Hypertension Genes Are Genetic Markers for Insulin Sensitivity and Resistance

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Abstract—Insulin resistance is a determinant of blood pressure variation and risk factor for hypertension. Because insulin resistance and blood pressure cosegregate in Mexican American families, we thus investigated the association between variations in 9 previously reported hypertension genes (ACE, AGT, AGTRI, ADD1, NPPA, ADDRB2, SCNN1A, GNB3, and NOS3) and insulin resistance. Families were ascertained via a coronary artery disease proband in the Mexican American Coronary Artery Disease Project. Individuals from 100 Mexican American families (n=656) were genotyped for 14 polymorphisms in the 9 genes and all adult offspring and offspring spouses were phenotyped for insulin sensitivity by hyperinsulinemic euglycemic clamp (n=449). AGT M235T and NOS3 A(−922)G and E298D polymorphisms were significantly associated with insulin sensitivity (P=0.018, 0.036, 0.039) but were not significant after adjusting for body mass index. ADD1 G460W was associated with insulin sensitivity only after adjusting for body mass index. The NPPA T2238C and SCNN1A A663T were associated with decreased fasting insulin levels after adjusting for body mass index (P=0.015 and 0.028). In conclusion, AGT, NOS3, NPPA, ADRB2, ADD1, and SCNN1A may well be genetic markers for insulin resistance, and adiposity was a potential modifier for only some gene/trait combinations. Our data support the hypothesis that genes in the blood pressure pathway may play a role in insulin resistance in Mexican Americans. (Hypertension. 2005;45[part 2]:799-803.)

Key Words: genetics ■ hypertension ■ insulin resistance ■ risk factors

Nearly 50 million individuals in the United States, ≈20% of the adult population aged 18 to 74 years of age, are hypertensive. This hypertension (HTN) is not benign; it is strongly associated with the development of atherosclerosis and left ventricular hypertrophy resulting in congestive heart failure, stroke, and age-related macular degeneration.1–2 The risk of complications increases with increasing blood pressure (BP), particularly systolic blood pressure (SBP).3,4 High BP is also a major antecedent for renal failure and, after diabetes, is the second most common cause of chronic renal failure in the adult population.1,5

Insulin resistance (IR) is a potentially important intermediate phenotype for HTN in certain populations, particularly in Mexican Americans (MAs). Cross-sectional studies have revealed correlations between IR and BP in this and other ethnic groups.6–8 Hyperinsulinemia preceded and predicted the development of HTN in an 8-year prospective study of MAs in San Antonio, Texas6 and has exhibited major gene effects in at least 3 ethnic groups.9–11 By path analysis, we have shown that BP and IR cosegregate and have a significant genetic component independent of any relationship to body mass index (BMI).12 This cosegregation was further confirmed in our genome scan linkage analysis, wherein we identified several regions of coincident linkage of insulin sensitivity and BP.13 For example, fasting insulin, SBP, and mean arterial blood pressure were all mapped to the same region on chromosome 7 (112 to 128 cM). Considered together, these findings provide strong support for the concept that IR is an intermediate and genetically regulated phenotype for HTN.

Although the aforementioned data may suggest that IR is a proximate cause of HTN, an alternative possibility is that both are pleiotropic manifestations of the same underlying susceptibility. In support of this latter hypothesis has been the observation that several large scale clinical trials of pharmacological agents whose targets are the renin-angiotensin system, namely angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers, have revealed a decreased frequency of new-onset cases of type 2 diabetes. These data raise the possibility that genes with recognized roles in BP regulation might have additional roles in the biologic pathways that result in IR.
A number of genes have been associated with HTN, including dipeptidyl carboxypeptidase 1 (DCPI or ACE), angiotensigenin (AGT), angiotensin II receptor type 1 (AGTR1), adducin 1 (ADD1), natriuretic peptide precursor A (NPPA), adrenergic receptor, B-2 (ADRB2), sodium channel, nonvoltage-gated 1 alpha (SCN1A), guanine nucleotide binding protein (G protein) beta polypeptide 3 (GNB3), and nitric oxide synthase 3 (NOS3). Here we investigate the correlation between BP and IR related traits, and evaluate the association between single nucleotide polymorphisms (SNPs) in these previously reported HTN genes and IR related traits. A positive association between HTN genes and IR-related traits would support a genetic basis for the cosegregation of BP and IR, and this may further disentangle the cause–effect pathway between those genes, HTN, and IR.

Methods

Subjects

The subjects studied were recruited through the Mexican American Coronary Artery Disease (MACAD) Project, an ongoing study of coronary artery disease and IR in MAs.14 Family samples were ascertained through a proband with documented coronary artery disease.14,15 Two generations were enrolled into the study: the proband and proband spouses (parental generation), and their adult (age 18 years or older) offspring and the spouses of those offspring (offspring generation). The offspring were free of diabetes and clinically manifest coronary artery disease, thus avoiding secondary changes in phenotype caused by overt disease. The data reported here are from the offspring generation, because they underwent detailed phenotyping for IR assessment. The study was approved by institutional review boards at UCLA and Cedars-Sinai Medical Center. All subjects gave informed consent before participation.

Genotyping

In this study, 14 polymorphisms in the 9 HTN-related genes were genotyped in 656 individuals from 100 MA families. EBV-transformed lymphoblastoid cell lines were established on each subject and DNA was isolated using standard methods.16 Alleles were determined by: (1) multiplex polymerase chain reaction with biotin labeling; (2) hybridizing to an array of sequence-specific oligonucleotide probes immobilized on nylon; and (3) visualizing with streptavidin-horseradish peroxidase conjugate (Roche Diagnostic Systems).17,18 Genotyping was performed as described in Hoppe et al.19

Phenotyping

All subjects underwent a basic clinical assessment, including age, sex, weight, height, BP, and standard biochemical measures such as fasting lipids, glucose, and insulin.

SBP and diastolic BP were measured in the sitting position using a Dinamap system (Critikon, Inc) after subjects had been sitting with legs dangling for >5 minutes. Three BP readings were taken at 5-minute intervals and were averaged for analysis. Width of the BP cuff was ≥80% of the arm circumference in each subject. The adult offspring and their spouses (n = 449) underwent a 3-day phenotyping protocol, which included indices of IR determined by euglycemic clamp. Several indices of insulin sensitivity were obtained, including fasting insulin, glucose infusion rate (GINF) over the last 30 minutes of steady-state insulin and glucose concentrations, and the insulin sensitivity index (SI), obtained by dividing GINF by body surface area and the increment in plasma insulin from baseline to steady state.14

Statistical Analysis

To study the relationship between BP and IR-related traits, the Pearson correlation coefficient was calculated using SAS and probability values were obtained via generalized estimating equations methods as implemented in the GENMOD procedure in SAS to account for familial correlations.20 Appropriate transformation was used when necessary for each of the BP and IR-related traits to reduce non-normality.

Association Analysis

To account for the dependency among family member, the generalized estimating equations methods20 was used in the association analysis by using family as the cluster factor, ie, members from the same family were assumed to be correlated and those from different families were assumed to be independent. An appropriate test, either the 2-degrees of freedom overall test of genotypic association or a specific genetic model (ie, dominant or recessive), was assumed depending on allele frequencies. Age and sex were included as covariates to adjust for possible confounding effects and analyses were repeated with further adjustment for BMI to evaluate the effect of adiposity.

Results

Table 1 gives the basic characteristics of the sample studied. The offspring generation had a mean age of 34.4 years and a mean BMI of 28.8 kg/m². The mean BP values were in the normal range. Males and females had comparable ages and BMIs, but females tended to have slightly lower BPs and higher IR (Table 1). Correlation analysis revealed that both GINF and SI were negatively correlated with SBP and diastolic BP (r = -0.21 and -0.07; P = 0.059 and 0.0002, respectively) and diastolic BP (r = -0.17 and -0.08; P = 0.002 and 0.02, respectively), whereas fasting insulin was positively correlated with SBP and diastolic BP (r = 0.18 and 0.14, P < 0.0001 for both).

### TABLE 1. Basic Characteristics of MACAD Offspring Cohort

<table>
<thead>
<tr>
<th>Trait</th>
<th>Male</th>
<th>Female</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (SE)</td>
<td>N</td>
</tr>
<tr>
<td>Age, y</td>
<td>187</td>
<td>34.2 (0.69)</td>
<td>264</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>187</td>
<td>28.7 (0.35)</td>
<td>263</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>187</td>
<td>120.3 (1.0)</td>
<td>263</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>187</td>
<td>71.2 (0.72)</td>
<td>263</td>
</tr>
<tr>
<td>Fasting insulin, μIU/mL</td>
<td>168</td>
<td>14.4 (0.67)</td>
<td>234</td>
</tr>
<tr>
<td>GINF, mg/kg per min</td>
<td>171</td>
<td>5.8 (0.22)</td>
<td>242</td>
</tr>
<tr>
<td>S_{IGF} mg/m²·min⁻¹·μIU⁻¹</td>
<td>162</td>
<td>2.3 (0.10)</td>
<td>228</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; DBP, diastolic blood pressure; GINF, glucose infusion rate; SBP, systolic blood pressure.

### Notes

- **BP and IR-related traits** were measured in the sitting position using a Dinamap system (Critikon, Inc) after subjects had been sitting with legs dangling for >5 minutes. The mean BP values were in the normal range. Males and females had comparable ages and BMIs, but females tended to have slightly lower BPs and higher IR (Table 1). Correlation analysis revealed that both GINF and SI were negatively correlated with SBP and diastolic BP (r = -0.21 and -0.07; P = 0.059 and 0.0002, respectively) and diastolic BP (r = -0.17 and -0.08; P = 0.002 and 0.02, respectively), whereas fasting insulin was positively correlated with SBP and diastolic BP (r = 0.18 and 0.14, P < 0.0001 for both).

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The characteristics of the 14 SNPs in the 9 HTN genes and the frequency of the minor allele for each SNP are given in Table 2. Most of the SNPs were common with the minor allele frequency >10%, except for 3 SNPs: G664A in the NPPA/ANP gene, W493R in the SCNN1A gene, and C(−690)T in the NOS3 gene. The low frequency of the minor alleles in these 3 SNPs may lead to unreliable statistical results and therefore were eliminated from further analysis. The genotype specific means and probability values for those SNPs that showed significant associations are shown in Figure. The association analysis revealed that the T allele of M235T in the AGT gene was significantly associated with increased IR, as measured by GINF (M allele carriers versus noncarriers: 4.95 ± 0.20 versus 5.52 ± 0.13; P = 0.036); the overall effect of a specific gene on IR ranged from 10% to 16%. No association was observed between the SNPs in the ACE, AGTR1, and GNB3 genes and IR-related traits. When >1 gene was associated with a trait, we further examined pair-wise gene–gene interactions, eg, the interaction between T2238C and A663T for fasting insulin, but no significant gene–gene interactions were detected for each of the 3 traits.

### Table 2. BP Gene SNPs and Minor Allele Frequencies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Polymorphism</th>
<th>dbSNP</th>
<th>Minor Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin-converting enzyme</td>
<td>D/I in intron 16</td>
<td>Rs1799752</td>
<td>0.425</td>
</tr>
<tr>
<td>ADD1</td>
<td>Adducin 1</td>
<td>G460W</td>
<td>Rs4961</td>
<td>0.173</td>
</tr>
<tr>
<td>ADRB2</td>
<td>Adrenergic receptor, beta-2</td>
<td>R16G</td>
<td>Rs1042713</td>
<td>0.422</td>
</tr>
<tr>
<td>AGT</td>
<td>Angiotensinogen</td>
<td>M235T</td>
<td>Rs699</td>
<td>0.310</td>
</tr>
<tr>
<td>AGTR1</td>
<td>Angiotensin II receptor, type 1</td>
<td>A1166C</td>
<td>Rs5186</td>
<td>0.268</td>
</tr>
<tr>
<td>GNB3</td>
<td>Guanine nucleotide binding protein (G protein), beta polypeptide 3</td>
<td>C825T</td>
<td>Rs5443</td>
<td>0.417</td>
</tr>
<tr>
<td>NPPA/ANP</td>
<td>Natriuretic peptide precursor A</td>
<td>G664A</td>
<td>Rs5063</td>
<td>0.051</td>
</tr>
<tr>
<td>NOS3</td>
<td>Nitric oxide synthase 3 (endothelial cell)</td>
<td>A(−922)G</td>
<td>Rs800779</td>
<td>0.237</td>
</tr>
<tr>
<td>SCNN1A</td>
<td>Sodium channel, nonvoltage-gated 1 alpha</td>
<td>W493R</td>
<td>Rs742912</td>
<td>0.031</td>
</tr>
</tbody>
</table>

SNP indicates single nucleotide polymorphism.

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### Discussion

Previous studies have shown that BP and IR cosegregate, and that IR is an intermediate and genetically regulated phenotype for HTN. In this study, we evaluated the phenotypic association between measures of IR and BPs and the genetic association between 14 polymorphisms in the 9 putative HTN genes (ACE, AGT, AGTR1, ADD1, NPPA, ADRB2, SCNN1A, GNB3, NOS3) and IR-related traits: fasting insulin, GINF, and SI. Our data revealed that a number of these HTN-related genes (AGT, ADRB2, ADD1, and NOS3) are associated with the direct measures of IR, ie, GINF and/or SI, whereas some (NPPA and SCNN1A) are associated with an indirect measure of IR, ie, fasting insulin only. Some of these genes (AGT and NOS3) seem to affect IR (direct measures) through their effect on adiposity as measured by BMI, whereas the others (ADD1, NPPA, ADRB2, and SCNN1A) interact with BMI to influence IR (either the direct measure GINF or the indirect measure fasting insulin). These data provided direct evidence that previous observations that BP and IR cosegregate can be explained in part by HTN genes, ie, that AGT, NOS3, NPPA, ADRB2, ADD1, and SCNN1A might be genetic markers for IR.

Additional data support this concept of BP genes influencing IR. NOS3 has been observed to affect insulin levels in
Probability values for the association analysis were obtained while adjusting for age and sex for M235T, A(/H11002, M235T, and 3 genotypes of ADRB2 R16G. Probability values for the association analysis were obtained when adjusting for age and sex for M235T; age, sex, and BMI were adjusted for in the association analysis. c, Mean glucose infusion rate (GINF: mg/kg per minute) classified by M allele carrier vs noncarriers of AGT M235T, and 3 genotypes of ADRB2 R16G.

No associations were observed between IR measures and variations in 3 (ACE, AGTR1, and GNB3) of the 9 genes. One possible explanation is that genetic heterogeneity exists between IR and BP/HTN. Some genes may affect the progress of HTN through affecting IR-related traits, whereas some affect the progression directly or through other pathways. Nevertheless, we cannot exclude the possibility of the existence of a true association because we studied only 1 SNP in each of these 3 genes, and it is quite possible that we have missed an association between the phenotype and the gene if not enough polymorphisms were studied. The genotyping technique we used here was set up for a fast screening of a large number of genes, in which markers were preselected based on the knowledge in the literature. This limits the number of markers in each gene and may decrease the ability to detect effects even if these genes are important players. However, against that interpretation is that the polymorphisms tested in the study herein were those specifically identified in previous studies, and thus genetic heterogeneity of the IR/BP relationship seems a more likely explanation.

Perspectives
Our data have shown that some genes involved in the susceptibility to hypertension are associated with IR, supporting the hypothesis that genes in the BP pathway play a role in IR in the MA population.

Acknowledgments
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References
4. Lloyd-Jones DM, Evans JC, Larson MG, O’Donnell CJ, Levy D. Differ-
ential impact of systolic and diastolic blood pressure levels on JNC-VI
staging. Joint National Committee on Prevention, Detection, Evaluation,
5. Maisonneuve P, Agodoa L, Gellert R, Stewart JH, Bucchiangi G,
Lowenfels AB, Wolfe RA, Jones E, Disney AP, Briggs D, Mccredie M,
Boyle P. Distribution of primary renal diseases leading to end-stage renal
failure in the United States, Europe, and Australia/New Zealand: results
from an international comparative study. Am J Kidney Dis. 2000;35:
157–165.
6. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, and
7. Falkner B, Hulman S, Tannenbaum J, Kushner H. Insulin resistance and
8. Fuh MM-T, Shieh S-M, Wu D-A, Chen Y-DI, Reaven GM. Abnor-
malities of carbohydrate and lipid metabolism in patients with hyper-
Tabulations and expectations regarding the genetics of human hyper-
10. Mitchell BD, Kammerer CM, Hixson JE, Arwood LD, Hackleman S,
Blanero J, Haffner SM, Stern MP, MacCluer JW. Evidence for a major
11. Prochazka M, Lillioja S, Tait JF, Knowler WC, Mott DM, Spraul M,
Inman J. Prospective analysis of the insulin-resistance syndrome
12. Chang TJ, Tsai MH, Jiang YD, Lee B, Lee KC, Lin YJ, Chiu KC, Tai TY,
Chuang LM. The Arg16Gly polymorphism of human beta2-adrenergic
receptor is associated with type 2 diabetes in Taiwanese people. Clin Endo-
Zimmann B; HOPE Study Investigators. Ramipril and the development of
14. Devereux RB, Dahlof B, Kjeldsen SE, Julius S, Aurup P, Beavers G,
Edelman JM, de Faire U, Fagard R, Feneberg F, Helse B, Hints T,
Kereiakes J, Leheballe-Pedersen O, Lindholm LH, Nieminen MS, Ovni P,
Paprot S, Snipins S, Wedel H; LIFE Study Group. Effects of losartan or atenolol
in hypertensive patients without clinically evident vascular disease: a substudy of the LIFE randomized trial. Ann Intern Med.
15. Pfeiffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, Michelson
EL, Olofsson B, Ostergren J, Yusuf S, Pocock S; CHARM Investigators and
Committees. Effects of candesartan on mortality and morbidity in patients
with chronic heart failure: the CHARM-Overall programme. Lancet.
16. Weber MA, Julius S, Kiebsd SE, Brunner HR, Ekman S, Hansson L,
Hua T, Laraa JH, McNes GT, Mitchell L, Plat F, Schork MA, Smith B,
Zanchetti A. Blood pressure dependent and independent effects of anti-
hypertensive treatment on clinical events in the VALUE Trial. Lancet.
17. DeFronzo RA, Goldberg M, Agus Z. The effects of glucose and insulin
18. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyper-
insulinemia produces sympathetic neural activation and vasodilation in
19. Wu HY, Jeng YY, Yue CJ, Chyu K-Y, Hsuwa WA, Chan TM. Endo-
thelial-dependent vascular effects of insulin and insulin-like growth factor
I in the perfused rat mesenteric artery and aortic ring. Diabetes. 1994;
43:1027–1032.
20. Laasko M, Edelman SV, Brechel G, Baron AD. Decreased effect
of insulin to stimulate skeletal muscle blood flow in obese man: a novel
21. Resnik L, Gruet P, Gruenbaum H, Alldman M, Laragh J. Hyper-
tensions and peripheral insulin resistance: mediating role of intracellular free mag-
22. Sowers JR. Insulin resistance, hyperinsulinemia, dyslipidemia, hyper-
539–553.
23. Mehain P, Buchanan TA, Shargill N, Hsuwa WA. Effect of chronic insulin
24. Brands MW, Hildebrandt DA, Mizzel HL, Hall JE. Sustained hyperinsu-
25. Buchanan TA, Sipos GF, Gadalah S, Yip Y-P, Marsh DJ, Hsuwa VA,
Bergman RN. Glucose tolerance and insulin action in rats with renol-