G Protein β3 Subunit Gene Variant and Blood Pressure Variation in Canadian Oji-Cree

Robert A. Hegele, Stewart B. Harris, Anthony J.G. Hanley, Henian Cao, Bernard Zinman

Abstract—The subunits of the heterotrimeric G proteins are attractive candidate gene products for both susceptibility to essential hypertension and interindividual variation in blood pressure. There is alternative splicing of exon 9 of the gene encoding the β3 subunit of heterotrimeric G proteins (GNB3) associated with a C→T change at nucleotide 825, which activates a cryptic splice site. The 825T allele results in a gene product that is 41 amino acids smaller than the wild-type gene product. G protein heterotrimers containing the shorter variant are more reactive than those containing the wild type, and the 825T allele appears to be associated with essential hypertension. To evaluate whether this variant is associated with hypertension or blood pressure in other human samples, we genotyped 447 young adult Oji-Cree for the GNB3 C825T variation. We found that the frequency of the GNB3 825T allele was 0.501 in the Oji-Cree, which is considerably higher than the frequency observed in whites. Furthermore, genetic variation of the GNB3 nucleotide 825 was significantly associated with variation in systolic pressure but not diastolic pressure. Specifically, subjects with the 825T/T genotype had significantly lower systolic pressure than subjects with the 825C/T and 825C/C genotypes; the association was independent of sex. Furthermore, the 825T allele frequency tended to be higher in subjects who took antihypertensive medications than in subjects who did not (0.571 versus 0.496; P=NS), although this young sample had relatively few subjects with hypertension. The findings support an association of variation in this gene with variation in blood pressure. (Hypertension. 1998;32:688-692.)

Key Words: ion channels ■ hypertension, genetic ■ linkage disequilibrium ■ genetics

There have been many reported associations between hypertension-related phenotypes and DNA markers of candidate genes.1 However, alleles of very few genes are consistently related to intermediate phenotypes across diverse populations. Part of the inconsistency may be due to the fact that most DNA markers studied thus far do not have a functional impact on the structure or expression of the gene product. Thus, most reported genetic associations have been attributed to linkage disequilibrium with putative functional changes elsewhere at the genetic locus. Since this may vary between populations, factors such as admixture can result in false conclusions about genetic associations. One strategy to reduce such confounding in association studies may be to select DNA markers that are proven, by various assays, to directly mark a functional change in the gene of interest. Additionally, identifying the genetic determinants of an intermediate quantitative phenotype, such as blood pressure (BP), may help to identify the genetic determinants of a disease, such as hypertension, which is defined by threshold values imposed on the quantitative trait.

Proteins involved in intracellular ion transport, such as the sodium-proton transporter, represent attractive candidates to study for interindividual genetic differences in susceptibility to hypertension.2 Altered sodium-proton transporter activity and enhanced G protein activation have been observed in immortalized cell lines taken from patients with essential hypertension.2-4 Recently, the molecular basis for altered intracellular signal transduction affecting ion transport in immortalized cells in vitro was found to be a single base change (C→T) at nucleotide 825 in exon 10 of the GNB3 gene on chromosome 12p13, which encodes the β3 subunit of heterotrimeric G proteins.5 The 825T allele was associated with the occurrence of a splice variant, which produced a 123-base pair deletion due to alternative splicing of exon 9.5 The resultant loss of 41 amino acids from the Gβ subunit encoded by the GNB3 825T allele was associated with increased stimulated binding of labeled GTP in cell lines from hypertensive patients and in transfected insect cells.5 Furthermore, an association analysis showed that the GNB3 825T allele, which had an allele frequency of 0.25 in the general, nonhypertensive German population, was significantly associated with essential hypertension (odds ratio, 1.44; 95% CI, 1.09 to 1.88).5

We previously reported a significant association between the angiotensinogen gene (AGT) T235 variant and both elevated systolic BP and hypertension in a sample of adult...
Canadian Oji-Cree. This is a very young study sample, with a low prevalence of hypertension compared with the rest of Canada. We have been interested in other genetic determinants of hypertension and related intermediate traits in the Oji-Cree. Given the reported functional impact of the $\text{GNB3 C825T}$ variation, we hypothesized that this variation would be associated with variation in BP in our aboriginal population sample.

Methods

Study Subjects
The isolated community of Sandy Lake, Ontario, is located ~2000 km northwest of Toronto, in the subarctic boreal forest region of central Canada. Historically, the ancestors of the contemporary residents of this region lived a nomadic, hunting-gathering subsistence typical of other native peoples of the northeastern subarctic. Since the development of the reservation and residential school systems, the lifestyle changed from very physically active to very sedentary. The primary source of food changed from wildlife with roots and berries to processed foods high in animal fats, which are supplied by a company store.

Seven hundred twenty-eight members of this community aged ≥10 years participated in the present study. Subjects answered a questionnaire for medical history, which included a question on the current use of antihypertensive drugs. Physical examination included determination of body mass index (BMI), defined as weight/height$^2$ (kilograms per meter squared) and 2 separate BP determinations in the right arm with the subject seated. Systolic BP was recorded to the nearest 2 mm Hg at the appearance of the first Korotkoff sound (phase I), and diastolic BP was recorded to the nearest 2 mm Hg at the disappearance of the fifth Korotkoff sound (phase V). Blood samples were obtained with informed consent after a 10-hour fasting period. Exclusion criteria included age <18 years, a past history of diabetes mellitus, and an inadequate blood sample for all determinations. The project was approved by the University of Toronto Ethics Review Committee.

Biochemical and Genetic Analyses
Blood for lipoprotein analyses was centrifuged at 2000 rpm for 30 minutes, and the plasma was stored at −70°C. Fasting plasma concentrations of lipoproteins and apolipoproteins were determined as described. Genotypes for $\text{GNB3 nucleotide 825}$ were determined with the use of primers, amplification conditions, digestion with $\text{BseDI}$ (Fermentas), and electrophoresis as described.

Statistical Analysis
SAS (version 6.1) was used for all statistical comparisons. The distributions of both systolic and diastolic BP were significantly nonnormal in this data set. Therefore, for parametric statistical analyses, each quantitative variable was transformed and subjected to analysis of normality as described. ANOVA was performed with the general linear models procedure to determine the sources of variation for systolic and diastolic BP, with F tests computed from the type III sums of squares. This form of sums of squares is applicable to unbalanced study designs and reports the effect of an independent variable after adjustment for all other variables included in the model. Dependent variables were transformed systolic and diastolic BP. Independent variables were age, sex, the natural logarithm of BMI, and current treatment with an antihypertensive medication. Also included as independent variables were the $\text{AGT codon 235 genotype}$ and the plasma concentration of apolipoprotein B, since these were previously shown to be significantly associated with variation in systolic and diastolic BP, respectively, in the Oji-Cree. In addition, the family identification number was included as a covariate. Finally, the $\text{GNB3 C825T genotype}$ was also included as an independent variable. To test for interactions between $\text{GNB3}$ C825T genotype and either sex, BMI, or $\text{AGT codon 235 genotype}$, interaction terms were included as covariates in separate post hoc ANOVA. BP differences between individuals classified by $\text{GNB3 genotype}$ were compared by means of an unpaired t test. In addition, association between $\text{GNB3 C825T genotype}$ and hypertension, defined as systolic BP >140 mm Hg and/or diastolic BP >90 mm Hg and/or current use of antihypertensive medication, was evaluated with $\chi^2$ analysis. The nominal level of significance was taken to be $P<0.05$.

Results

Baseline Phenotypes in Whole Sample
Sufficient DNA and phenotypic information were obtained from 447 adult subjects who had no history of diabetes. Of these, 55% were women. Baseline clinical features for the overall study sample are shown in Table 1. Twenty-eight subjects took medication for hypertension; almost all of these took angiotensin-converting enzyme inhibitors.

Allele and Genotype Frequencies
The observed frequency of the $\text{GNB3 825T allele}$ was 0.501, which was more than twice that reported in Germans. The observed frequencies for genotypes C/C, C/T, and T/T were 0.207, 0.586, and 0.208; these did not deviate significantly from the frequencies predicted by the Hardy-Weinberg equation.

Genetic Determinants of Variation in Systolic and Diastolic BP
The results of the ANOVA are shown in Table 2. One ANOVA was performed for systolic BP and 1 for diastolic BP. Since ANOVA takes multiple comparisons into account, we did not adjust the levels of nominal significance. For systolic but not diastolic BP, a significant association with $\text{GNB3 C825T genotype}$ was seen ($P=0.022$). None of the other genetic associations was significant at $P<0.05$.

Pairwise comparisons of least square means for each genotypic class for the overall study sample are shown in Table 3. The 93 homozygotes for the $\text{GNB3 825T allele}$ had significantly lower systolic BP than the 262 heterozygotes and the 92 homozygotes for the 825C allele ($P=0.033$ and $P=0.0037$, respectively). There was no difference in systolic BP between the heterozygotes and homozygotes for the 825C allele ($P=0.11$). None of the pairwise comparisons for diastolic BP indicated significant differences between the genotypes.

Pairwise comparisons of least square means for each genotypic class for each sex are shown in Tables 4 and 5. For both men and women, the homozygotes for the $\text{GNB3 825T allele}$ had significantly lower systolic BP than the heterozygotes and the homozygotes for the 825C allele. There were no
G Protein β3 Variant and Blood Pressure

TABLE 2. ANOVA in Sandy Lake Oji-Cree

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Logarithm of Systolic BP</th>
<th>Logarithm of Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F Value</td>
<td>P&gt;F</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>25.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>31.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family of origin</td>
<td>138</td>
<td>0.93</td>
<td>NS (0.70)</td>
</tr>
<tr>
<td>Logarithm of BMI</td>
<td>1</td>
<td>20.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Antihypertensive treatment</td>
<td>1</td>
<td>25.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Logarithm of apo B</td>
<td>1</td>
<td>0.15</td>
<td>NS (0.69)</td>
</tr>
<tr>
<td>AGT codon 235 genotype</td>
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<td>2.08</td>
<td>NS (0.11)</td>
</tr>
<tr>
<td>GNB3 nt 825 genotype</td>
<td>2</td>
<td>3.86</td>
<td>0.022</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; P>F, probability of a greater between-group F value using ANOVA; BMI, body mass index; apo, apolipoprotein; AGT, gene encoding angiotensinogen; GNB3, gene encoding β3 subunit of heterotrimeric G proteins; and nt, nucleotide.

Differences in systolic BP between the heterozygotes and homozygotes for the 825C allele. None of the pairwise comparisons for diastolic BP in either sex indicated significant differences between the genotypes.

In a separate ANOVA performed post hoc for systolic BP, there was no significant interaction term for GNB3 genotype and sex (P=0.17), for GNB3 genotype and BMI (P=0.78), or for GNB3 genotype and AGT genotype (P=0.28). In a separate ANOVA performed post hoc for diastolic BP, there was no significant interaction term for GNB3 genotype and sex (P=0.75), for GNB3 genotype and BMI (P=0.30), or for GNB3 genotype and AGT genotype (P=0.50).

While the frequency of the GNB3 825T allele in subjects who took antihypertensive medications tended to be higher than in subjects not on medication (0.571 versus 0.496), this trend was not nominally significant (Table 6; P=0.32 [NS]). While the odds ratio for taking antihypertensive medication for T/T homozygotes versus C/C homozygotes was 1.6, this was not significantly different from 1.0 (95% CI, 0.61 to 5.72). However, our study was admittedly underpowered with respect to both the numbers of subjects with a diagnosis of hypertension and those taking antihypertensive medications to detect differences in frequencies of this magnitude, which are similar to those found in larger samples of hypertensive subjects.

Discussion

In this study of a young and largely normotensive sample of aboriginal Canadians, we found (1) a very high prevalence of the GNB3 825T allele; (2) a significant association between variation in systolic BP and variation in the GNB3 gene; (3) a significantly lower systolic BP in subjects homozygous for GNB3 825T than in subjects with the other 2 genotypes; and (4) no significant relationship between a diagnosis of hypertension and the GNB3 C825T genotype. This latter finding is not unexpected because the study sample was very young and there was a low prevalence of hypertension. Thus, our power to detect a statistically significant association was limited. Nevertheless, our findings indicate that the GNB3 C825T genotype, which marks a genomic variant that has functional consequences in vitro, is significantly associated with variation in systolic BP. Specifically, among Oji-Cree, those who were homozygous for this variant had significantly lower systolic BP than subjects with the other 2 genotypes. There was no evidence of any interaction between the GNB3 genotype and either sex, BMI, or AGT genotype.

Our finding in Oji-Cree is inconsistent with the expectation based on the functional impact of the mutation in vitro and the association with hypertension in German subjects. Given the increased stimulated binding of GTP to cells from hypertensive subjects with GNB3 825T and to insect cells transfected with GNB3 825T, we were not surprised to observe an association between variation at this site with systolic BP in the ANOVA. However, we were surprised by the counterintuitive association revealed in the pairwise comparisons. There are several possible explanations for the disparity with the association analysis in the German hypertensive subjects.

First, the phenotype under study was different in the 2 samples: the German cases were ascertained on the basis of a diagnosis of essential hypertension, whereas the Oji-Cree were ascertained on the basis of community-wide screening. Thus, the phenotypes were not directly comparable. Furthermore, the mean age of the German subjects was more than 22 years greater than the mean age of the Oji-Cree; this addi-
genetic and/or environmental factors on the development of hypertension. In addition, the functional impact of the \( \text{GNB3} \) 825T allele may differ according to age. Furthermore, the impact could be different when hypertension has become an established phenotype. For example, the tendency of \( \text{GNB3} \) 825T to predispose to elevated BP at the cellular level could be adequately overcome, or perhaps even overcompensated for, by robust counterregulatory mechanisms in younger subjects. Such possible overcompensation might initially result in lower mean BP in subjects with \( \text{GNB3} \) 825T. However, with the passage of time, and with physical and metabolic changes in the patient, it is possible that such putative counterregulatory mechanisms may become fatigued and/or may fail outright. Thus, the phenotype associated with the \( \text{GNB3} \) 825T allele in older subjects, such as the Germans studied by Siffert et al., might be hypertension, which could represent the end point resulting from failure of counterregulatory mechanisms. However, there would be few precedents in human pathophysiology for such an explanation.

Alternatively, the genomic change at \( \text{GNB3} \) position 825 may not have functional relevance in the Oji-Cree but may instead be in linkage disequilibrium with another genetic change at this locus, which would be the actual molecular basis for the association with variation in systolic BP. While our hope had been to demonstrate that this marker for altered \( \text{GNB3} \) AGT function would be associated unequivocally with biologically plausible phenotypes, our results do not exclude the possibility of linkage disequilibrium with other functional DNA changes within or near \( \text{GNB3} \). Even Siffert et al. could not fully discount the possibility of linkage disequilibrium between \( \text{GNB3} \) 825T and another functional variant at this locus.

Despite a frequency of \( \text{GNB3} \) 825T of >0.5, only 28 members of this study group were prescribed antihypertensive medications. The frequency of the \( \text{GNB3} \) 825T allele in the subset who were taking antihypertensive medications was 0.571, which tended to be higher than the frequency of 0.496 seen in the subset who were not taking antihypertensive medications. However, the small numbers of affected subjects limited our ability to detect a statistically significant association between \( \text{GNB3} \) 825T and hypertension. The very young age of the Oji-Cree study sample may have been another limiting factor. In any event, the current prevalence of hypertension in this community is lower than that seen in the rest of Canada. Given the very high frequencies of both \( \text{GNB3} \) 825T and \( \text{AGT} \) T235 alleles, it would be of great interest to follow this community prospectively to observe the future development of hypertension, especially now that the \( \text{GNB3} \) and \( \text{AGT} \) genotypes are known.

It is also of interest that the significance of the association between the \( \text{AGT} \) T235 allele and systolic BP was reduced by including the second genetic variable, namely \( \text{GNB3} \) 825T genotype, in the multivariate ANOVA. However, the association between the \( \text{AGT} \) T235 allele and systolic BP is clearly significant when it is the only genetic variable included in the ANOVA (data not shown). Furthermore, the nonsignificant interaction term composed of \( \text{AGT} \) and \( \text{GNB3} \) genotypes suggests that there was no epistasis between the \( \text{AGT} \) and \( \text{GNB3} \) variation in this study sample. These findings highlight a possible limitation of multivariate statistical analysis: it may be that for small genetic effects on a phenotype, a multivariate model can soon become crowded with too many variables, and this could affect the levels of significance for an independent variable that is a modest determinant of variation in a dependent variable. Rather than trying to overfit a model with several genetic variables simultaneously, it may be more appropriate to perform several analyses using one genetic variable at a time and to adjust the overall level of significance for multiple comparisons.

The very high \( \text{GNB3} \) 825T allele frequency in Sandy Lake compared with other samples might have resulted from founder effects involving the ancestors of the contemporary community. Archaeological studies suggested that hunter-gatherers inhabited the Sandy Lake region 6000 years ago. The current inhabitants of the Sandy Lake region lived a hunter-gatherer subsistence until \( \approx 70 \) years ago. The present community is largely descend- ed from 1 clan, which established the present reservation. Alternatively, selection pressure from a possible advantage of the allele harboring the \( \text{GNB3} \) 825T variant may have produced the present allele frequencies.

In summary, we have observed that the presence of the \( \text{GNB3} \) 825T variant was associated with lower systolic BP in a young, essentially normotensive study sample of aboriginal people. This modest association might have been due to a direct physiological effect of the genetic variation or to linkage disequilibrium with another functional change at the \( \text{GNB3} \) locus. Secondary genetic or environmental factors may influence the association of the \( \text{GNB3} \) variation with BP-related phenotypes. Understanding the background of genetic predisposition to abnormal phenotypes may be important in native populations, which appear to develop an increased prevalence of metabolic diseases as their lifestyles change.

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References
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