

# Association of the $G_s\alpha$ Gene With Essential Hypertension and Response to $\beta$ -Blockade

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**Abstract**—We examined whether the *GNAS1* locus, encoding the  $G_s$  protein  $\alpha$ -subunit ( $G_s\alpha$ ), is implicated in the genetic causes of essential hypertension. A common silent polymorphism (ATT→ATC, Ile<sup>131</sup>) was identified in exon 5 of the  $G_s\alpha$  gene by single-strand conformation polymorphism analysis and DNA sequencing. This polymorphism consists of the presence (+) or absence (−) of a restriction site for *FokI*. Only 1 other rare allele was found in the coding region; the high GC content of the 5′ noncoding sequence prevented mutation scanning of the promoter region of the gene. There was a significant difference in frequency of the *FokI* alleles between 268 white hypertensives (*FokI*+:*FokI*−, 51%:49%) and a matched group of 231 control subjects (*FokI*+:*FokI*−, 58%:42%) ( $P=0.02$ ). Multiple regression analysis showed that the *FokI* genotype was independently related to the level of untreated systolic blood pressure in 294 well-characterized white hypertensives ( $P=0.01$ ) but not in normotensives. The influence of the *FokI* allele on blood pressure (BP) response to  $\beta$ -blockade was examined in 114 of the patients randomly assigned to this class of drug. Significant differences in frequency of the *FokI* allele were observed in the good responders (*FokI*+:*FokI*−, 62.5%:37.5%,  $n=36$ ) versus the poor responders (*FokI*+:*FokI*−, 41.7%:58.3%,  $n=30$ ) after  $\beta$ -blocker therapy ( $P=0.02$ ). In a multiple regression analysis, the  $G_s\alpha$  genotype was the only independent predictor of BP response. These results suggest that the *GNAS1* locus might carry a functional variant that influences BP variation and response to  $\beta$ -blockade in essential hypertension. (*Hypertension*. 1999;34:8-14.)

**Key Words:** G proteins ■ hypertension, essential ■ genetics ■ polymorphism

The  $\beta$ -adrenoceptor ( $\beta$ AR)– $G_s$  protein system is essential to the activation of adenylyl cyclase in cardiac and vascular smooth muscle and therefore plays an important role in neuroendocrine regulation of cardiac output and peripheral resistance.<sup>1,2</sup> Abnormalities of  $G_s$  proteins have been an obvious target for investigation in essential hypertension (EH).

The G proteins mediate signal transduction across cell membranes.<sup>3</sup> The unique  $\alpha$ -subunit of each heterotrimeric G protein contains the guanine nucleotide-binding site, has intrinsic guanosine triphosphatase activity, and confers the functional specificity on each G protein that allows it to discriminate among multiple receptors and effectors. In the cardiovascular system, the  $\alpha$ -subunit of  $G_s$  ( $G_s\alpha$ ) couples  $\beta_1$ AR and  $\beta_2$ AR to the stimulation of cAMP production.

Several pharmacological and biochemical abnormalities have been reported in the  $G_s$  protein-linked pathway in animal models and patients with EH. First, in spontaneously hypertensive rats, the reduced relaxation by  $\beta$ AR agonists of femoral arteries is due to a functional loss of  $G_s$  protein.<sup>2</sup> Second, in the renal vasculature of young spontaneously hypertensive rats, vasodilators are ineffective in counteracting the vasoconstrictor effect of angiotensin II, and this defect is related to reduced coupling of the vasodilator receptors to

$G_s$  proteins.<sup>4</sup> Third, in Milan hypertensive strain rats, the amount of  $G_s\alpha$  protein is reduced in vascular membranes<sup>5</sup>; paradoxically, increased response of adenylyl cyclase activity in the membranes of vascular smooth muscle cells derived from thoracic aortas seems to reflect increased  $\beta$ AR coupling to  $G_s$  protein.<sup>6</sup> Finally, studies of lymphocyte membranes from untreated hypertensive (HT) patients demonstrate reduced functional activity of  $G_s$  protein and functional uncoupling of  $\beta$ AR from  $G_s$  protein.<sup>7-9</sup> These studies, although not entirely consistent, have pointed to alterations in  $G_s\alpha$  protein as 1 of the possible mechanisms responsible for the pathogenesis of EH. The studies alone, however, cannot distinguish between a primary phenomenon and a process secondary to the HT state itself. Large-scale studies of cardiac or vascular  $G_s\alpha$  in hypertensive humans are not feasible. Studies of the  $G_s\alpha$  gene provide a better chance to discern primary changes. Indeed, a significant linkage of the  $G_s\alpha$  locus to hypertension has recently been shown in a quantitative trait locus study in experimental hypertension.<sup>10,11</sup>

In the present study, therefore, we examined whether the  $G_s\alpha$  gene might be implicated in human EH. We first looked for genetic variability in the coding region of the  $G_s\alpha$  gene in a group of HT patients. A frequent silent polymorphism was

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**TABLE 1. Oligonucleotide Primers for Amplification of Exons and Splice Sites of the  $G_s\alpha$  Gene**

Exon	Primer Sequence	Primer Position	PCR Product, bp
1A	F 5'-TCC TCC TCC TCC TCC TTC CAC-3'	-92*	566
	R 5'-CGG TAG ACC TGC TTG TCC TTC TGC-3'	49†	
1B	F 5'-AGA AGG CGC AGC GTG AGG CCA AC-3'	70†	367
	R 5'-GCG AGA GAA GAG AGA GAC ATG AGC-3'	+252‡	
2	F 5'-AAC AGC AGA CCT CCC TGC CCA A-3'	-50*	190
	R 5'-TCT GTT CCT CTT ACT TGG TGC-3'	+26‡	
3	F 5'-GAG ACT GTG TGG GGT TTG TGT-3'	-52*	195
	R 5'-TCT GGC CTC AGT TTC CCA GTA-3'	+58‡	
4-5	F 5'-CTC CTA ACT GAC ATG GTG CAA-3'	-34*	345
	R 5'-TAA GGC CAC ACA AGT CGG GGT-3'	+10‡	
6	F 5'-AGT TCA AGC TCT TGC CTT TCT C-3'	-26*	183
	R 5'-TGT TTT ATG TGC TGA TGG GTT GGG-3'	+15‡	
7-8	F 5'-CTG TGA ACA CCC CAC GTG TCT TTC-3'	-13*	357
	R 5'-TGG GGT GAA TGT CAA GAA ACC ATG-3'	+36‡	
9-10	F 5'-TCC CTC TGG AAT AAC CAG CTG TCC-3'	-14*	385
	R 5'-CTA TAA ACA GTG CAG ACC AGG GCC-3'	+41‡	
11	F 5'-CGC TTC TCC CAA GCA TTC ACA CGG-3'	-16*	203
	R 5'-TGA ACA GCC AGC AAG AGT GGA AGC-3'	+10‡	
12	F 5'-TTC AGG AGC TAC AGA GAT GCT AGC-3'	-57*	192
	R 5'-AAG AGG AGG AAC AAG AGA GGA AAC-3'	+21‡	
13	F 5'-ACT GAC AAG TCC CCT TGT TTG TGC-3'	-7*	269
	R 5'-GCC CTA TGG TGG GTG ATT AAC TGC-3'	+69§	

Annealing of primers to DNA template was performed at 65°C except for exon 12, which was optimal at 60°C. F indicates forward primer; R, reverse primer.

\*Primer position (3' end) upstream of the beginning of the exon.

†Primer position (3' end) upstream of the end of the exon 1.

‡Primer position (3' end) downstream of the end of the exon.

§Primer position (3' end) downstream of the termination codon.

detected by single-strand conformation polymorphism (SSCP) and DNA sequencing analyses in 1 of the exons of the  $G_s\alpha$  gene. This was investigated for possible association with the disease by comparison of allele frequencies in a large group of HT patients and age- and gender-matched normotensive (NT) control subjects in whom accurate untreated BP levels had been recorded. We also investigated the influence of the allelic variation on quantitative difference in BP and a response to anti-HT drug by a  $\beta$ -blocker.

## Methods

### Study Population

#### HT Patients

Two hundred sixty-eight unrelated white study subjects were drawn from the Hypertension Research Clinic (HRC) at Addenbrooke's Hospital, Cambridge, England, which has recruited untreated patients since 1986.<sup>12</sup> Eligibility criteria for entry to the HRC were an age of 18 to 75 years; systolic blood pressure (SBP) >160 mm Hg and/or diastolic blood pressure (DBP) >90 mm Hg (from a Datascope) on 3 occasions over 3 months; and absence of secondary causes of hypertension. Patients in the HRC were randomly allocated by computer to open-labeled treatment with 1 of 4 standard antihypertensive drugs given initially as monotherapy for 4 weeks. Of the 663 HT patients on the HRC database, 160 previously untreated white HTs were randomized to  $\beta$ -blocker therapy, and of these

patients, 92 were available for study. An additional 22 untreated white HTs recruited since 1993 to a second rotational study of the drug groups were also included in the study.<sup>13</sup>

#### NT Subjects

Two hundred thirty-one unrelated white NT control subjects with BP <150/90 mm Hg were selected from the database of a large community survey of cardiovascular risk factors conducted in the same region between 1990 and 1994. Thirty-two thousand subjects 40 to 70 years old with no history of cardiovascular or other diseases were recruited from 43 local general practices. An average of 3 BP readings from the same Datascope were recorded along with demographic details. The present study was approved by the district ethical committee, and the subjects recruited gave informed consent.

### Detection and Identification of Polymorphism of the $G_s\alpha$ Gene

#### Polymerase Chain Reaction-SSCP Analysis

Genomic DNA was extracted from peripheral blood leukocytes according to a standard protocol.<sup>14</sup> The human  $G_s\alpha$  gene on chromosome 20q13.2-13.3 includes 13 exons and 12 introns.<sup>15,16</sup> The primers (Table 1) for polymerase chain reactions (PCR) were designed within intronic sequences to flank either 1 or 2 exons. Reactions were performed with 100 ng genomic DNA in a total volume of 25  $\mu$ L that contained 1  $\mu$ mol/L of each primer, 0.2 mmol/L of each dNTP, 1.5 mmol/L MgCl<sub>2</sub>, 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 0.1% Triton X-100, and 1 U *Taq* DNA polymerase. Samples underwent 32 to 35 cycles of PCR. Aliquots of

PCR products were diluted 1:2 in 98% formamide, heat-denatured, and subjected to nonradioactive SSCP analysis with the PhastSystem (Pharmacia LKB).

#### Direct DNA Sequencing of SSCP Variants

Any exon showing SSCP variant bands was sequenced, together with control samples, in both DNA strands. Direct DNA sequencing was performed with an ABI Dye Terminator Cycle Sequencing kit (Perkin Elmer), and subjected to an ABI DNA sequencer.

#### PCR and DNA Sequencing of GC-Rich Exon 1

The GC-rich exon 1 of the  $G_s\alpha$  gene was amplified by including the nucleotide analogue 7-deaza-2'-dGTP ( $dc^7GTP$ )<sup>17</sup> because standard PCR was hampered by the generation of nonspecific products. The sequencing of the PCR products was analyzed as above.

#### Restriction Enzyme Analysis of Polymorphisms

Polymorphisms detected in exons were confirmed with an independent technique by prediction and detection of an altered restriction site. In the case of both the Ile<sup>131</sup> and Asn<sup>371</sup> polymorphisms detected in exons 5 and 13, respectively, enzyme digestion was performed in a 15- $\mu$ L reaction containing 10  $\mu$ L PCR product and 3 U *FokI* (Promega) at 37°C for 16 hours.

#### Statistical Analysis

Data were analyzed with the SPSS for Windows (Release 6.1) statistical package. Differences in genotype and allele frequencies between 2 groups were assessed by the Pearson  $\chi^2$  test. Two sets of continuous variables were compared by the Student *t* test or the Mann-Whitney *U* test for nonnormally distributed variables. Potential confounding variables for predicting BP and  $\Delta$ BP were assessed by a multiple-linear regression analysis. A further analysis on  $\Delta$ BP values was performed after use of Oldham's transformation  $\{\Delta BP / [(BP1 + BP2)/2]\}$  to correct for correlation with pretreatment BP.<sup>18</sup> A value of  $P < 0.05$  was considered significant.

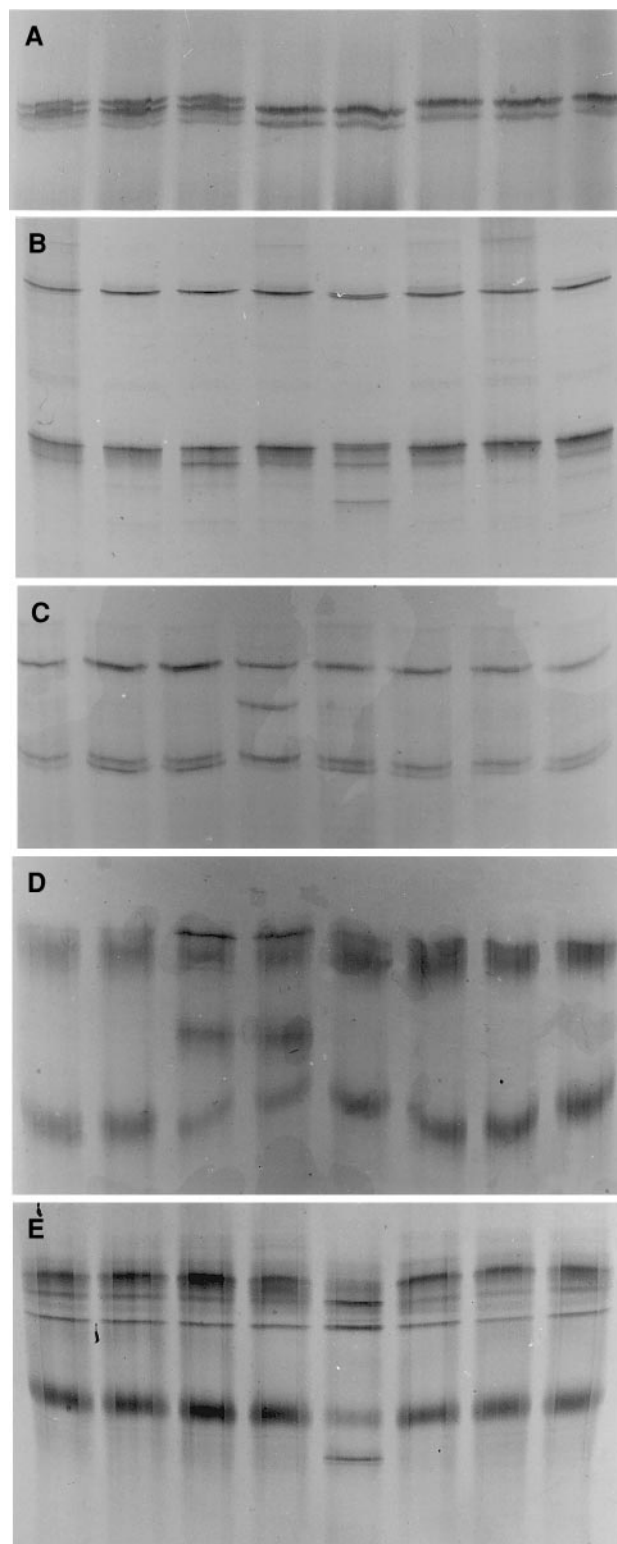
## Results

### Molecular Scanning of the $G_s\alpha$ Gene in EH

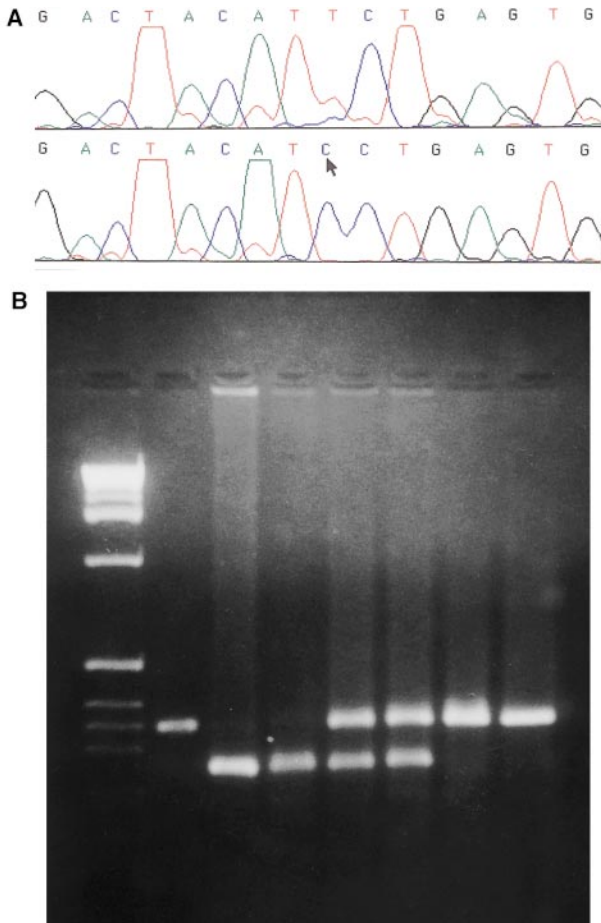
SSCP analysis of exons 2 to 13 and their splice sites was obtained by PCR-amplified fragments from genomic DNA of 40 HT subjects, and 5 different aberrant migration patterns were revealed (Figure 1). Subsequent DNA sequencing of the PCR products identified 5 base substitutions, 2 of which were within the coding regions of the  $G_s\alpha$  gene. The T→C transition (Figure 2A) within codon 131 (ATT→ATC, Ile) of exon 5 creates a target site for the endonuclease *FokI* (Figure 2B). The frequency of the *FokI*+ allele was 53% in 40 cases. By coincidence, the single base change within codon 371 (AAC→AAT, Asn) of exon 13 from 1 heterozygous patient abolishes a recognition sequence of the *FokI*. Another 3 rare nucleotide substitutions were detected in the flanking intron sequences but not in the splice junctions. Satisfactory PCR-SSCP analysis of the GC-rich exon 1 could not be obtained. The modified PCR including the nucleotide analogue  $dc^7GTP$  achieved specific amplification of this region. Subsequently, direct DNA sequencing was undertaken from HT patients homozygous for the exon 5 polymorphism. No polymorphisms were found in exon 1 from these samples.

### Association of $G_s\alpha$ Exon 5 Polymorphism With EH

On the basis of the hypothesis that EH in humans might be influenced by a functional mutation within the  $G_s\alpha$  gene, which is in linkage disequilibrium with 1 of the silent polymorphisms in the  $G_s\alpha$  locus, we performed a case-control study with the



**Figure 1.** SSCP analysis of PCR-amplified fragments of the  $G_s\alpha$  gene. Samples were run on PhastGel at 4°C and 15°C and silver-stained. A, 2 extra bands in lanes 1 to 3 and 2 bandshifts in lanes 4 and 5 that were detected only at 4°C. B, Extra band in lane 5 detected only at 4°C. C, Extra band in lane 4 detected only at 15°C. D, Extra band in lanes 3 and 4 detected only at 4°C. E, Extra band in lane 5 detected only at 4°C.



**Figure 2.** A, Direct sequence analysis of the exon 5 polymorphism. Samples were denatured at 90°C and analyzed with an ABI 373A DNA sequencer. The sequencing electropherograms are from 2 homozygous individuals. The top electropherogram shows T at position 393 encoding Ile<sup>131</sup>, and the bottom shows polymorphic base C (arrow) at position 393 encoding Ile<sup>131</sup>. B, *FokI* digestion confirmation of the exon 5 polymorphism. Samples were digested with *FokI* at 37°C for 16 hours and separated by a 1.7% agarose gel electrophoresis. Lane M is 1-kb DNA ladder, and lane 1 is undigested control. Lanes 2 and 3 show the presence of the cut band at 259 bp in length (*FokI*+ genotype), lanes 6 and 7 show the presence of the uncut band at 345 bp in length (*FokI*- genotype), and lanes 4 and 5 show the presence of both the uncut and the cut bands (*FokI*+ genotype).

most frequent exon 5 polymorphism. Baseline parameters from the HT and NT populations are presented in Table 2. Both groups were in Hardy-Weinberg equilibrium. Significant differences were observed in the frequencies of the *FokI* alleles ( $\chi^2_1=5.37$ ,  $P=0.02$ ) and genotypes ( $\chi^2_2=6.51$ ,  $P=0.04$ ) between the HT and NT groups. Interestingly, separate  $\chi^2$  analysis of frequencies of the *FokI*- and *FokI*+ alleles of each age group between the HT and NT subjects showed a significant difference only in the >59-year age group (53%:47% versus 38%:62%,  $\chi^2_1=5.02$ ,  $P=0.03$ ), but not in the age groups 40 to 49 and 50 to 59 years ( $\chi^2_1<1.0$ ,  $P>0.3$ ). The results of multiple regression analysis of baseline BP on age, gender, BMI, alcohol intake, and *G<sub>s</sub>α* genotype are shown in Table 3. In the control group, *G<sub>s</sub>α* genotype did not influence BP variation. By contrast, *G<sub>s</sub>α* genotype ( $P=0.03$ ) was second only to age ( $P=0.00001$ ) as

**TABLE 2. Baseline Characteristics and Genotype and Allele Frequencies of Hypertensive Patients and Normotensive Control Subjects**

Variable	Hypertensive	Normotensive	<i>P</i> *
Subjects, n	268	231	...
Gender, n (male/female)	165/103	127/104	NS†
Age, y	53.6 (0.5)	53.4 (0.5)	NS
BMI, kg/m <sup>2</sup>	26.4 (0.3)	25.5 (0.2)	0.01
Alcohol, u/wk	4.0 (0–84)‡	4.0 (0–42)‡	NS§
Cholesterol, mmol/L	6.0 (0.1)	5.8 (0.1)	0.01
SBP, mm Hg	172.8 (1.1)	123.7 (0.8)	0.0001
DBP, mm Hg	106.1 (0.6)	75.5 (0.5)	0.0001
<i>G<sub>s</sub>α</i> genotypes			
<i>FokI</i> - -	59 (22%)	32 (14%)	
<i>FokI</i> - +	144 (54%)	128 (55%)	
<i>FokI</i> + +	65 (24%)	71 (31%)	0.04†
<i>G<sub>s</sub>α</i> alleles			
<i>FokI</i> -	262 (49%)	192 (42%)	
<i>FokI</i> +	274 (51%)	270 (58%)	0.02†

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; *FokI*- +, absence/presence of *FokI* site; and NS, not significant with nominal  $P<0.05$ . One unit of alcohol is defined as 1 glass of wine. Values are expressed as mean (SEM) unless otherwise stated.

\*By Student *t* test unless otherwise stated.

† $\chi^2$  analysis.

‡Median (range).

§Mann-Whitney *U* test.

||Genotype and allele distributions are indicated in absolute values (percentages).

significantly independent predictors of SBP variation in the 268 HRC patients. The effect of *G<sub>s</sub>α* genotype on SBP variation was even more significant ( $P=0.01$ ) in a similar analysis of all 294 HT patients (HRC plus 26 young patients, age<40 years) and together with age accounted for 11% of SBP variation ( $P=0.00001$ ). In the analysis of DBP, alcohol consumption ( $P=0.04$ ) was the only weakly positive variable.

### Relationship of *G<sub>s</sub>α* Genotype With Variation in BP Response to $\beta$ -Blockers

To test for a possible relationship between variation in BP response to  $\beta$ -blockers and *G<sub>s</sub>α* genotype, 114 HT patients taking  $\beta$ -blockers were chosen for the study (Table 4). Among these patients, 36 subjects were classified as good responders, and 30 were classified as poor responders defined by a fall in mean arterial pressure >15 or <11 mm Hg, respectively. These values approximated to the top and bottom quartiles of the distribution of BP response to  $\beta$ -blockade in the cohort, expanded slightly to permit sufficient power for comparing their genotypes. The HRC randomization was concerned with drug class ( $\beta$ -blocker, diuretic, angiotensin-converting enzyme inhibitor, or  $\alpha$ -blocker) and not with individual drugs. However, there were no significant differences in the allocations of  $\beta$ -blocker types (non-selective or  $\beta_1$ -selective) between good and poor responders ( $P>0.05$ ). The *FokI*+ allele was much more common than the *FokI*- allele in the good responders (62.5% versus 37.5%), whereas the *FokI*- allele was more common

**TABLE 3. Multiple Regression Analysis of Baseline Blood Pressure**

	$\beta$ -Coefficient	P
Normotensives (n=231)		
SBP		
Age	0.35	0.00001
Gender	-0.13	0.04
BMI	0.13	0.04
Alcohol	-0.07	NS
G <sub>s</sub> $\alpha$ genotype	-0.003	NS
DBP		
Age	0.17	0.01
Gender	-0.26	0.0001
BMI	0.17	0.01
Alcohol	-0.03	NS
G <sub>s</sub> $\alpha$ genotype	0.03	NS
Hypertensives (n=294)		
SBP		
Age	0.31	0.00001
Gender	0.09	NS
BMI	0.10	NS
Alcohol	0.03	NS
G <sub>s</sub> $\alpha$ genotype	0.14	0.01
DBP		
Age	0.09	NS
Gender	0.02	NS
BMI	-0.08	NS
Alcohol	0.12	0.04
G <sub>s</sub> $\alpha$ genotype	0.08	NS

Abbreviations are as in Table 2. G<sub>s</sub> $\alpha$  genotype was coded 0, 1, or 2 with reference to the number of *FokI*+ allele.

in the poor responders (58.3% versus 41.7%) ( $\chi^2_1=5.70$ ,  $P=0.02$ ). The difference between the groups was even more marked for genotype, with 37% of the poor responders being *FokI*- - versus 11% of good responders; by contrast, almost twice as many of the latter were *FokI*++ versus the poor responders ( $\chi^2_2=6.48$ ,  $P=0.04$ , Table 4). In a multiple regression analysis of  $\Delta$ BP on age, gender, BMI, alcohol consumption,  $\beta$ -blocker type, and G<sub>s</sub> $\alpha$  genotype performed in the 66 good and poor responders, G<sub>s</sub> $\alpha$  genotype significantly influenced  $\Delta$ SBP ( $R^2=7.3\%$ ,  $P=0.03$ ) and  $\Delta$ DBP ( $R^2=8.6\%$ ,  $P=0.02$ ).  $\beta$ -Blocker type had only a minor effect on  $\Delta$ DBP ( $R^2=4.9\%$ ;  $P=0.04$ ). The significant relationships of G<sub>s</sub> $\alpha$  genotype with  $\Delta$ BP remained when the Oldham-corrected  $\Delta$ BP were entered into the multiple regression analysis. In a similar analysis of all 114 patients treated with  $\beta$ -blockers, the influence of G<sub>s</sub> $\alpha$  genotype was no longer a significantly independent predictor of  $\Delta$ BP, although the  $\beta$ -coefficient for genotype remained greater than for any other variables.

### Discussion

The gene encoding G<sub>s</sub> $\alpha$ , GNAS1, was the first candidate that we chose to study with the use of systematic mutation scanning before proceeding to a case-control association

study. This is important to emphasize because of the need with subsequent candidate genes to apply a correction for performing multiple comparisons.<sup>19</sup> The collection of large cohorts of untreated HT patients and NT control subjects over 10 years also permitted the influence of each allele or genotype on BP variation to be investigated quantitatively.

In the absence of functional data to indicate the explanation for the association we found with hypertension, it is unclear which of the alleles, *FokI*+ or *FokI*-, is implicated. Unlike linkage within families, which is due to a paucity of genetic recombination over 2 to 3 generations, genetic association in populations arises over hundreds of generations and may not extend far beyond the gene for which a disease association is found. However, we cannot say at present whether the silent *FokI* polymorphism is in linkage disequilibrium with a functional mutation in the G<sub>s</sub> $\alpha$  gene, and, if so, whether the phenotype might be increased production of cAMP in the heart, or reduced production in resistance arteries. As described in the Results, we performed modified PCR and DNA sequencing analysis of the GC-rich exon 1 only on HT patients homozygous for 1 of the *FokI* alleles. We were not able to exclude variants in the highly GC-rich 5' noncoding region or promoter region; this is an important omission because it is more likely that complex disorders such as essential hypertension are caused by multiple quantitative rather than single qualitative differences in phenotype and that the functional genetic variants therefore reside in regulatory rather than coding regions. The hypothesis that the silent polymorphism is in linkage disequilibrium with a functional variant in the G<sub>s</sub> $\alpha$  gene could be tested by determining the influence of the polymorphism on an intermediate phenotype, such as G<sub>s</sub>-dependent production of cAMP. Meanwhile, the association between the *FokI* polymorphism and  $\beta$ -blocker responsiveness provides some indirect evidence that there is a functional variant in the G<sub>s</sub> $\alpha$  gene itself. The locus for this on chromosome 20q13.2-13.3 has been mapped near that of the endothelin-3 gene,<sup>20</sup> but the distance between the 2 genes is not known.

The categorical comparison of genotype and allele frequency between hypertensives and control subjects showed an excess of the *FokI*- allele in hypertension, whereas the quantitative study of BP found the *FokI*+ allele to track with higher BP. The third finding was that the *FokI*+ and *FokI*- alleles were associated with good and poor responsiveness to  $\beta$ -blockade, respectively. One possibility is that the *FokI*- allele is associated with a functional variant that contributes to hypertension or reduced responsiveness, perhaps due to reduced cAMP-mediated relaxation of resistance arteries. Alternatively, increased pressure and responsiveness to  $\beta$  blockade may be due to a functional variant in association with the *FokI*+ allele, perhaps causing increased cAMP-mediated cardiac contractility.

Because the finding of a positive association between the insertion allele of the angiotensin-converting enzyme gene with EH has been attributed to an age-dependent decrease in the frequencies of the deletion allele in the HT patients,<sup>21</sup> we also investigated the frequencies of the *FokI* alleles in different age groups. Interestingly, the differences in allele frequencies between HT and NT subjects appeared to be age

**TABLE 4. Clinical Characteristics and Genotype and Allele Frequencies of the Subjects Receiving  $\beta$ -Blockers**

Variable	Total	Good Responder	Poor Responder	<i>P</i> *
Subject, n	114	36	30	...
Gender, n (male/female)	68/46	20/16	19/11	NS†
Age, y	55.1 (1.0)	55.3 (1.8)	54.4 (2.1)	NS
BMI, kg/m <sup>2</sup>	26.3 (0.4)	26.0 (0.6)	26.2 (0.8)	NS
Alcohol, u/wk	3.0 (0–70)‡	4.0 (0–70)‡	3.5 (0–60)‡	NS§
Cholesterol, mmol/L	5.9 (0.1)	5.9 (0.2)	5.7 (0.2)	NS
Decrease in SBP, mm Hg	20.4 (1.4)	36.9 (1.8)	5.7 (1.4)	0.0001
Decrease in DBP, mm Hg	14.6 (0.9)	23.9 (1.3)	4.5 (0.8)	0.0001
$G_s\alpha$ genotypes				
<i>FokI</i> —	29 (25.5%)	4 (11%)	11 (37%)	
<i>FokI</i> —+	56 (49%)	19 (53%)	13 (43%)	
<i>FokI</i> ++	29 (25.5%)	13 (36%)	6 (20%)	0.04†
$G_s\alpha$ alleles				
<i>FokI</i> —	114 (50%)	27 (37.5%)	35 (58.3%)	
<i>FokI</i> +	114 (50%)	45 (62.5%)	25 (41.7%)	0.02†

Abbreviations are as in Table 2. Values are expressed as mean (SEM) unless otherwise stated.

\*Comparison of the good and poor responder groups by Student *t* test unless otherwise stated.

† $\chi^2$  analysis.

‡Median (range).

§Mann-Whitney *U* test.

||Genotype and allele distributions are indicated in absolute values (percentages).

specific, and the proportion of *FokI*+ to *FokI*— alleles reverses with increasing age. The influence of age was not itself statistically significant, but in  $\chi^2$  comparisons of HT and NT the value of  $\chi^2$  was many times higher in the oldest decade of patients. The relative excess in the frequency of *FokI*— alleles only in the elderly HT patients may suggest that the subjects with the *FokI*— allele tended to have a late onset of hypertension, consistent with the poorer response, in some studies, of older versus younger patients to  $\beta$ -blockade.<sup>22</sup> Alternatively, it is conceivable that, if *FokI*++ homozygosity is associated with increased cardiac cAMP production or more severe hypertension, such patients would be underrepresented in our cohorts of relatively well and previously untreated patients; they might be at risk of premature death or earlier treatment.

The identification of subgroups of the HT population that are more sensitive to certain anti-HT agents may help to uncover genetic determinants of the responsiveness and, in turn, help to recognize the susceptibility genes that are involved in the genetic causes of EH. There is considerable interindividual variation in BP response to  $\beta$ -blockers, which cannot be fully explained by differences in metabolism.<sup>13</sup> The relationship between  $G_s\alpha$  genotype and  $\beta$ -blocker response was not significant in the entire cohort. Although further studies in large cohorts are clearly necessary, we should consider the possibility that there is a rare allele at the GNAS1 locus with noncontinuous influence on BP responsiveness. In Liddle syndrome<sup>23</sup> or apparent mineralocorticoid excess,<sup>24</sup> the rare alleles would be found only at the extreme right of the distribution of response to amiloride or spironolactone, respectively. Similarly, the relatively weak associa-

tion between the *FokI* polymorphism of the  $G_s\alpha$  gene and EH could reflect either a small contribution from a common functional trait to BP variation in a substantial population of EH or a large contribution from a rare trait in a small number of patients. In either case, the influence of such a trait locus is unlikely to be detected by linkage analysis. A marker at the human GNAS1 locus has recently been shown to have no significant linkage in an affected sib-pair study.<sup>11</sup> This discrepancy might reflect the low heterozygosity of the marker used and low power of the linkage analysis to detect small gene effects and therefore does not exclude a positive association of the *FokI* polymorphism with hypertension.

In conclusion, the findings in the present study provide evidence in favor of an association of a genetic marker at the GNAS1 locus with EH in East Anglian subjects and point to a functional trait locus on chromosome 20q lying in or near the  $G_s\alpha$  gene that may contribute to EH. The GNAS1 locus may also be involved in the genetic control of BP responsiveness in the subgroup that received  $\beta$ -blockers. Despite the greater power of the association approach, our study has illustrated the great care that is required to appreciate selection bias in samples of patients.

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