Absence of Linkage of the Epithelial Sodium Channel to Hypertension in Black Caribbeans

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Hypertensives of African origin have low-renin, sodium-sensitive blood pressure and respond poorly to treatment with angiotensin converting enzyme inhibitors. The epithelial sodium channel may be important in the pathogenesis of essential hypertension in this population. This is supported by the identification of mutations within this channel, which lead to excess sodium reabsorption and hypertension in Liddle’s syndrome. In this study we tested whether there was linkage of the genes encoding the three subunits of the epithelial sodium channel to essential hypertension in 63 affected sibling pairs of West African origin from St. Vincent and the Grenadines. We found no support for linkage of the epithelial sodium channel to essential hypertension in this population. However, further studies will be needed in larger populations of African ancestry to exclude a contribution of the genes encoding the epithelial sodium channel to hypertension. Am J Hypertens 1998;11:942–945 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Epithelial sodium channel, black Caribbeans, genetic linkage, human essential hypertension.

In the elucidation of the genetic basis of human hypertension the greatest progress has been made in determining the causes of the rare Mendelian forms where physiologic features permitted definition of distinct hypertensive phenotypes.1–3 The importance of sodium balance in hypertension is exemplified by Liddle’s syndrome. This disorder is characterized by suppression of the renin angiotensin system, blunted aldosterone secretion with hypokalemia, and response to triamterene.4 These features suggest that the epithelial sodium channel expressed within the distal nephron is an important candidate for this syndrome.4 The channel is regulated by aldosterone and is comprised of an α subunit that supports sodium conductance and β and γ subunits that greatly augment the activity of the α subunit, but only support very low levels of sodium conductance.5 Recently, mutations that lead to constitutive overactivity possibly through inhibiting degradation of this sodium chan-
nel have been discovered within the genes encoding the $\beta$ and $\gamma$ subunits located on chromosome 16. Hypertension among peoples of West African ancestry may exhibit low plasma renin activity, enhanced sodium sensitivity, and a blunted response of aldosterone to stimulus. This raises the possibility that more subtle susceptibility variants may exist within genes encoding the epithelial sodium channel, which lead to sodium retention and essential hypertension. In this study we set out to test whether any of the three subunits comprising the epithelial sodium channel are linked to essential hypertension in affected sibling pairs from St. Vincent and The Grenadines.

**MATERIALS AND METHODS**

**Study Population** After ethical approval, 63 families comprising 134 individuals (100 affected women and 34 affected men), based upon at least one affected sibling pair, were identified through the primary care clinic network on the island of St. Vincent. All subjects were of African ancestry defined by grandparental and parental ethnicity. The affected relatives had sitting diastolic blood pressures of $\geq 95$ mm Hg or were receiving treatment for essential hypertension and had documented evidence of diastolic blood pressures $>95$ mm Hg. Families with relatives with secondary hypertension and diabetes were excluded. A random control population of 50 individuals from the same practices were recruited to provide population-based allele frequencies. Family pedigrees, age, body mass index, alcohol consumption (units/week), smoking habit, and blood pressure were recorded for all individuals.

**Genetic Analysis** Genomic DNA was extracted from white cells using phenol-chloroform extraction methods. Two highly polymorphic microsatellite markers closely linked to the three genes encoding the subunits of the epithelial sodium channel were genotyped in all the hypertensive siblings and random control population. The close physical proximity of the $\beta$- and $\gamma$-subunits on chromosome 16p12.2–13.11 enabled a single microsatellite marker (D16S420) to test for linkage of both subunits. The $\alpha$ subunit is located on chromosome 12p13.1–pter; D12S889 was used to test for linkage of the gene encoding this subunit in our affected sibling pairs.

DNA was amplified by the polymerase chain reaction (PCR) in 96-well microtiter plates (Hybaid, Teddington, Middlesex, England). Each well contained 20 to 50 ng of genomic DNA; 1.5 mmol/L magnesium chloride; 1X reaction buffer (Advanced Biotechnologies, Leatherhead, Surrey, England); 200 $\mu$mol/L of deoxynucleotides of guanine, adenine, cytosine, and thymidine; 50 ng of each primer, one of which was fluorescein-labeled; and 0.2 units of Red Hot DNA polymerase (Advanced Biotechnologies), in a total volume of 15 $\mu$L. The PCR reaction was performed in a thermocycler (Hybaid Omnigene) and reaction conditions consisted of initial denaturation at 94°C for 2.5 min, followed by 30 cycles of 94°C for 35 sec, then annealing of primers at 55°C for 30 sec, and elongation at 72°C for 15 sec, which was extended to 2 min for the final cycle. The products of this PCR were then separated by electrophoresis on 6% denaturing polyacrylamide gels for 3 to 4 h at a rate-limiting voltage of 1100 V using a 373A DNA sequencer (Applied Biosystems, Warrington, Cheshire, England). Analysis of the allele sizes was carried out using Genescan 672 (version 1.2) and Genotype version 1.1 software (Applied Biosystems). Genescan –500 TAMRA was used as a size standard. To assure quality control the assignment of genotypes by the computer software was checked manually by inspection of the electropherogram.

**Statistical Analysis** The affected pedigree member (APM) method of linkage analysis was used for data analysis. This method computes a $T$ statistic, which tests whether affected relatives share alleles more often than would be expected by chance at each of the genetic markers. The $T$ statistics were weighted to allow the excess sharing of rare alleles to outweigh the importance of common alleles. The recommended intermediate weighting function, $1/\sqrt{p}$ where $p$ denotes the control allele frequency, was used for this study. Allelic distributions of the dinucleotide repeats in the 50 random controls were used to define these population allele frequencies.

**RESULTS**

Sixty-three families, including 134 affected relatives, from 12 primary care clinics on the island of St. Vincent were genotyped for the genetic markers D16S420 and D12S889. The demographic characteristics of these families are summarized in Table 1. The informativeness of the genetic markers as measured by the heterozygosity in the 50 Vincentian controls was 0.867.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median (Interquartile Range)</th>
</tr>
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<tbody>
<tr>
<td>Age in years</td>
<td>65 (58–72)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>27.3 (23.8–31.4)</td>
</tr>
<tr>
<td>Alcohol consumption (units/week)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>180 (160–190)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>100 (100–110)</td>
</tr>
</tbody>
</table>
for D16S420 and 0.826 for D12S889, which indicates adequate informativity for this linkage study.

There was no evidence to support linkage of either the β- or γ-subunits on chromosome 16p12.2–13.11 in our 63 families using the affected pedigree member method and applying the intermediate weighting scales (t statistic = 1.06, P = .14). Genotypes for marker D12S889, which is close to the α-subunit, could not be accurately assigned for two of the 63 families and these were excluded from further analysis. Linkage analysis in the remaining 61 families did not indicate any support for linkage of the α-subunit to hypertension (t statistic = 1.30, P = .10).

**DISCUSSION**

There is increasing support from observational population and migrant studies for a role of sodium intake in the elevation of blood pressure. Even within populations with high sodium intake the blood pressure distribution indicates that at least 60% of people are normotensive. This finding has led to speculation that genetic susceptibility to the influences of sodium loading may be important in some hypertensives. This is supported by studies in black hypertensives, which indicate that a high proportion have blood pressure responses to sodium loading and depletion.

Recent data from black families imply that there are heritable components to salt-responsive blood pressure. These heritable influences are largely independent of age and are particularly associated with systolic blood pressure. The characterization of mutations within the epithelial sodium channel in Liddle’s syndrome is of particular interest because the features of low-renin hypertension and suppressed aldosterone levels are analogous to those seen in some people of African origin with hypertension.

In this study we did not find support for linkage of the epithelial sodium channel to hypertension in black Caribbeans from St. Vincent. This might be because our affected sibling pairs were recruited based on diastolic blood pressure criteria, which correlates less well with heritable factors for salt sensitivity than systolic blood pressure. It was not possible to characterize our black Caribbean families for blood pressure response to sodium loading or depletion, so we cannot evaluate our data according to salt sensitivity.

The existence of more subtle alterations in the carboxy terminal region of the β subunit that harbors the Liddle’s mutation has been explored by direct sequencing in Japanese subjects, revealing a single polymorphism that did not alter the amino acid sequence. No alterations were found in the proline-rich region of the carboxy terminus of the β-subunit, which might impair degradation of the epithelial channel and lead to overactivity. The γ-subunit, which has also been implicated in Liddle’s syndrome, was not sequenced in this study and the patients were not characterized in terms of sodium sensitivity.

There have been several studies of the epithelial sodium channel subunits in hypertensive rat models that have not demonstrated support for linkage of any subunits of the epithelial sodium channel to hypertension. Interestingly, the chromosomal interval containing the β- and γ-subunits of the rodent epithelial sodium channel is on rat chromosome 1 within a region containing the SA gene, which has been linked to hypertension in sodium-responsive hypertensive rat models. Indeed, linkage analysis from one of these studies suggested that a quantitative trait locus in the spontaneously hypertensive rat that might influence blood pressure mapped 4.4 centimorgans (approximately 4.4 million bases) away from the β- and γ-subunits of the epithelial sodium channel. Furthermore, sequencing strategies directed at the β- and γ-subunits in rat models have only detected polymorphisms within the γ subunit that do not affect the sodium channel activity when expressed in *Xenopus* oocytes.

The results reported here do not exclude a role for genes encoding the subunits of the human epithelial sodium channel in the genetic basis of human hypertension because our study had limited power. We have estimated that the families studied here would offer 40% power to detect a P = .01, which would be accepted as “suggestive of linkage” in a single-locus model. This takes into account the population prevalence of hypertension, the risk of recurrence of hypertension to a sibling, and the heterozygosity of each marker. Because it is widely accepted that hypertension is a multilocus disorder, it is clear that this can only be regarded as an exploratory study and further studies will be needed to exclude this locus from a role in human essential hypertension.

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