of ELAC2 in prostate cancer, suggest moderate familial risk, and estimate that risk genotypes in ELAC2 may cause 2% of prostate cancer in the general population.

NICOLA J. CAMP1 AND SEAN V. TAVTIGIAN2
1Genetic Epidemiology, Department of Medical Informatics, University of Utah Medical School, and 2Genetic Cancer Susceptibility Unit, International Agency for Research of Cancer, Lyon, France

Electronic-Database Information
Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for prostate cancer [MIM 176807]), HPC2/ELAC2 [MIM 605367], Ser-to-Leu change at amino acid 217 [MIM 605367.0001], and Ala-to-Thr change at amino acid 541 [MIM 605367.0002]

References

Address for correspondence and reprints: Dr. Nicola J. Camp, Genetic Epidemiology, Department of Medical Informatics, University of Utah School of Medicine, 391 Chipeta Way, Suite D, Salt Lake City, UT 84108.
E-mail: nicki@genepi.med.utah.edu
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Regarding “Testing for Population Subdivision and Association in Four Case-Control Studies”

To the Editor:
Ardlie et al. (2002) recently found no evidence for population structure in separate case-control studies of type 2 diabetes and hypertension in U.S. whites and only weak evidence of structure in a case-control study of hypertension in African Americans. These results are consistent with the theoretical results of Wacholder et al. (2000), who found that the magnitude of bias due to unrecognized population stratification is likely to be small under most plausible scenarios. To further evaluate the potential bias due to stratification for these and other conditions, we conducted a series of case-control studies for six common phenotypes in a population-based sample of U.S. adults.

The study population included 444 unrelated adults (231 African Americans and 213 non-Hispanic whites) randomly selected from five U.S. communities as part of the Hypertension Genetic Epidemiology Network (HyperGEN) of the National Heart, Lung, and Blood Institute (NHLBI) Family Blood Pressure Program (Williams et al. 2000). The study was approved by the institutional review boards at each institution, and appropriate informed consent was obtained from human subjects. Phenotypes measured included: (1) obesity (BMI ≥ 30), (2) hypercholesterolemia (total plasma cholesterol ≥ 240 mg/dl or current use of medications to lower cholesterol), (3) hypertension (systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or current use of medications to lower blood pressure), (4) diabetes (fasting serum glucose ≥ 126 mg/dl, nonfasting glucose ≥ 200 mg/dl, self-reported physician diagnosis of diabetes, or current use of hypoglycemic medications), (5) renal dysfunction (serum creatinine ≥ sex-specific 90th percentile [1.4 mg/dl in men and 1.1 mg/dl in women]), and (6) cardiovascular disease (self-reported history of heart attack, stroke, or coronary artery bypass surgery). For each

phenotype, those who did not meet the case definition served as control individuals.

We constructed contingency tables and performed \( \chi^2 \) tests of association for these six phenotypes with each of 368 STR markers typed by the NHLBI Mammalian Genotyping Service at Marshfield, WI (screening set 10). Like Ardlie et al. (2002), we then computed a statistic, \( \chi^2 \), to test for overall differences in allele frequencies between each set of case individuals and control individuals (Pritchard and Rosenberg 1999). To simplify the analysis and ensure that expected values in contingency tables were sufficiently large (> 5) for the classical \( \chi^2 \) test, we converted each STR marker to a biallelic marker by selecting one index allele for each marker and then collapsing all other alleles for that marker into a single alternative allele. Index alleles for each marker were selected by first choosing alleles with allele frequencies of at least 15% in both African Americans and whites and then selecting the allele that demonstrated the largest absolute difference in allele frequencies between racial groups.

The prevalence of several of the phenotypes differed substantially between racial groups (table 1). In crude analysis pooling both racial groups, the percentage of markers nominally associated \( (P < .05) \) with each phenotype was higher than expected, under the null hypothesis, for diabetes (8.4%), hypertension (7.9%), renal dysfunction (7.6%), and hypercholesterolemia (5.4%) but not for cardiovascular disease (4.9%) or obesity (4.9%). The summary test for stratification incorporating all 368 markers \( (i.e., 368 \text{ df}) \) was statistically significant for diabetes, renal dysfunction, and hypertension (table 1), indicating overall differences in allele frequencies between case individuals and control individuals. However, after adjustment for race or stratification by race, there was no evidence of cryptic stratification for any of the six phenotypes, with the possible exception of obesity in whites.

Our results provide further evidence that hidden or unrecognized population stratification is unlikely to be a serious threat to the validity of case-control designs that appropriately account for ethnicity in either the design or analysis phase of the study (Wacholder et al. 2000; Ardlie et al. 2002). Because of the large number of markers tested, it is likely that our study was even more sensitive to subtle background genetic differences between case individuals and control individuals than that conducted by Ardlie et al. (2002), which included only 9 STR markers and 35 SNP markers. We think that other factors, such as selection bias, chance, publication bias, gene-environment interactions, and differences in linkage disequilibrium patterns across study populations, are more plausible explanations for inconsistency of results between genetic association studies.

Acknowledgments

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Table 1

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>NO. OF CASE SUBJECTS (PREVALENCE)</th>
<th>ALL SUBJECTS, UNADJUSTED</th>
<th>ALL SUBJECTS, ADJUSTED FOR RACE</th>
<th>AFRICAN AMERICANS ONLY</th>
<th>WHITES ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>African Americans</td>
<td>Whites</td>
<td>( \chi^2 )</td>
<td>( P )</td>
<td>( \chi^2 )</td>
</tr>
<tr>
<td>Obesity</td>
<td>110 (48%)</td>
<td>82 (39%)</td>
<td>379.5</td>
<td>.33</td>
<td>336.8</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>44 (19%)</td>
<td>55 (26%)</td>
<td>401.4</td>
<td>.11</td>
<td>360.9</td>
</tr>
<tr>
<td>Hypertension</td>
<td>130 (56%)</td>
<td>91 (43%)</td>
<td>456.2</td>
<td>.001</td>
<td>371.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>46 (20%)</td>
<td>23 (11%)</td>
<td>507.2</td>
<td>&lt;.001</td>
<td>395.7</td>
</tr>
<tr>
<td>Renal dysfunction</td>
<td>29 (13%)</td>
<td>11 (5%)</td>
<td>442.5</td>
<td>.005</td>
<td>374.2</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>30 (13%)</td>
<td>20 (9%)</td>
<td>378.6</td>
<td>.34</td>
<td>373.2</td>
</tr>
</tbody>
</table>
and Blood Institute: Susan E. Old, Millicent Higgins (retired), Cashell Jaquish, Martha Lundberg, and Mariana Gerschenson.

JAMES S. PANKOW,1 MICHAEL A. PROVINCE,2 STEVEN C. HUNT,3 AND DONNA K. ARNETT1
1Division of Epidemiology, University of Minnesota, Minneapolis; 2Division of Biostatistics, Washington University, St. Louis; and 3 Cardiovascular Genetics, University of Utah, Salt Lake City

References


Address for correspondence and reprints: Dr. James S. Pankow, Division of Epidemiology, University of Minnesota, 1300 South Second Street, Suite 300, Minneapolis, MN 55454. E-mail: pankow@epi.umn.edu
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The ABCA4 Gene in Autosomal Recessive Cone-Rod Dystrophies

To the Editor:

Recently, Maugeri et al. (2000) reported on the screening of the ABCA4 gene in 5 patients with autosomal recessive cone-rod dystrophies (CRD) and 15 patients with sporadic CRD originating from Germany and the Netherlands. The identification of mutations in 13/20 patients (65%) led the authors to speculate that “Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy.”

The present study was undertaken to evaluate the prevalence of ABCA4 mutations in a cohort of 55 patients affected with autosomal recessive or sporadic CRD.

Within the huge family of inherited retinal dystrophies, the CRD phenotype indicates a specific form of retinal degeneration in which the cone degeneration appears early in life with a central involvement of the retina, followed by a degeneration of rods several years later (Klevering et al. 2002). This particular form of retinal dystrophy has long been regarded as “inverse retinitis pigmentosa” (RP) and can be misdiagnosed as macular dystrophy in the first stages of the disease.

Indeed, the main symptoms at onset of the disease are decrease of visual acuity, loss of color discrimination, and photophobia. The b-wave of the photopic ERG (cone response) is severely reduced, although the b-wave of the scotopic ERG is still normal. As the disease progresses, nystagmus, progressive peripheral visual field deficit, and decreasing scotopic electroretinogram (ERG) amplitudes are observed.

Four genes (CRX [MIM 602225], GUCY2D [MIM 600179], GCAP1 [MIM 600364], and HRG4 [MIM 604011]) and two loci have been implicated in autosomal dominant CRD (CORD5 [MIM 600977] and CORD7 [MIM 603649]), whereas two other loci were reported for autosomal recessive CRD (CORD9 [Danciger et al. 2001] and CORD8 [MIM 605549]) and one for X-linked CRD (RPGR [MIM 312610]).

Conversely, the ABCA4 gene, which was identified in 1997 as the Stargardt-causing gene, was later recognized as responsible for some forms of RP (RP19) and some CRD, depending on the nature of the ABCA4 mutations and on the remaining protein activity (Allikmets et al. 1997; Martinez-Mir et al. 1997; Cremers et al. 1998; Gerber et al. 1998; Rozet et al. 1998, 1999).

Sixty-one individuals affected with CRD and 40 healthy relatives belonging to 55 families of various origin were recruited from genetic and ophthalmologic consultations. In 29/55 families, the disease was undoubtedly inherited as an autosomal recessive condition—23 multiplex families (11/23 consanguineous) and six simplex patients born to consanguineous parents. In the 26/55 remaining families, the patients were simplex cases. The time course of the disease was determined by interviewing at least one patient per family and, whenever possible, all affected siblings of the family. Minimal criteria for inclusion in the study were initial cone dysfunction and subsequent progressive peripheral disease.

In one affected patient per family, we screened for mutations the 50 exons of the ABCA4 gene, as well as the flanking intrinsic sequences, using denaturing high-pressure liquid chromatography. On the basis of the secondary structure of each exon, the screening was performed at 1 or 2 temperatures (mutation detection rate estimated to be at least 0.98). Exons showing a shift were directly sequenced.

Sixteen different mutant alleles were identified in 13/55 patients (i.e., 23.6% of all cases). Among these 13 patients, 2 were homozygotes (from two consanguineous families), 4 were compound heterozygotes, and 7 were...