G-Protein β3 Subunit Gene (*GNB3*) Variant in Causation of Essential Hypertension

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Abstract—Essential hypertensives display enhanced signal transduction through pertussis toxin–sensitive G proteins. The *T* allele of a C825T variant in exon 10 of the G protein β3 subunit gene (*GNB3*) induces formation of a splice variant (Gβ3-s) with enhanced activity. The *T* allele of *GNB3* was shown recently to be associated with hypertension in unselected German patients (frequency=0.31 versus 0.25 in control). To confirm and extend this finding in a different setting, we performed an association study in Australian white hypertensives. This involved an extensively examined cohort of 110 hypertensives, each of whom were the offspring of 2 hypertensive parents, and 189 normotensives whose parents were both normotensive beyond age 50 years. Genotyping was performed by polymerase chain reaction and digestion with *Bse*DI, which either cut (*C* allele) or did not cut (*T* allele) the 268-bp polymerase chain reaction product. *T* allele frequency in the hypertensive group was 0.43 compared with 0.25 in the normotensive group (χ^2=22; *P*=0.00002; odds ratio=2.3; 95% CI=1.7 to 3.3). The *T* allele tracked with higher pretreatment blood pressure: diastolic=105±7, 109±16, and 128±28 mm Hg (mean±SD) for *CC*, *CT*, and *TT*, respectively (*P*=0.001 by 1-way ANOVA). Blood pressures were higher in female hypertensives with a *T* allele (*P*=0.006 for systolic and 0.0003 for diastolic by ANOVA) than they were in male hypertensives. In conclusion, the present study of a group with strong family history supports a role for a genetically determined, physiologically active splice variant of the G protein β3 subunit gene in the causation of essential hypertension. ***(Hypertension.* 1998;32:1094-1097.)**

Key Words: race ■ hypertension, essential ■ blood pressure ■ G protein ■ genetics

Increased activity of the Na^+-H^+ exchanger has been noted in up to half of patients with essential hypertension.[1,2] Since this protein is expressed in cells throughout the body, it has been studied most conveniently in lymphoblasts and fibroblasts. The data suggest that the higher Ca^{2+} mobilization and DNA synthesis in these cells[3] is not due to an alteration in the Na^+-H^+ exchanger or its expression but has its origin instead in altered pertussin toxin–sensitive G protein signal transduction.[4] G proteins are heterotrimeric, consisting of α-, β-, and γ-subunits, of which there are at least 17, 5, and 6 known isoforms, respectively. Genetic variation in Gα_i2, Gα_i3, Gβ1, and Gβ2 was excluded,[4] but a recent study has found a significant association of a polymorphism in the ubiquitously expressed, pertussis toxin–sensitive G protein β3 gene (*GNB3*; chromosome 12p13 [Reference 5]) with essential hypertension in 426 unselected patients in Germany.[6] The variant was seen after sequencing *GNB3* reverse transcriptase–polymerase chain reaction (PCR) products and involved a C/T polymorphism at nucleotide 825, which is in the exon 10 region of the *GNB3* cDNA. The nucleotide substitution results in the use of a cryptic splice acceptor site (AG: nucleotides 619 and 620) and cryptic branch site (nucleotides 579 to 585), which leads to alternative splicing within exon 9 (nucleotides 498 to 699), such that nucleotides 498 to 620 are deleted. As a result, amino acids 167 to 197 are missing from the encoded protein (designated Gβ3-s). The missing region resides within a set of 7 Trp-Asp (WD) repeats, each of which consists of ≈40 highly conserved amino acids (the C-terminal ones being Trp-Asp), and together these 7 WD repeats span amino acids 43 to 340 (the latter residue being at the COH terminus of the protein) to form a β-propeller structure.[7,8] In Gβ3-s 1 complete blade of the propeller is lost, comprising all of the fourth WD repeat apart from the last 5 amino acids and the last 4 amino acids of the third WD repeat. It thus appeared that a nucleotide outside of the splice donor and acceptor sites is responsible for the generation of this alternatively spliced transcript, and preliminary evidence for a possible influence of the C→T substitution on secondary structure of the pre-mRNA was obtained.[6] Cell lines and Sf9 insect cells expressing Gβ3-s, along with Gα_i2 and Gγ5, displayed much higher binding of [^{35}S]GTPγS when stimulated.[6] The enhanced G protein activation conferred by Gβ3-s may occur only in combination with certain $G_{\alpha i}$ and Gγ subunits, or it could be that Gβ3-s

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alters the coordinated formation of $G\beta\gamma$ heterodimers and their interaction with $G\alpha$.[6] A recent model predicts that loss of this blade results in an alteration in the position of critical β-propeller residues that contact the lip in $G\alpha$ that guards the likely GDP exit route.[9] This may account for the enhanced activity.

The preliminary genetic findings of Siffert and coworkers[6] provide support for suggestions that the onset of hypertension may work through a pertussin toxin–sensitive mechanism that produces gradual vascular hypertrophy rather than through vasoconstrictor mechanisms, since the latter involve effects of vasoconstrictor hormones on pertussin toxin–insensitive G proteins.

Since the validity of any genetic finding requires confirmation in different settings, the aim of the present study was to test for association of the *GNB3* variant in Australian white hypertensive patients. This involved hypertensives with a strong genetic background (2 hypertensive parents), a subset, representing ≈10% of the hypertensive population,[10] that have a greater likelihood of revealing an existing association than those with only 1 affected first-degree relative,[11] or an unselected hypertensive group, as used by Siffert and coworkers.[6] The group with 2 affected parents has, moreover, been the subject of a number of previous molecular genetic studies of hypertension.[9,10,12-14]

Methods

Subjects

The subjects used for the study were 110 unrelated, age- and sex-matched, nondiabetic, treated white essential hypertensive patients who had 2 hypertensive parents, and a control group of 189 normotensive subjects whose parents were normotensive past the age of 50 years. The research was approved by the University of Sydney Human Ethics Committee. Ascertainment details of the study groups have been described previously.[9,11,12] Characteristics of each group are shown in Table 1.

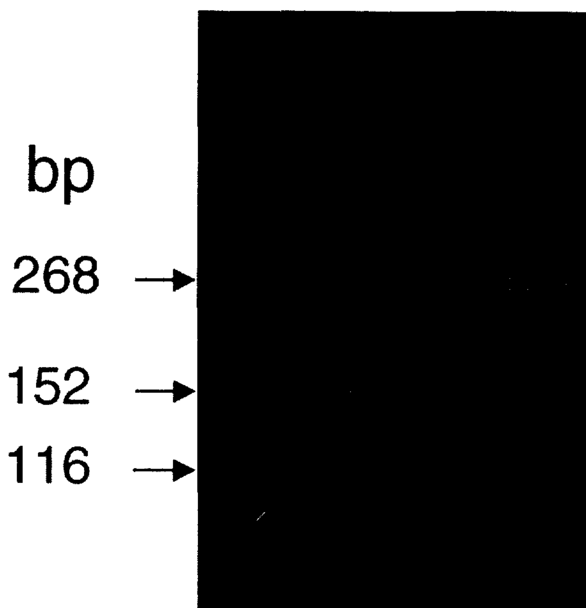
Genotyping

DNA was isolated from whole blood by a modified salting-out method.[15] Genotypes for the C825T polymorphism were determined by PCR with the use of the following primers: sense, 5′-TGA CCC ACT TGC CAC CCG TGC-3′; antisense, 5′-GCA GCA GCC CAG GGC TGG C-3′ (synthesized by Bresatec, Adelaide, South Australia). After an initial denaturation step at 94°C for 5 minutes, 35 cycles of 94°C for 1 minute, 60°C for 45 seconds, and 72°C for 1 minute were performed with a 15-μL reaction mixture that contained 0.3 pmol each primer, 0.2 mmol/L each dNTP, 0.1 U AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, Conn), 56 mmol/L KCl, 11 mmol/L Tris-HCl, pH 8.3, and 2 mmol/L $MgCl_2$. To confirm results, samples were also subjected to a "hot-start" PCR protocol that involved 10 cycles of 94°C, 65°C, and 72°C for 1 minute each, followed by 15 cycles of 94°C, 60°C, and 72°C for 1 minute each, and finally 20 cycles of 94°C, 58°C, and 72°C for 1 minute each, finishing with a step at 72°C for 30 minutes. PCR products (15 μL) were then incubated with 0.1 U *Bse*DI (Fermentas, Vilnius, Lithuania) in 2.2 mmol/L Tris acetate, 0.7 mmol/L Mg acetate, 4.4 mmol/L K acetate, and 7 μg/mL BSA in a final volume of 19.4 μL at 60°C for 3 hours. The digests were then electrophoresed on a 2.5% agarose gel and visualized under UV light by ethidium bromide staining. *Bse*DI cuts at 5′-C ↓ CNNGG-3′ to give bands of 116 and 152 bp (*C* allele) or does not cut, leaving a 268 bp band (*T* allele).

TABLE 1. Demographic Parameters for Normotensives and Hypertensives

Parameter	n	NT	n	HT
Male/female	189	109/80	110	50/60
Age, y	189	48±10	116	52±12
BMI, kg/m²	189	26.0±4.3	105	26.1±4.6
Pretreatment systolic blood pressure, mm Hg	189	120±11	91	176±25*
Pretreatment diastolic blood pressure, mm Hg	189	73±8	91	110±18*

NT indicates normotensives; HT, hypertensives. Values are mean±SD.
*$P<0.0001$ vs NT group by *t* test.



Example of genotyping result for C825T polymorphism of *GNB3*. Shown is pattern of bands on ethidium bromide–stained gel after electrophoresis of *Bse*DI-digested PCR products from subjects homozygous for the *C* allele (152 and 116 bp), homozygous for the *T* allele (268 bp), and *CT* heterozygotes (268, 152, and 116 bp). *Hpa*II-cut pUC19 size marker (lane not shown) was used for each gel. The position and size of bands are shown on the left.

Plasma Assays

The methods used for determination of plasma lipid profile,[16] plasma renin concentration,[17-19] plasma angiotensinogen concentration,[20] and plasma angiotensin-converting enzyme activity[10] were as described previously.

Statistical Analyses

Total alleles on all chromosomes were calculated from genotype data, and χ^2 analysis was performed with StatView (Abacus Concepts). Comparison of different parameters across genotypes was by 1-way ANOVA.

Results

An example of the appearance of an ethidium bromide–stained gel on which *Bse*DI digests of PCR products were run is shown in the Figure. Genotypes were assigned on the basis of the pattern of bands on electrophoretic gels. The frequency of each genotype in each group was found to conform with Hardy-Weinberg equilibrium.[21] Genotype and derived allele

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TABLE 2. Comparison of Genotype and Allele Frequencies of the C825T Polymorphism of *GNB3* Between Hypertensive and Normotensive Groups

	Genotypes (Frequency)					Total Alleles on All Chromosomes (Frequency)			
Group	*CC*	*CT*	*TT*	χ^2	*P*	*C*	*T*	χ^2	*P*
HT	27	71	12			131	95		
	(0.25)	(0.65)	(0.11)			(0.57)	(0.43)		
				27	1.7×10^{-6}			22	1.6×10^{-5}
NT	101	82	6			284	94		
	(0.53)	(0.43)	(0.03)			(0.75)	(0.25)		

HT indicates hypertensive group; NT, normotensive group.

frequencies are shown in Table 2. Frequency of the minor allele (*T*) was 0.25 in the normotensive group and 0.43 in the hypertensive group. The difference was highly significant (Yates corrected $\chi^2=22$, $P=2.0\times10^{-5}$). *T* allele frequency was, moreover, similar in male (0.46) and female (0.41) hypertensives. Since the appearance of an association with hypertension can in certain cases be caused by a genotypic contribution to enhanced mortality or survival,[10] we also looked for differences in relation to age. In the 34 older (≥60 years) hypertensives, *T* allele frequency was 0.47, compared with 0.41 in the 76 aged <60 years ($\chi^2=0.6$, $P=0.4$; comparison with normotensives gave $\chi^2=14$ and $\chi^2=14.3$, $P=0.0002$ and $P=0.00015$, respectively). For those above or below the mean age (52 years), *T* allele frequency was also similar (0.42 and 0.44, respectively). Age for *CC*, *CT*, and *TT* was also similar: 51±11, 52±12, and 49±13 years, respectively, as was the case for those aged <60 or ≥60 years (67±7, 66±5, and 65±5 years).

Comparison of blood pressures between each genotype in the hypertensive group indicated tracking of both systolic and diastolic pressure with the *T* allele (Table 3). This was clearly evident in women but much less so in men and, for those

TABLE 3. Pretreatment Blood Pressure for Each Genotype of the *GNB3* Variant in Hypertensives With 2 Hypertensive Parents

	CC	*CT*	*TT*	F*	*P*
Systolic pressure					
Whole group	166±21	176±26	187±26	2.5	0.085
	(n=23)	(n=59)	(n=12)		
Men	174±21	168±20	175±16	0.6	0.6
	(n=9)	(n=25)	(n=6)		
Women	161±20	183±28	210±30	5.6	0.0064
	(n=14)	(n=34)	(n=3)		
Diastolic pressure					
Whole group	105±7	109±16	128±28	7.2	0.0013
	(n=23)	(n=59)	(n=9)		
Men	106±9	109±17	119±21	1.2	0.3
	(n=9)	(n=25)	(n=6)		
Women	104±6	109±15	145±38	5.6	0.0003
	(n=14)	(n=34)	(n=3)		

Values are mean±SD, expressed in millimeters of mercury.
*Results shown are from 1-way ANOVA.

women aged above the mean age, was similar to those aged below the mean age (data not shown).

Since body mass index (BMI) can affect blood pressure, we compared data for lean (BMI <26 kg/m², n=54) with obese (BMI ≥26 kg/m², n=51) hypertensives. No difference, however, was seen in genotype ($\chi^2=3.7$, $P=0.2$) or allele ($\chi^2=2.6$, $P=0.1$) frequencies. Moreover, BMI did not differ between each genotype in the hypertensive group, either for the group as a whole ($P=0.2$ by ANOVA) or for just men ($P=0.3$), for just women ($P=0.6$), or for lean ($P=0.9$) or obese ($P=0.4$) subgroups. In the obese hypertensive subgroup, systolic pressures were 165±8, 176±28, and 196±22 mm Hg for *CC*, *CT*, and *TT* ($P=0.08$), and diastolic pressures were 110±6, 113±19, and 129±31 mm Hg ($P=0.1$), whereas in the lean hypertensives only diastolic pressure tracked with the *T* allele: 104±6, 107±11, and 125±21 mm Hg, respectively ($P=0.02$).

Age of onset of hypertension for hypertensives with 2 affected parents was 32±10 (SD) years, and this did not differ between each genotype. Plasma lipids (mean±SE) in the normotensive and hypertensive groups were, respectively (mmol/L): cholesterol, 5.2±0.1 and 5.8±0.1 ($P=0.0001$ by *t* test); triglyceride, 1.5±0.08 and 2.5±0.2 ($P=0.0001$); HDL cholesterol, 1.3±0.04 and 1.1±0.06 ($P=0.006$); and LDL cholesterol, 3.2±0.09 and 3.6±0.1 ($P=0.008$). Plasma renin (mean±SE) was 8.3±0.6 and 9.9±1.2 pmol angiotensin I per hour per milliliter in the normotensive and treated hypertensive groups, respectively ($P=0.2$); angiotensin-converting enzyme was 85±2.3 and 87±5 nmol Gly-Gly per minute per milliliter, respectively ($P=0.7$); and angiotensinogen was 1166±19 and 1318±36 pmol/mL, respectively ($P=0.0001$). All of the various plasma parameters were similar for each genotype (data not shown).

Discussion

The present study has found a highly significant association of the C825T polymorphism of *GNB3* with essential hypertension. This result therefore confirms and extends the findings of Siffert and coworkers.[6] The *T* allele frequency in our normotensive group was 0.25, which is identical to that observed by Siffert et al. In contrast to these workers, we used a group of hypertensives who were strongly selected for genetic predisposition, and we were able to demonstrate a much higher frequency of the hypertension-associated *T* allele of 0.43 compared with the value

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of 0.31 obtained in the Siffert study. The latter used patients from Berlin, Essen, and Heidelberg who were not specifically selected for family history of hypertension. Consequently, the *P* value we obtained (0.000016) by χ^2 analysis was more highly significant than that of Siffert et al (0.008),[9] even though we tested 110 hypertensive patients compared with their number of 426. Our results thus lend further support to our previous findings that indicate a not unexpected greater likelihood of demonstrating an existing genetic association with hypertension when a population with a strong family history (2 hypertensive parents) is used.[11]

Further evidence of a role of the C825T variant in hypertension was provided by our observation of significant tracking of blood pressure with the *T* allele. This was significant in the women. It could be that female hypertensives exhibit a greater response to hormonal or some other sex-specific parameter(s) that affect receptor mechanisms linked to pertussis toxin–sensitive G proteins, so as to lead to an amplification of any effect involving Gβ3-s in vascular smooth muscle or other relevant tissues. It was also clear that the effect on blood pressure was influenced by the number of *T* alleles, with *TT* having higher blood pressure than *CT*. It is possible that *T* is a recessive allele.

To further confirm the results of association studies, linkage analyses can be performed. However, for determination of correlated transmission within a pedigree, ie, concordant inheritance,[22,23] as opposed to correlated occurrence of a disease and an allele in a population, the use of highly informative markers, such as microsatellites, is preferred because of their high heterozygosity ($\approx$0.8; compared to 0.43 for the C825T biallelic variant). Since *GNB3* has been mapped physically[5] but not genetically, any such linkage study will require the testing of a number of markers in the vicinity of chromosome 12p13, and we have commenced such an analysis, involving several microsatellites in affected sib pairs. A positive linkage finding applies to a broader region of DNA[24] than is the case for an association result, because in the latter linkage disequilibrium extends over very short distances in an old population,[23] ie, a linkage region will contain numerous genes, and it then remains to be determined which one (or more) is the cause of the disease.

In the present study, the age of our hypertensives (52$\pm$12 years) was lower than the age of hypertensives used by Siffert et al (57$\pm$14 years),[6] consistent with an earlier age of onset (32$\pm$10 years) and more severe hypertension (176$\pm$25/110$\pm$18 mm Hg) for our hypertensives with 2 hypertensive parents compared with the unselected hypertensive population used by Siffert et al (159$\pm$22/99$\pm$14 mm Hg).[6]

In conclusion, the present study provides strong evidence in favor of an association of the functionally significant *T* allele of *GNB3*, and thus the splice variant, Gβ3-s, in the causation of hypertension.

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