G Protein β3 Subunit Variant and Essential Hypertension in Japanese

Norihiro Kato, Takao Sugiyama, Hiroyuki Morita, Hiroki Kurihara, Yukio Yamori, Yoshio Yazaki

Abstract—Enhanced G protein activation has been implicated to underlie the increased sodium-proton transport in blood cells, an impaired characteristic observed in 30% to 50% of patients with essential hypertension. Recently, significant association between a C825T polymorphism of the gene encoding the G protein β3 subunit and hypertension was demonstrated in a white population, together with the finding that the T825 variant might be related to alternative splicing through unidentified mechanisms. We therefore investigated the disease relevance of this candidate gene by conducting an association study in a relatively large Japanese population. Participants comprised 718 hypertensive case subjects (without diabetes mellitus), 515 normotensive control subjects, and 191 hypertensive subjects with borderline or established diabetes mellitus; all individuals were recruited at a single institution. Genotype distribution of the C825T polymorphism was compared between hypertensive subjects, with or without diabetes, and the control group with χ² statistics. No significant association was observed in the present study. Results were still not significant when the case group was subdivided according to more stringent classification criteria. Allele frequencies of T825 proved to be almost concordant among the 3 study groups and higher in Japanese (49.0% to 49.6%) compared with a reported prevalence of 25% to 31% in whites. Our data suggest that the T825 variant of the G protein β3 subunit gene is unlikely to constitute major susceptibility for essential hypertension in the Japanese population studied. However, further investigation is required to answer the question of whether the lack of association reflects ethnic differences in the nature of genetic susceptibility loci. (Hypertension. 1998;32:935-938.)

Key Words: hypertension, essential ■ association ■ case-control studies ■ G proteins ■ genetics

A variety of approaches have been attempted to elucidate the genetic basis of essential hypertension, which is thought to be of multifactorial origin. One of the logical and promising strategies is the investigation of candidate genes that encode key components of the physiological mechanisms impaired in (or characteristic of) hypertensive patients. Among these mechanisms, enhanced signal transduction via pertussis toxin–sensitive G proteins has been demonstrated in immortalized lymphoblasts from hypertensive patients, which may underlie the sodium-proton transport abnormality in blood cells of a subgroup of hypertensive subjects. In the search for structural changes in the α, β, or γ subunit of heterotrimeric G proteins, Siffert et al have recently shown that a C825T polymorphism of the gene encoding the G protein β3 subunit (GNB3) is significantly associated with essential hypertension in a white population; the T825 allele had a higher frequency in hypertensive subjects than in normotensive subjects. Of note is the fact that the T825 allele was also associated with the occurrence of alternative splicing, which caused the loss of 41 amino acids within highly conserved repeating units of GNB3. The splice variant appeared to be predominantly expressed in cell lines with the TC or TT genotype. Therefore, it has been implicated that the T825 variant (or another unidentified mutation) of the GNB3 gene may predispose to essential hypertension. Independent replications would help to confirm this positive association. In addition, some studies have shown the likely ethnic variation in the nature of genetic susceptibility loci for hypertension— an important issue that should be further addressed. We have thus performed a case-control association study for the GNB3 locus in a group of 1233 Japanese (718 hypertensive and 515 normotensive subjects), where all participants were recruited at the same institute with relatively clear classification criteria. Allele frequencies of the T825 variant proved to be higher in Japanese (49.0% to 49.6%) compared with a reported prevalence of 25% to 31% in whites. Although the present study does not provide any support for the GNB3 association as previously reported, the lack of association has to be carefully interpreted, which may simply reflect the ethnic differences between the 2 ethnic groups.

Methods

Study Population
This study was approved by an institutional review committee. Participants in the present study comprised a total of 1424 individu-
Association Study of G Protein β3 Subunit

TABLE 1. Clinical Characteristics of Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive Subjects</th>
<th>Hypertensive Subjects</th>
<th>Hypertensive Subjects With IGT or NIDDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (Men/women)</td>
<td>515 (310/205)</td>
<td>718 (391/327)</td>
<td>191 (121/70)</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.8±11.3</td>
<td>63.2±9.8†</td>
<td>66.3±9.9†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.5±2.8</td>
<td>23.7±3.0†</td>
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<td>BP, mm Hg</td>
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<td></td>
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<tr>
<td>Systolic</td>
<td>117.2±12.7</td>
<td>160.5±19.1†</td>
<td>158.1±20.1†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.0±9.2</td>
<td>98.0±10.5†</td>
<td>96.1±12.4†</td>
</tr>
<tr>
<td>FPG, mg/dL</td>
<td>91.4±9.5</td>
<td>93.0±9.6†</td>
<td>127.8±36.5†</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>5.5±0.44</td>
<td>5.56±0.39</td>
<td>6.62±1.20†</td>
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Values are mean±SD unless otherwise indicated. IGT indicates impaired glucose tolerance; NIDDM, non–insulin-dependent diabetes mellitus; FPG, fasting plasma glucose; HbA₁c, glycosylated hemoglobin. Because HbA₁c was not measured in all normotensive subjects, values are calculated on available data alone (175 individuals) in the control group.

*p<0.01, †P<0.0001, normotensive vs hypertensive subjects.

Discussion

The present study is the first conducted to date to assess the relevance of the GNB3 gene to essential hypertension in a nonwhite population. Our failure to reproduce the positive association originally demonstrated in whites‡ has to be interpreted with considerable caution. Confounding factors such as population stratification and misclassification are known to frequently cause false-positive and false-negative results in case-control study.¹⁴,¹⁵ Although the Japanese are thought to be monoracial, some phylogenetic studies have

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**Discussion**

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reported that there is a moderate spectrum of genetic variation among persons living in different parts of the country.\textsuperscript{16,17} All the participants were therefore recruited at the same institute to minimize the chance of mixing populations with inherently diverse allele frequencies of a susceptibility gene. In addition, diagnosis of hypertension was basically determined after long-term clinical follow-up, which enabled us to reduce the risk of misclassification to some extent. Furthermore, our trial size involving over 1200 participants in the case-control study should have decreased the substantial sampling variation (or selection bias) that is generally unavoidable in case-control association strategy. This thereby lends us an appropriate, though far from sufficient, statistical power to evaluate a susceptibility gene exerting principal effects on BP regulation.

Three interpretations can be proposed to explain the lack of observed association in the Japanese population. First, the contribution of \textit{GNB3} to the pathogenesis of hypertension may be less significant than originally implicated\textsuperscript{4} in a given population, partly because of the population-specific combination of genetic and environmental factors. In the assessment of this interpretation, our data can be examined as follows. The odds ratio for the TT+TC versus CC genotype is 1.44 (95\% CI, 1.09 to 1.88) in the reported association study,\textsuperscript{4} while the corresponding odds ratio is 0.94 (95\% CI, 0.72 to 1.22) in the present study; both CIs partially overlap. If results for these 2 studies potentially represent the same likely than the hypothesis of “population-specificity”), the sample size required to confirm the susceptibility in question will be very large. For example, it is roughly estimated that >1000 individuals are required in each group of cases and controls for detecting odds ratio \( \approx 1.2 \) with 80\% power at a 5\% type I error probability.\textsuperscript{18} Thus, no conclusive claims can be made from our data because statistical power is insufficient based on this assumption. On the other hand, the higher frequency of T825 allele in Japanese may have hampered the detection of the potential association, although our trial size is larger than the previous study. With these arguments considered together, the T825 variant at least is unlikely to constitute major susceptibility for essential hypertension in the population studied.

Second, detailed characterization of the pathophysiological mechanisms may help further investigate the disease association of \textit{GNB3}. Given the moderate effects of \textit{GNB3} on hypertension as assumed above, an intermediate phenotype, which the molecular variant of the \textit{GNB3} gene itself represents, would allow for more appropriate evaluation of this candidate gene by dissecting the genetic heterogeneity of essential hypertension. There is also the possibility that a “true” mutation as yet unidentified, which is in linkage disequilibrium with the T825 variant, may exist in the \textit{GNB3} locus. If so, a search for other base-substitution polymorphisms, together with haplotype analysis,\textsuperscript{19} will provide a better chance to detect the potential association.

Third, the lack of association may result from an ethnic variation. Some of the phenotypic characteristics related to hypertension are known to show ethnic differences. For example, the prevalence of salt sensitivity is estimated to be higher in blacks than in whites.\textsuperscript{20} The likely presence of ethnic variation has been also indicated in the disease relevance of the angiotensinogen gene\textsuperscript{7–9}; that is, despite a number of studies supporting positive linkage and/or association as originally reported by Jeunemaitre et al\textsuperscript{21}, lack of linkage in Chinese\textsuperscript{6} and lack of association in a few black populations\textsuperscript{7,8} have been demonstrated. Accordingly, the issue of ethnic variation remains to be extensively explored for the \textit{GNB3} gene.

In summary, the present study does not replicate the association between the T825 variant of \textit{GNB3} and essential hypertension in the Japanese population, although there is a possibility that this particular polymorphism is uninformative in the Japanese. Use of an intermediate phenotype and/or haplotype analysis, as well as a genetic linkage analysis, would allow for further investigation of this candidate gene in relation to hypertension, and thereby the question of whether the lack of association in the Japanese population simply reflects ethnic differences can be answered.

\textbf{Acknowledgment}

We gratefully acknowledge Chie Fujinami for assisting us in DNA preparation and data arrangement.

\textbf{References}


\begin{table}[h]
\centering
\caption{C825T Genotypes in Each Group}
\begin{tabular}{lccc}
\hline
Genotype & Normotensive Subjects & Hypertensive Subjects* & Hypertensive Subjects With IGT or NIDDM \\
\hline
TT & 124 & 172 (138) & 50 \\
TC & 263 & 359 (272) & 87 \\
CC & 128 & 187 (143) & 54 \\
Frequency of T allele & 0.496 & 0.490 (0.492) & 0.490 \\
\hline
\end{tabular}
\end{table}


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