INTRODUCTION

OBSTRUCTIVE SLEEP APNEA (OSA) HAS A CLEAR GENETIC BASIS,1 AND SUSCEPTIBILITY TO ITS CONSEQUENCES, INCLUDING HYPERTENSION, ALSO APPEAR TO BE INFLUENCED BY ONE’S GENETIC MAKEUP.2 Recently, a common insertion/deletion (I/D) polymorphism in the ACE gene has been suggested as a susceptibility locus both for OSA itself,2 as well as for apnea-induced hypertension.2 We sought to confirm these reported associations using a family-based cohort, the Cleveland Family Study, created for the purpose of unraveling the genetic basis of OSA and its consequences.

METHODS

Participants and Genotyping

The Cleveland Family Study is a longitudinal genetic epidemiologic study of OSA in families.1 Index probands with a diagnosis of OSA (apnea-hypopnea index [AHI] > 30 events/h or on treatment with positive airway pressure), and at least 2 first-degree relatives available to be studied were recruited along with their families. Neighborhood control families were also initially recruited for the study. Further details on recruitment have been previously published.2 Analyses for this study were restricted to participants older than 18 years of age. Blood samples were collected after an overnight fast. ACE I/D genotype was determined using standard protocols.4

Phenotyping

Overnight in-home sleep monitoring was performed with an Edentrace I or II monitor (Eden Prairie, Minn) measuring airflow (nasal/oral oximetry), chest wall impedance, pulse oximetry, and heart rate. Respiratory events were defined as cessations (apneas) or discrete reductions (hypopneas) in airflow or chest impedance, lasting at least 10 seconds and associated with at least a 3% fall in oxygen saturation. Sleep time was estimated from visual inspection of the sleep record, correlated with a sleep diary completed by the subject. The AHI was determined by dividing the number of respiratory events by the estimated hours of sleep time. The AHI measured in this manner has been previously shown in our cohort to correlate closely with AHI obtained from full laboratory polysomnography (intraclass correlation = 0.83).5

Height and weight were directly measured to compute body mass index. Seated blood pressure was measured twice in the evening prior to polysomnography and averaged; hypertension was defined as systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, or current antihypertensive medication use.

Statistical Analysis

The software program Haploview v.3.3.2 (Cambridge, Mass) was used to calculate allele frequencies among founders and to test for Hardy-Weinberg equilibrium. In order to account for the familial nature of the data, mixed-effects modeling was used in all models as implemented in the ASSOC program of the statisti-
cal package S.A.G.E. v. 5.0.3 (Cleveland, Ohio). Models included independent variance terms to account for random polygenic, marital, sibship, and individual effects. AHI was log-transformed prior to analysis in order to approximate a normal distribution; the logit function was used for binary outcomes.

RESULTS

A total of 972 individuals from 273 families were included in this analysis. Mean ± SD age was 45.6 ± 15.9 years, 55.3% were women, 55.5% Caucasian, and mean ± SD body mass index was 32.4 ± 8.6 kg/m². The prevalences of severe OSA (AHI > 30) and hypertension were 24.3% and 35.6%, respectively. The allele frequencies among Caucasian (I: 0.44, D: 0.56) and African American founders (I: 0.39, D: 0.61) were similar (p = .34), so analyses were not stratified by ethnicity. The distribution of the 3 genotypes (II, ID, DD) was consistent with Hardy-Weinberg equilibrium (p = .27).

No association was found between ACE genotype and sleep-disordered breathing. The prevalence of severe OSA by genotype was 26.8% in II subjects, 23.3% in ID subjects, and 24.3% in DD subjects (p = .47). This finding was not altered by adjusting for age, sex, race, and body mass index. Multivariate modeling of log(AHI) as a continuous trait revealed similar nonsignificant findings.

The association between ACE genotype and hypertension status is presented in Table 1. In the unstratified analyses, a trend was found for a decreased risk of hypertension with increasing number of D alleles. In analyses adjusting for age, sex, race, and body mass index, DD genotype is associated with a 37% reduction in the odds of hypertension, as compared with the II genotype (p = .03). When analyses are stratified by sleep-apnea severity, the trend in odds-ratio estimates suggests that the protective effect of the D allele is primarily present in individuals with severe OSA. In the comparisons of both ID to II individuals and DD to II individuals, the point estimates of the odds ratio for hypertension diminish with increasing OSA severity, though a test of trend is only significant in the ID genotype (p = .01). It should be noted that the confidence intervals for each of these stratified groups is fairly wide such that only the comparison of ID to II in the AHI >30 group was statistically significant using a 5% type I error threshold (odds ratio = 0.47 with 95% confidence interval [0.22–1.00]).

DISCUSSION

The ACE D/I polymorphism is known to be functional, with the D allele leading to higher serum ACE levels. Although an early study suggested that the D allele may be a risk factor for OSA, subsequent studies have not replicated this finding. Our data also support the notion that the ACE D/I polymorphism has no effect on OSA risk. The conflicting results may be a result of the different ethnic backgrounds studied.

Recently, the work by Lin et al from the Wisconsin Sleep Cohort study suggested that the ACE D/I polymorphism may interact with OSA status to affect the risk of developing hypertension. These investigators reported that, in those with intermediate levels of sleep-disordered breathing (AHI 5-30), the D allele conferred an increased risk of hypertension. In this group with mild to moderate apnea, the odds ratios for hypertension were 2.2 and 4.7 for the comparisons of ID to II and DD to II genotypes, respectively. Our results are in direct contrast to this previous report because we found no significant association between ACE genotype and hypertension in subjects with mild to moderate apnea. If anything, a trend toward decreased risk was found in those with 1 or more D alleles. These differences may be due to differences in characteristics of the 2 cohorts, including factors that might influence angiotensin responses. The Cleveland Family Study enrolled a younger population with a higher proportion of African Americans. In addition, by recruiting based on affected probands, the Cleveland Family Study may have selected for individuals with a greater genetic predisposition to OSA than would be found in a general OSA population.

In contrast to the conflicting findings in subjects with mild to moderate apnea, both our work and results from the Wisconsin Sleep Cohort suggest that among subjects with severe apnea (AHI > 30), the D allele is associated with a reduced risk of hypertension. The Lin study reported odds ratios of 0.61 and 0.38 for the comparisons of ID to II and DD to II, respectively. While not statistically significant, in this cohort in which only 71 of 1100 individuals had severe OSA, the odds ratios are of

<table>
<thead>
<tr>
<th>Table 1—Relationship Between Angiotensin Converting Enzyme (ACE) Genotype and Hypertension Stratified by Sleep ApneaSeverity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Hypertension, %</td>
</tr>
<tr>
<td>Unadjusted</td>
</tr>
<tr>
<td>ID vs II</td>
</tr>
<tr>
<td>Adjusted</td>
</tr>
<tr>
<td>ID vs II</td>
</tr>
</tbody>
</table>

Data are presented as odds ratio (95% confidence interval). AHI refers to apnea hypopnea index.

Model 1: adjusted for age, sex, ethnicity, and body mass index
Model 2: adjusted for age, sex, ethnicity, body mass index, sleep apnea severity, and interaction effects between sleep apnea severity and ACE genotype.
the same magnitude and direction as the estimates found in our cohort (0.47 and 0.57 for ID to II and DD to II, respectively). Furthermore, data from a Chinese cohort found the frequency of the D allele to be significantly lower among hypertensives with AHI > 20 compared to hypertensive patients with mild or no apnea.1,2 In aggregate, these data suggest that the ACE deletion polymorphism may have a protective effect on hypertension development in those with severe OSA. Angiotensin in the carotid body has been found to increase the sympathetic response to hypoxia.3,4 One may speculate that ACE polymorphisms may differentially mediate hypertension susceptibility in individuals with different exposures to intermittent hypoxia through effects on carotid-body activity.

Among those without apnea, the odds-ratio estimates for hypertension were greater than 1 in those with ID genotype but less than 1 for the DD genotype. Given the broad and overlapping confidence intervals in these estimates, as well as the unlikely plausibility for a biologic effect to increase hypertension risk with 1 D allele but reduce it with 2 alleles, we believe this finding most likely represents stochastic variability.

It should be noted that, although the relationship between the ACE I/D polymorphism and hypertension in the general population has been controversial, the debate has centered on whether the D allele is a risk factor for hypertension. Our findings are novel in that they suggest, in the setting of OSA, that the D allele may actually be protective. The possibility that the same allele may promote hypertension in one setting and be protective in another may reflect the multifactorial effects of this gene. Increased activation of plasma angiotensin may promote hypertension development, but a reduced autonomic response to hypoxia may result in an overall protective effect of this allele in those with severe OSA. This complex biology may explain the difficulty in finding any relationship between ACE genotype and hypertension in studies that do not assess sleep-disordered breathing.

Clearly, these findings need to be interpreted cautiously given the wide confidence intervals. However, the correspondence of findings across studies argues for future research into the effect of the D ACE allele on cardiovascular consequences in OSA, both through the study of other epidemiologic cohorts as well as through further physiologic investigation into the role of the renin-angiotensin system in mediating the hypertensive effect of OSA. The use of more-refined blood pressure phenotypes such as 24-hour measurements, as well as better measures of the apnea exposure including age of onset and duration of OSA, might improve ability to detect associations. The limited power of both this work and the Lin et al study despite sample sizes on the order of 1000 subjects emphasizes the need for the development of large multicenter cohorts for future research on the genetics of OSA and its consequences. Finally, demonstration that the genetic polymorphism has a functional effect predisposing to hypertension will be needed to infer causality.

ACKNOWLEDGMENTS

This work was supported by NIH grants HL081385, HL046380, and HL071515. Some of the results presented were obtained by using the program package S.A.G.E., which is supported by a U.S. Public Health Service Resource Grant (RR03655) from the National Center for Research Resources.

REFERENCES