

Obesity reveals an association between blood pressure and the G-protein $\beta 3$ -subunit gene: a study of female dizygotic twins

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The 825C>T polymorphism of the G-protein $\beta 3$ -subunit gene (*GNB3*) has been associated with hypertension, although results are not entirely consistent. In a sample of 282 female Caucasian dizygotic twins aged 21–80 years, we aimed to investigate the associations between blood pressure and five single nucleotide polymorphisms (SNPs) including the 825C>T and haplotypes of the *GNB3* gene. The polymorphisms (–350A>G, 657A>T, 814G>A, 825C>T and 1429C>T) were genotyped by polymerase chain reaction-restriction enzyme assays. Regular association tests did not show a significant effect on blood pressure for any of the five SNPs. However, strongly significant interactions between the A-350G, 825C>T and 1429C>T loci and adiposity (both body mass index and waist circumference) were observed for systolic blood pressure ($P < 0.01$) as well as diastolic blood pressure ($P < 0.05$), suggesting increases in adiposity amplify the effects of the SNPs on blood pressure. Haplotype analyses confirmed the effects of the *GNB3* gene–obesity interaction on hypertension risk. Additionally, sib-transmission disequilibrium tests (sib-TDTs) showed significant associations with blood pressure for the 825C>T and 1429C>T loci. The presence of obesity reveals an

association between blood pressure and the *GNB3* gene in White females. Our data suggest that adiposity is a final pathway through which gene–lifestyle interactions may exert their effects on the development of hypertension. Our results from the combined SNP, haplotype and sib-TDT analyses also support the hypothesis that the 825C>T is a susceptibility locus for hypertension, whereas effects of other loci on blood pressure may result from their strong linkage disequilibrium with the 825C>T locus. *Pharmacogenetics* 14:1–9 © 2004 Lippincott Williams & Wilkins

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Introduction

The heterotrimeric G-proteins mediate intracellular signal transduction and are present in all cells. A polymorphism, 825C>T, in the $\beta 3$ subunit of the pertussis toxin-sensitive G_i -type proteins (*GNB3*) has been described both *in vitro* and *in vivo* [1]. The 825T allele was associated with alternative splicing, which shortens the protein by 41 amino acids. This results in enhanced G protein-mediated signal transduction, thus providing a multiplicity of mechanisms of relevance to the pathogenesis of hypertension [2].

There have been numerous studies indicating that the 825T allele is a determinant of hypertension. However, the currently available genetic association studies are not entirely consistent [3]. This is not unexpected for a multifactorial disease such as hypertension. There might be several reasons for this. The first is that only the 825C>T polymorphism has been investigated in detail. Roskopf *et al.* [4,5] described additional single nucleotide

polymorphisms (SNPs) and the haplotype structure of the *GNB3* gene, showing that the 825C>T polymorphism is part of a complex haplotype. Recent findings suggest that use of haplotypes can reduce inconsistencies observed in single marker analysis and may improve power to detect hypertension susceptibility genes [6]. However, *GNB3* haplotypes have not yet been used in association studies of hypertension or blood pressure.

The second reason might be population stratification, which can be controlled for using within-family association tests known as transmission disequilibrium tests (TDTs). Extensions of the classic twin study design to include measured genotypes have been recently described [7,8]. Use of dizygotic (DZ) twins has numerous advantages for the study of the genetics of common complex traits such as blood pressure. DZ twins are sib-pairs that are naturally matched for age and a range of possible environmental confounders, and are thus ideally suited for sib-TDTs.

Furthermore, the action of the 825T allele on blood pressure may be dependent on other factors such as age, sex, obesity or lifestyle, constituting a final possible reason for the lack of consistency in association studies [9–12]. For example, a recent study of 737 men and 775 women from a Caucasian population found clear sex differences, with effects of the 825C>T polymorphism on a range of cardiovascular and metabolic phenotypes in men, but not in women. In particular, higher daytime ambulatory systolic blood pressure (SBP) and diastolic blood pressure (DBP) was observed in TT homozygous males, but not in females [10].

The main purpose of the present study was to examine the associations of multiple SNPs and haplotype variation of the *GNB3* gene with blood pressure in a sample of 282 female Caucasian dizygotic twins. We also investigated whether there were interactions between individual SNPs and haplotypes of the *GNB3* gene with age, and adiposity measured by body mass index (BMI) and waist circumference.

Methods

Subjects

A total of 282 female Caucasian DZ twins (137 pairs and eight single twins) aged 21–80 years from the St Thomas' UK Adult Twin Registry were available for this study. Twins from the registry were ascertained from the general population through national media campaigns in the UK [13]. All twins participated in a comprehensive screening for risk factors of common chronic disease between March and November of 1999 and were unaware of the specific hypotheses tested. Informed consent was obtained from all subjects and the study was approved by the St Thomas' Hospital Trust Ethics' Committee. Zygosity was determined by standardized questionnaire and DNA fingerprinting was used for confirmation [13]. Information on medical history, medication use, lifestyle and demographic variables was obtained by standardized nurse administered questionnaire.

Measures

Subjects were interviewed and studied by trained research nurses. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Weight (light clothing only) was measured to the nearest 0.1 kg using digital scales. BMI was used as a measure of general adiposity and calculated as weight divided by height squared (kg/m^2). Waist circumference (cm) was used as an index of central adiposity and measured at the level midway between the lower rib margin and the iliac crest. Brachial blood pressure was measured using an automated cuff sphygmomanometer (OMRON HEM713C; Omron Healthcare (UK) Ltd, Henfield, UK) in the seated position under standardized conditions. SBP and DBP were measured three times. The

last two readings were highly correlated (0.90 for SBP and 0.92 for DBP) and averaged.

Genotyping

DNA was extracted from blood according to standard procedures. All the *GNB3* polymorphisms were detected by polymerase chain reaction (PCR) followed by restriction enzyme digestion assays, as previously described with minor modifications [4]. Genotypes were confirmed by direct sequence analysis with the use of a dye terminator kit on an ABI-377 automated sequencer. Genotyping was carried out in batches and each batch contained appropriate and verified allelic controls. To prevent observer bias, the investigators were unaware of sample origin and all gels were cross-checked by a separate investigator.

Analytical approach

The main purpose of our analyses was to test the association between SNP and haplotype variation in the *GNB3* gene with SBP and DBP. We further investigated whether the effect of the *GNB3* gene on blood pressure was dependent on age and/or adiposity by testing interactions of individual SNPs and haplotypes of the *GNB3* gene with these variables.

Association analysis

All regular association analyses were performed within a regression framework using generalized estimating equations, which takes the non-independency of twin data into account and yields unbiased *P*-values [14,15]. Analyses were performed separately for each of the SNPs and followed up by haplotype analyses. Both codominant (three genotype groups) and completely dominant (carriers versus non-carriers of rare allele) models for each SNP were tested. Age and adiposity (either BMI or waist circumference) were included as covariates in the models, as were their interactions with individual SNPs and haplotypes. To test the association of statistically inferred haplotypes with the continuous blood pressure traits, we used the haplotype trend regression (HTR) approach as outlined by Zaykin *et al.* [16]. Assuming additive effects of the haplotypes on the trait, the HTR approach tests for the contribution of individual haplotypes rather than haplotype pairs. HTR is more powerful than analysis of variance methods and naturally extends to the case where haplotype frequencies are not directly observed [16]. Briefly, HTR is based on the regression of a trait on a design matrix that includes the expected proportions of haplotypes. The contributions of haplotypes are weighted with the design matrix, such that unambiguous pairs of haplotypes are coded 1 for the haplotypes of homozygotes and 0.5 for each of the haplotypes of a heterozygote. All other haplotypes are coded as 0. However, the contributions of ambiguous pairs of haplotypes in the design matrix are based on the probabilities of haplotype pairs (divided by 2) as estimated by PHASE

2.0 software [17,18]. Haplotypes with estimated frequencies below 3% were pooled together and included in the model as one term. The most frequent haplotype was used as the baseline haplotype with which effects of the other haplotypes were contrasted [19].

Sib-TDTs

Sib-TDTs for quantitative traits were performed using structural equation modelling as described elsewhere [20,21]. Parameters were estimated by normal-theory maximum-likelihood, where the models were fitted to the raw data. Only DZ pairs discordant for their genotype are informative for the sib-TDTs. The locus effect on the quantitative trait was modelled using a parameterization in which a score a is assigned to A_1A_1 subjects, d to A_1A_2 subjects, and $-a$ to A_2A_2 subjects. Codominant (a and d estimated), additive (only a estimated, $d = 0$) and completely dominant (d equals a) models were tested for each locus. Comparing the full model including the genotype effect with the reduced model in which the effect of the genotype was set to zero, gives a chi-square test with 2 for the codominant and a chi-square test with 1 d.f. for the additive and dominant models. Analyses were adjusted for the effect of adiposity (BMI or waist circumference) by incorporating these variables as a linear regression on the trait value within the model. DZ twins are naturally matched for age in the sib-TDTs. Sib-TDTs were limited to SNPs because we are not aware of a sib-TDT method that allows for haplotype phase uncertainty by incorporating haplotype pair probabilities as used in the regular association test described above.

Statistical analysis

SBP and DBP were both log-transformed to obtain better approximations of the normal distribution. Preliminary analyses were performed using STATA 8 (StataCorp, College Station, Texas, USA). Hardy–Weinberg equilibrium was investigated by a chi-square test with 1 d.f. in one twin of each pair chosen at random to prevent inflated significance. Pairwise linkage disequilibrium (LD) coefficients were calculated using 2 LD [22] and reported as the ratio of the unstandardized coefficient to the maximal value ($D' = D/D_{\max}$) [23]. Haplotype frequencies for the five *GNB3* SNPs were estimated using PHASE 2.0 [17,18]. Both DZ twins of a pair were used for estimates of haplotype frequencies and D' . Ancestral relationships between inferred haplotypes with frequency > 1% were investigated using the reduced median (RM) network algorithm [24] with NETWORK 2.0 software (<http://www.fluxus-engineering.com>). Sib-TDTs were performed with the statistical software Mx [25].

Results

General characteristics of the female twin subjects are shown in Table 1. Almost 15% ($n = 42$) reported

current use of antihypertensive medication and these subjects were excluded from further quantitative trait analyses. An additional 18 subjects were diagnosed with untreated hypertension (mean SBP ≥ 140 mmHg and/or mean DBP ≥ 90 mmHg). Accordingly, a total of 60 women (21.3%) had hypertension. In an effort to use the potentially informative 42 subjects on antihypertensive medication, we compared SNP allele and haplotype frequencies between hypertensive cases ($n = 60$) and controls ($n = 222$) but found no significant differences (data not shown). For the 103 complete DZ twin pairs who did not use antihypertensive medication, twin correlations were 0.29 and 0.25 for SBP and DBP, respectively ($P < 0.01$). These results are in accordance with the majority of twin studies reporting SBP and DBP heritabilities between 40% and 60% [26,27].

Table 2 shows allele frequencies and pairwise LD coefficients (D') of the five *GNB3* polymorphisms in all subjects. The 657A>T and 814G> are relatively rare with minor allele frequencies of 2% and 6%, respectively. Apart from a non-significant D' between these two rare polymorphisms, strong LD is observed between all *GNB3* loci. None of the loci showed deviation from Hardy–Weinberg equilibrium.

Results for the regular association test for all five *GNB3* SNPs on both SBP and DBP are shown in Table 3. For the 657A>T locus, no homozygotes for the rare allele were observed. Only two individuals were homozygote for the 814A allele, and they were combined with the heterozygote group for the analysis. For the other three more common SNPs, codominant models (Table 3) as well as dominant models (data not shown) were tested, adjusted for either age and BMI, or age and waist circumference. In spite of some suggestive trends, none of main effects of the SNPs reached a statistical significance. Furthermore, none of the interactions between age and individual SNPs were significant. However, strongly significant interactions between the A-350G, 825C>T and 1429C>T loci and adiposity (both

Table 1 General characteristics of subjects

Variables	Values
n (subjects)	282
Age (years)	49.2 \pm 11.1
Height (m)	1.62 \pm 0.06
Weight (kg)	67.5 \pm 13.5
BMI (kg/m ²)	25.6 \pm 4.8
Waist circumference (cm)	80.2 \pm 10.4
SBP (mmHg)	118.23 \pm 15.13
DBP (mmHg)	74.67 \pm 10.28
Antihypertensive medication (%)	14.89
Hypertension (%) ^a	21.28

Data are mean \pm SD, unless stated otherwise. ^aSBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or antihypertensive medication. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2 Allele frequencies and pairwise linkage disequilibrium coefficients of *GNB3* polymorphisms in all subjects (D' below diagonal and P-value above diagonal)

	Physical distance (bp) ^a	Allele frequency	-350A>G	657A>T	814G>A	825C>T	1429C>T
-350A>G	-	0.33/0.67	-	0.020	0.000	0.000	0.000
657A>T	4075	0.02/0.98	-1.000	-	0.685	0.000	0.019
814G>A	1764	0.94/0.06	-0.513	0.486	-	0.000	0.008
825C>T	11	0.68/0.32	0.830	-0.999	-0.997	-	0.000
1429C>T	1587	0.73/0.27	0.832	-0.423	-0.742	0.864	-

^aPhysical distances between neighbouring single nucleotide polymorphisms.

Table 3 Results for the association tests between blood pressure and *GNB3* polymorphisms

Locus	Genotype	n	SBP (mmHg)	P ^a	DBP (mmHg)	P ^a
-350A>G	AA	30	115.3 ± 16.9	NS/NS	71.9 ± 10.8	NS/NS
	AG	98	116.3 ± 13.3		74.2 ± 9.4	
	GG	112	117.1 ± 13.9		73.9 ± 10.1	
657A>T	TT	232	116.8 ± 14.1	NS/NS	73.8 ± 9.9	NS/NS
	AT	8	109.9 ± 8.3		72.0 ± 8.6	
814G>A	GG	215	117.2 ± 14.1	NS/NS	74.0 ± 10.0	NS/NS
	GA + AA	25	110.5 ± 12.1		72.0 ± 8.4	
825C>T	CC	116	115.7 ± 13.3	NS/NS	73.3 ± 9.5	NS/NS
	CT	97	116.9 ± 14.8		73.9 ± 9.9	
	TT	27	118.8 ± 14.4		75.4 ± 11.5	
1429C>T	CC	130	115.6 ± 11.8	NS/NS	73.3 ± 8.8	NS/NS
	CT	96	117.3 ± 16.6		74.1 ± 11.3	
	TT	14	120.4 ± 13.9		76.5 ± 9.2	

^aAdjusted for: age and BMI/age and waist. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not significant.

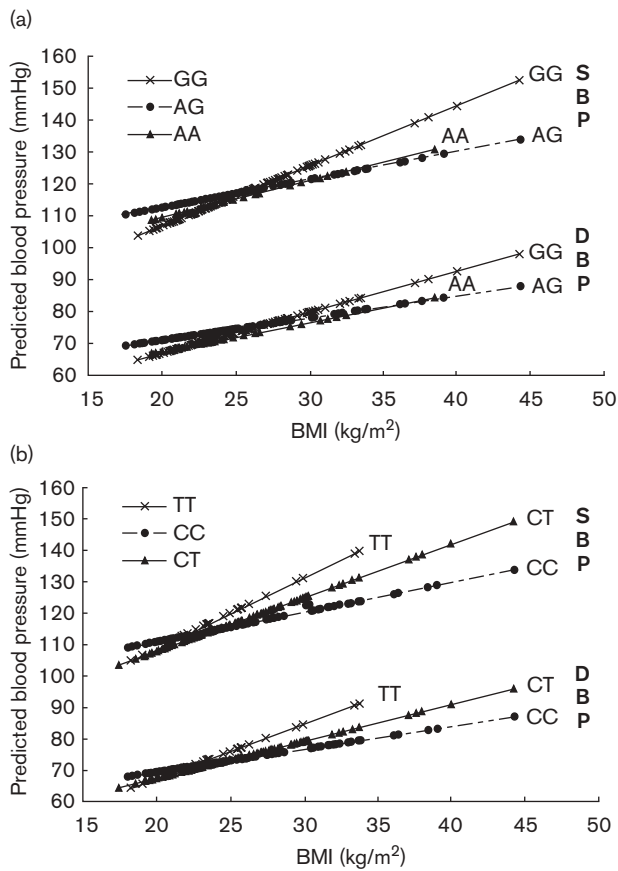
BMI and waist circumference) were observed for SBP ($P < 0.01$) as well as DBP ($P < 0.05$). GG homozygotes of the -350A>G locus always showed a steeper increase in SBP and DBP compared to AG heterozygotes and AA homozygotes with increases in BMI (Fig. 1a) or waist circumference (data not shown). For the 825C>T locus, TT homozygotes showed the steepest, and CC homozygotes the slowest, increase in SBP and DBP with increases in BMI (Fig. 1b) or waist circumference (not shown). Heterozygote individuals showed an intermediate increase. A similar pattern of interaction with general and central adiposity was observed for the 1429C>T locus, with carriers of the T allele showing the steepest increase in SBP or DBP with increases in BMI or waist circumference (data not shown). Further analyses splitting the sample in a normal weight (BMI < 25, $n = 137$) and an overweight (BMI ≥ 25 , $n = 103$) group showed that the effects of the -350A>G, 825C>T and 1429C>T loci on blood pressure was limited to the overweight group (Table 4).

Table 5 shows inferred haplotype frequencies of *GNB3* polymorphisms in all DZ twin subjects. Only three truly common haplotypes were observed, comprising 83% of the total. Two additional haplotypes (4 and 5) had frequencies of approximately 5% whereas the remaining eight haplotypes were rare (< 2%). Figure 2 shows a phylogeny of the eight most common (> 1%) inferred *GNB3* haplotypes in Table 5, allowing insight

into their ancestral relationships. Every haplotype is connected to the haplotype most similar to itself and represented by a circle whose area represents the overall frequency of that haplotype in the sample. SNPs that are different between haplotypes are shown on the connecting lines. Reticulations often suggest recombinant haplotypes. For example, haplotype 4 is best explained as a recombinant between the highly frequent haplotypes 1 and 3. An analysis of nonhuman primates [4] indicated that the ancestral *GNB3* gene harbored the -350G, 814G, 825C and 1429C alleles. Thus, the most common haplotype 1 is the ancestral haplotype.

Because the 657A>T polymorphism showed no variation among the seven most common haplotypes, this locus was excluded from the haplotype association analyses. That is, haplotype frequencies were re-estimated using only the -350A>G, 814G>, 825C>T and 1429C>T SNPs. The results of these haplotype analyses are shown in Table 6. No significant main effects of *GNB3* haplotypes on blood pressure were found. The inclusion of haplotype-adiposity interactions always led to a significant improvement of the overall model for both SBP and DBP with haplotypes GGTT and GGTC responsible for the effect (Table 6). The beta coefficient for the GGTT-BMI interaction ($P = 0.054$) on SBP was 1.5, which means that individuals homozygous for this haplotype show a 1.5 mmHg

Fig. 1



(a) $-350A>G$ genotype and body mass index (BMI) interaction on systolic ($P = 0.001$) and diastolic blood pressure ($P = 0.014$). (b) $825C>T$ genotype and BMI interaction on systolic ($P = 0.008$) and diastolic blood pressure ($P = 0.015$).

steeper increase in SBP per unit increase of BMI compared to the most common reference haplotype. Similarly, the beta coefficient for the GGTC–waist interaction ($P = 0.042$) on DBP was 0.75 (i.e. individuals homozygous for GGTC show a 0.75 mmHg

steeper increase in DBP per cm increase in waist circumference compared to the baseline haplotype). The differentiating characteristic of both these haplotypes is the 825T allele at position 3 compared to the most common haplotype GGCC. Based on simulations, Lake *et al.* [19] suggest that haplotype frequencies need to be at least 5% to avoid biased regression parameters. However, pooling the AACC haplotype (3.8%) with the other rare haplotypes in a rest category did not change the results (data not shown).

For the 657A>T and 814G> loci, very few DZ pairs were informative for the sib-TDT, such that analyses were only performed for the three common SNPs (Table 7). Only additive (a) and completely dominant ($d = a$) models are shown, because dominance deviation (d) in the codominant models ($a + d$) was never significant (i.e. $d = 0$). The 825C>T polymorphism showed a significant association with SBP in both additive and dominant models, irrespective of adjustment for adiposity. Association with DBP in the dominant model was of borderline significance. The same pattern was found for the association between the 1429C>T locus and SBP, although the significance was only borderline for the raw data (i.e. unadjusted for adiposity).

Discussion

In this cohort of female twins, we comprehensively examined the influence of GNB3 polymorphisms ($-350A>G$, 657A>T, 814G>, 825C>T and 1429C>T) and informative haplotypes on SBP and DBP. Regular association tests did not show any significant findings between these polymorphisms and blood pressure. However, strong interactions between the three common polymorphisms ($-350A>G$, 825C>T and 1429C>T) and adiposity were observed for SBP as well as DBP. Increases in both BMI and waist circumference amplified the effects of the SNPs on blood pressure in a dose–response fashion for T allele carriers of both the 825C>T and 1429C>T loci. For the $-350A>G$ promoter polymorphism, GG homozygotes

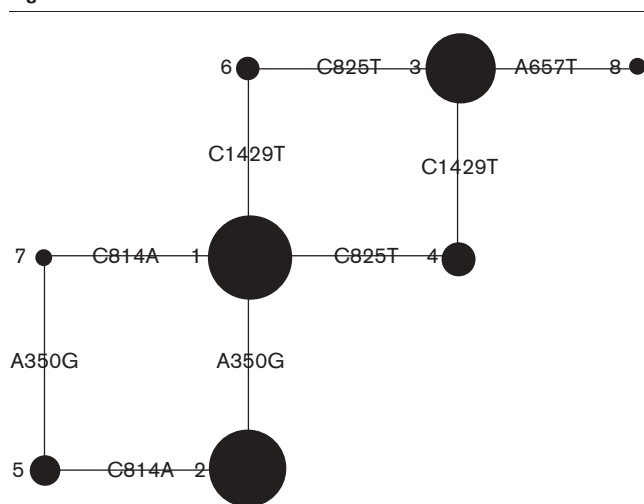
Table 4 Results for the association tests between blood pressure and $-350A>G$, 825C>T and 1429C>T according to BMI category

Locus	Genotype	<i>n</i>	BMI < 25 kg/m ²			BMI ≥ 25 kg/m ²					
			SBP (mmHg)	<i>P</i> ^a	DBP (mmHg)	<i>P</i> ^a	<i>N</i>	SBP (mmHg)	<i>P</i> ^a	DBP (mmHg)	<i>P</i> ^a
$-350A>G$	AA	20	111.1 ± 15.1		68.2 ± 8.5		10	123.6 ± 18.1		79.4 ± 11.5	
	AG	59	114.2 ± 12.5		72.6 ± 9.3		39	119.5 ± 14.0		76.6 ± 9.2	
	GG	58	110.7 ± 10.0	NS	70.1 ± 8.3	NS	54	123.9 ± 14.4	0.051	78.0 ± 10.2	NS
825C>T	CC	71	113.8 ± 13.1		71.6 ± 9.1		45	118.6 ± 13.3		76.1 ± 9.5	
	CT	51	110.9 ± 11.2		70.6 ± 8.9		46	123.6 ± 15.5		77.4 ± 9.9	
	TT	15	109.6 ± 6.9	NS	68.7 ± 7.6	NS	12	130.2 ± 13.1	< 0.025	83.3 ± 10.0	0.052
1429C>T	CC	73	113.8 ± 11.9		71.4 ± 8.4		57	117.8 ± 11.5		75.6 ± 8.9	
	CT	55	110.4 ± 12.5		70.2 ± 10.0		41	126.6 ± 17.0		79.4 ± 10.8	
	TT	9	111.6 ± 7.5	NS	71.7 ± 5.4	NS	5	136.3 ± 4.7	< 0.01	85.3 ± 8.1	< 0.05

^aAdjusted for age. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not significant.

Table 5 Inferred haplotype frequencies of *GNB3* polymorphisms in all subjects ($n = 282$)

Haplotype	-350A>G	657A>T	814G>A	825C>T	1429C>T	Frequency ($n = 564$)
1	G	T	G	C	C	0.3369
2	A	T	G	C	C	0.2677
3	G	T	G	T	T	0.2270
4	G	T	G	T	C	0.0567
5	A	T	A	C	C	0.0426
6	G	T	G	C	T	0.0195
7	G	T	A	C	C	0.0142
8	G	A	G	T	T	0.0124
9	A	T	G	T	C	0.0071
10	G	A	G	T	C	0.0071
11	A	T	G	T	T	0.0053
12	A	T	A	C	T	0.0018
13	A	T	G	C	T	0.0018

Fig. 2

Phylogeny of *GNB3* haplotypes. Each haplotype is represented by a circle whose area represents the overall frequency of that haplotype in the sample. The haplotype numbers correspond to those in Table 4. The site differences between haplotypes are shown on the connecting lines. Only haplotypes observed in $> 1\%$ of the sample are displayed.

showed a steeper blood pressure increase compared to A allele carriers. Stratified analysis confirmed that association with blood pressure for these SNPs was only observed in overweight subjects.

We have confirmed findings by Roszkopf *et al.* [4,5] that most polymorphisms within the *GNB3* gene are in strong LD, especially in Caucasians. In particular, the additional polymorphisms selected in this study ($-350A>G$, $657A>T$, $814G>$ and $1429C>T$) were all in strong LD with the $825C>T$. Based on comparison of observed and predicted genotype distributions (chi squared tests) between pairs of loci, Roszkopf *et al.* [5] deduced two typical *GNB3* haplotypes in Caucasians, a C haplotype (including $-350G$, C825 and C1429) and a

T haplotype (including A-350, 825T and 1429T). However, the frequencies of these major haplotypes were not given. Estimates of haplotype frequencies in our Caucasian sample showed three common haplotypes with frequencies $> 20\%$, two with frequencies of approximately 5% and eight rare haplotypes ($< 2\%$). The C haplotype was the most common one in our data (34%), and analyses of nonhuman primates [4] indicate that this is the ancestral *GNB3* haplotype. Interestingly, the T haplotype, more specifically the combination of A-350 and 825T, was extremely rare in our British females (1.24%). This was in agreement with a low haplotype frequency of 4.46% in Germans, as based on the contingency table data presented by Roszkopf *et al.* [5] and estimated using 2 LD [22]. The explanation is that the G rather than the A allele of the $-350A>G$ promoter polymorphism is in LD with the 825T allele, resulting in a considerable frequency of this $-350G/825T$ combination in our data ($> 28\%$, haplotypes 3, 4, 8 and 10) (Table 4), as well as the German data (27%). Thus, haplotype 3 in our data (GTGTT, Table 4) can be considered the T haplotype, which showed marked differences in predicted secondary structure of the pre-mRNA, potentially resulting in alternative splicing [5], and is thus most likely to show an effect on outcome variables.

To the best of our knowledge, this study is the first to perform haplotype association analyses for the *GNB3* gene. The results of these haplotype analyses offered further insight into the *GNB3*-obesity interaction observed in the single SNP analyses. After exclusion of the rare and uninformative $657A>T$ locus, haplotypes 3 (GGTT) and 4 (GGTC) were found to be associated with higher blood pressure increases with increasing levels of adiposity. The promoter $-350G$ allele did not differ between these haplotypes and the most common baseline haplotype GGCC. This is in agreement with evidence from reporter gene assays showing that the $-350A>G$ polymorphism did not change the *GNB3*

Table 6 Results of haplotype analysis using four single nucleotide polymorphisms in GNB3 gene including –350A>G, 814G>A, 825C>T and 1429C>T

Effects	Haplotype	Frequency	P-value BMI model		P-value waist model	
			SBP	DBP	SBP	DBP
Main	Age		0.000	0.056	0.001	0.220
	BMI/waist		0.008	0.011	0.008	0.002
	AGCC	0.263	NS	NS	NS	NS
	GGTT	0.234	NS	NS	0.081	NS
	GGTC	0.065	NS	NS	NS	0.057
	AACC	0.038	NS	NS	NS	NS
Interaction	AGCC–BMI/waist		NS	NS	NS	NS
	GGTT–BMI/waist		0.054	0.099	0.040	NS
	GGTC–BMI/waist		NS	NS	NS	0.042
	AACC–BMI/waist		NS	NS	NS	NS
Overall P for interactions			< 0.01	< 0.05	< 0.05	< 0.05

Results are shown for models that include haplotype interactions with either BMI or waist circumference. Estimates are contrasts with the most common haplotype GGCC (frequency = 0.341). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not significant.

Table 7 Results for the sib-transmission disequilibrium test (sib-TDT) between blood pressure and three common GNB3 polymorphisms

	<i>n</i> ^a	Genotype 11	Genotype 12	Genotype 22	<i>P</i> ^b	
					Additive model	Dominant model
–350A>G						
SBP	48	112.0	115.8	112.8	NS/NS/NS	NS/NS/NS
DBP	48	71.1	74.4	70.8	NS/NS/NS	NS/NS/NS
825C>T						
SBP	42	111.7	117.4	118.6	< 0.05/< 0.05/< 0.05	< 0.05/< 0.05/< 0.05
DBP	42	71.0	74.2	73.8	NS/NS/NS	0.056/0.060/< 0.05
1429C>T						
SBP	48	112.6	116.2	120.3	0.079/< 0.05/< 0.05	0.090/0.054/< 0.05
DBP	48	71.5	72.6	75.9	NS/NS/NS	NS/NS/NS

^a*n* is the total number of informative dizygotic twin pairs included in the sib-TDT analysis. ^b*P*-value for raw data/adjusted for BMI/adjusted for waist. NS, Not significant.

promoter activity and is unlikely to be functional. The more rapid rise in blood pressure with increasing adiposity in GG homozygotes as shown by the single SNP analysis (Fig. 1a) can probably be attributed to LD between the –350G and 825T alleles. Interestingly, both these haplotypes (3 and 4) carried the 825T allele at position 3, but differed in their 1429C>T allele (position 4). This indicates that the 1429T allele might not be necessary for a cooperative effect on the GNB3 pre-mRNA structure that would favour use of the alternative splice site[4].

Although we were unable to show significant results of the overall association tests, sib-TDTs showed associations with blood pressure for both the 825C>T and 1429C>T SNPs. However, the association was more convincing and significant for the 825C>T locus. These results are interesting, because they rule out a spurious association caused by hidden population stratification. They are also unexpected, because only DZ pairs discordant for their genotype are informative for

the sib-TDT, which reduces the effective sample size (and power) for this test [20]. We infer that using DZ twins for the sib-TDT may have added value. In addition to partial matching for genetic background effects, DZ twins are naturally matched for age and a range of possible environmental confounders. The within-pair difference as used in the sib-TDT may therefore provide a more precise estimate of the genetic effect.

The combination of our single SNP, haplotype and sib-TDT results supports the hypothesis that the 825C>T locus is mainly responsible for the association with blood pressure. The effects of the other loci appear to be secondary, and can efficiently be explained by their strong LD with the 825T allele.

The 825T allele has also been highlighted as a risk factor for obesity. In addition to BMI and waist circumference, measures of total body fat and central fat based on dual-energy X-ray absorptiometry were

available in most of our subjects [28]. SNP, haplotype and sib-TDT analyses of these traits were not presented in this paper as none of the results reached significance. These findings appear to be in agreement with the literature where effects of the 825C>T polymorphism have mostly been limited to males [10,29] and may only become apparent in females in the presence of adverse environmental factors such as a sedentary lifestyle [30]. Similar mechanism may explain why effects of the 825C>T locus on hypertension and blood pressure have been stronger in men than in women [9,10]. Women may be protected against the hypertensive action of the 825T allele, and additional factors such as a strong family history of essential hypertension [31] or obesity, as in our study, may be needed to unmask the effect of the polymorphism.

At least four possible pathways may explain why increasing levels of obesity potentiate the effect of the *GNB3* gene on hypertension risk. The first pathway consists of the sympathetic nervous system and its effect on vasoconstriction. Obese individuals show increased levels of leptin and free fatty acids, which stimulate sympathetic activity and the production of vasoconstrictive agents, including norepinephrine, endothelin-1 and angiotensin II, that act via G-protein coupled receptors. For example, Baumgart *et al.* [32] found an almost two-fold enhanced α_2 -adrenoceptor-mediated vasoconstriction in 825T allele carriers, and a recent study [33] observed enhanced vasoconstriction in the skin microcirculation in response to infusion of noradrenaline, endothelin-1 and angiotensin II in carriers of the 825T allele. Direct effects of leptin on the vascular wall may constitute the second pathway. Experimental models show that leptin promotes vascular cell calcification and smooth muscle cell proliferation and migration, which may lead to the intimal thickening and impaired arterial distensibility [34,35]. Further evidence from prospective and animal studies confirms the link between the hyperleptinemia of obesity with cardiovascular disease and hypertension [36,37]. Insulin resistance is the third pathway. Siffert [38] speculated that the 825T allele predisposes to hypertension via hyperinsulinemia. Obesity-induced insulin resistance and subsequent endothelial dysfunction may amplify the vasoconstrictor response in T allele carriers. Finally, obesity may increase renal tubular sodium reabsorption through hyperactivity of the renin-angiotensin system, and possibly by an alteration of intrarenal physical forces, thereby enhancing the renal action of the T allele. There is evidence that altered intracellular signal transduction in 825T allele carriers increases $\text{Na}^+ - \text{H}^+$ exchanger activity [39], which may lead to increased renal tubular sodium reabsorption and volume expansion. This view is strengthened by the association between the 825T allele and lower levels of plasma renin in a population-based study [40].

In summary, obesity reveals an association between the *GNB3* gene and blood pressure in our cohort of adult females. Women may display initial protection against the hypertensive effect of the 825T allele or the 'T haplotype' of the *GNB3* gene, and this effect is unmasked by additional adverse genetic (e.g. a family history of hypertension) or environmental factors such as a sedentary lifestyle. These findings highlight the importance of adiposity as a final pathway through which gene-lifestyle interactions may exert their effects on the development of hypertension.

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