732–44221	CA	0.481	101	107.4	2.17	0.104	
	TA	0.217	35	40.3	2.96	0.078	
	CG	0.302	86	74.2	8.49	**0.0002**	**0.0038**
ACE							
11599–15990–20060	AAA	0.233	67.1	58.9	4.4	**0.006**	
	AGA	0.339	65.3	72.0	3.2	0.056	
	AAG	0.154	39.2	38.4	0.07	0.736	
	GAG	0.242	48.8	51.5	0.75	0.388	
	AGG	0.028	6.5	6.5	0.01	0.906	0.138
−239–11599–15990–20060	AAAA	0.174	55	46.8	5.01	**0.0002**	
	TAAA	0.06	11.3	13.1	1.03	0.11	
	AAGA	0.17	32.8	34.8	0.56	0.426	
	TAGA	0.17	32.2	36.5	1.89	**0.043**	
	AAAG	0.117	33.2	29.4	2.04	**0.049**	
	TAAG	0.035	5.9	8.0	2.46	**0.011**	
	AGAG	0.18	35.5	35.6	0.0	0.982	
	TGAG	0.062	12.1	14.6	2.49	0.116	
	AAGG	0.022	4.4	3.5	1.48	0.108	**0.047**
European Americans							
Renin							
−4021− −3212–5795–10377	CCGC	0.257	30	32.5	0.81	0.354	
	CCTC	0.497	61.4	54.1	3.97	**0.01**	
	TCTC	0.038	3	5.8	2.83	0.0065	
	TTTC	0.076	4.6	6.4	1.24	0.224	
	CCTT	0.115	8.6	9.9	0.49	0.26	**0.032**
ACE							
10593–11599–12292	AAA	0.451	39	45.9	4.76	**0.0025**	
	GGG	0.540	71	64.1	4.84	**0.0021**	**0.0023**
−239–11599–15990–20060	AAGA	0.441	37	44	4.41	**0.021**	
	AGAG	0.142	14	13	0.19	0.675	
	TGAG	0.394	56	50.3	3.0	**0.05**	0.144
Pooled*							
Renin							
−4021−−3212	CC	0.788	253	264.6	7.15	**0.019**	
	TC	0.094	39	32.7	3.73	0.089	
	TT	0.118	42	36.8	2.35	0.137	**0.045**
AGTR1							
43732–44221	CA	0.478	161	168.8	1.99	0.177	
	TA	0.325	85	94.1	4.08	0.147	
	CG	0.198	88	71.1	15.3	**0.003**	**0.006**
ACE							
−239–11599–15990–0060	AAAA	0.099	53.5	42.5	8.72	**0.025**	
	TAAA	0.038	12.2	12.4	0.02	0.898	
	AAGA	0.294	73.3	87.1	10.55	**0.019**	
	TAGA	0.094	31.4	32.3	0.08	0.853	
	AAAG	0.072	33.0	27.3	4.17	0.086	
	TAAG	0.022	6.5	7.6	0.74	0.417	
	AGAG	0.160	47.9	46.9	0.08	0.784	
	TGAG	0.199	70.0	71.9	0.241	0.835	0.066

Haplotypes were tested in each block. Only the minimum SNPs required to represent most haplotypes in a block were used.
**P* values of pooled sample are based on 2000 replications as described in Methods.

cantly different from controls only for a block consisting of C43732T and A44221G of *AGTR1* in African Americans. However, a strong association between haplotype blocks and hypertension was found when haplotype TDT analysis with this block information was performed. In African Americans, *REN*, *AGTR1*, and *ACE* showed significant evidence of association with hypertension. *REN* and *ACE* genes also showed significant evidence in European Americans. No significant evidence for a role of the *AGTR1* gene was found in European Americans, perhaps due to the small sample size and the rarity of this haplotype contributing to hypertension. Pooling African Americans and European Americans also resulted in significant associations between *RAS* genes and hypertension.

In the single-locus TDT analysis, we found different transmission distortions associated with the *ACE* A-239T polymorphisms in African Americans and European Americans. This inconsistent result can be explained in the haplotype analysis. From the pooled result in Table 4, it is apparent that haplotype *AAAA* of the *ACE* gene is present primarily among African Americans because its observed count in the pooled data is almost equal to that in African Americans (53.5 vs 55). Haplotype *AAAA* is overtransmitted to affected offspring. Therefore, the overtransmission of allele *A* of A-239T to affected offspring in African Americans is due to the overtransmission of haplotype *AAAA*. Haplotype *AAGA* is contributed by both African Americans and European Americans and is undertransmitted to affected offspring. Hence, the undertransmission of allele *A* of A-239T to affected offspring in European Americans is due to the undertransmission of haplotype *AAGA*. This observation further supports the contention that the different associations observed in the single-marker analyses in the 2 populations might be attributable to inheritance patterns of different haplotypes on which the marker resides.

The family-based TDT method is apparently more powerful than the unrelated case-control analysis in our data sets. First, family-based TDT uses all affected sibs, but only 1 affected sib was selected in the unrelated case-control analysis. The sample size of a family-based TDT is therefore larger than that of unrelated case-control analysis. Second, family-based TDT uses untransmitted alleles as controls; environmental factors are therefore better matched between cases and controls. In comparison, unrelated case-control analysis might be inadequately matched for factors such as age, body mass index, and other important factors associated with hypertension.

Perspectives

Our findings suggest that using haplotype blocks can reduce inconsistencies observed in single-marker analysis. When we compared the frequency of a single marker in the cases and controls, we did not have sufficient power to detect genetic associations, with the exception of the polymorphism A44221G in *AGTR1*. However, using haplotype blocks dramatically improved the power to detect a disease susceptibility region. With access to very large numbers of SNPs[44,45] and improved understanding of the haplotype structure and linkage disequilibrium patterns of the genome,[36–38,46] haplotype-based association analysis, ranging from the whole genome to large sets of candidate genes, should greatly aid in the dissection and characterization of the genetic basis of common diseases.

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