



## PAPER

# G-protein $\beta 3$ subunit gene splice variant in obesity and overweight

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**OBJECTIVE:** To determine whether the C825T polymorphism of the G-protein  $\beta 3$  subunit gene (*GNB3*) is associated with overweight and obesity. This polymorphism leads to a splice variant ( $G\beta 3$ -s) with higher activity and very strong association with essential hypertension.

**DESIGN:** A cross-sectional case-control study.

**SUBJECTS:** The sets of affected and control British/European Caucasian subjects used were: (i) an obesity clinic group most of whom had 'morbid obesity' (mean body mass index (BMI) for group =  $43 \pm 8$  kg/m<sup>2</sup>) and non-obese controls (BMI  $\leq 30$ ); (ii) a group of overweight/obese healthy normotensive community volunteers (BMI  $> 25$ ; mean  $29 \pm 5$ ) and controls (BMI  $\leq 25$ ; mean  $23 \pm 1$ ); (iii) a group of overweight/obese hypertensive patients (BMI  $> 25$ ; mean  $30 \pm 4$ ) and lean hypertensive controls (BMI  $\leq 25$ ; mean =  $23 \pm 2$ ).

**MEASUREMENTS:** BMI, blood pressure, serum lipids, alleles of *GNB3* polymorphism.

**RESULTS:** Compared with control, frequency of the *T* allele in obese subjects was higher by 12% in (i), 17% in (ii) and 28% in (iii), but the differences were not statistically significant. Slight tracking of the *T* allele with elevation in BMI was, however, observed, in the obesity clinic group ( $P = 0.018$ ).

**CONCLUSION:** The C825T splice variant of *GNB3* makes little if any contribution to obesity in the groups we tested.

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## Introduction

Activation of G proteins is the main mechanism through which heptahelical receptors generate changes in intracellular function in response to ligand binding. Because of their crucial role in the function of many types of cells, genetic abnormalities in G-protein subunits have the potential to be involved in the aetiology of a wide range of clinical conditions.

A C825T polymorphism in exon 10 of the pertussis toxin-sensitive G protein  $\beta 3$  subunit gene (*GNB3*) results in a splice variant ( $G\beta 3$ -s) in which 41 amino acids of exon 9 are lost along with the fourth Trp-Asp (WD) repeat of the seven WD repeats which form a  $\beta$ -propeller structure.<sup>1</sup> Such a structural

change could alter the position of critical  $\beta$ -propeller residues that contact the lip in the  $\alpha$  subunit of the  $\alpha\beta\gamma$  subunit complex, and so help guide the likely GDP exit route,<sup>2</sup> thereby accounting for the enhanced activity of  $G\beta 3$ -s. This variant was shown initially to be associated with essential hypertension,<sup>1</sup> and the association was found by us to be particularly strong in those with strong family history (two affected parents) and consequent early onset disease.<sup>3</sup>

The mechanism by which pertussis toxin-sensitive G proteins produce hypertension could involve stimulation of cell proliferation leading to vascular hypertrophy.<sup>1</sup> An effect of G-protein subunits on adipogenesis has also been observed.<sup>4,5</sup> Could  $G\beta 3$ -s therefore contribute to the aetiology of obesity? Moreover, since increased body mass index (BMI) is associated with increased risk for hypertension, the possibility has been raised that association of the *GNB3* *T* allele with hypertension could be from a primary association with obesity.<sup>6</sup> Accordingly, studies have been conducted to test the C825T variant in obesity, and significant associations have been found.<sup>6,7</sup> More recently the *GNB3* *T* allele has also

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been associated with decreased weight loss after pregnancy.<sup>8</sup> The maternal *T* allele has shown an association with low birth weight,<sup>9</sup> and there have been many associations of low birth weight with later cardiovascular disease and type 2 diabetes in adult life (Barker hypothesis).

In the studies that claimed association of the *GNB3* variant with obesity, there were, however, only 35 subjects with BMI > 27 kg/m<sup>2</sup> in one,<sup>7</sup> and in the other there were only 22 that were truly obese (BMI > 30 kg/m<sup>2</sup>).<sup>6</sup> We therefore decided to test the *GNB3* variant for association with obesity, and degree of increase in BMI, using a much larger cohort, selected specifically for obesity, most of whom possessed the extreme phenotype of morbid obesity. We also examined association of *Gβ3-s* with overweight in subjects from our previous hypertension study that had shown a highly significant association with high blood pressure.<sup>3</sup>

## Methods

### Subjects

All subjects were unrelated Australian Caucasians of British or Northern European ancestry who were resident in Sydney. Obesity and overweight were defined according to WHO criteria, *viz* BMI > 30 kg/m<sup>2</sup> for obesity and BMI > 25 but ≤ 30 kg/m<sup>2</sup> for pre-obesity. Three sets of obese and control subjects were tested. In set (i) the obese group were recruited by Metabolism and Obesity Services, Royal Prince Alfred Hospital, Sydney, while attending the clinic for initial assessment for obesity. Their BMIs ranged from 33 to 75 kg/m<sup>2</sup>, and on average fell into the category of Grade III ('morbid') obesity (BMI > 40 kg/m<sup>2</sup>). These were compared with a non-obese control group that consisted of 184 sub-

jects who had BMI ≤ 30 kg/m<sup>2</sup> and who were healthy volunteers recruited from the Australian Red Cross Blood Bank, Sydney. Sets (ii) and (iii) were previously described<sup>10</sup> overweight/obese and normal weight/lean (BMI > or ≤ 25 kg/m<sup>2</sup>, respectively) hypertensive (set (i)) and healthy normotensive (set (ii)) volunteers from the general community. The research was approved by The University of Sydney Human Ethics Committee.

### Genotyping and lipid measurements

DNA was isolated from peripheral blood leukocytes by a Nucleon Bacc3 kit (Amersham Life Science, England) in the case of the obese subjects, and by a QIAamp DNA Mini Kit (Valencia, CA, USA) for all other subjects. Genotypes for the *GNB3* polymorphism were determined by PCR followed by *Bsa*JI digestion and agarose gel electrophoresis as described previously.<sup>3</sup> Genotyping was performed on at least two separate occasions and digestion with restriction endonuclease was extended to 6 h to check that results for heterozygotes did not represent incomplete digestion of PCR products. For all, repeat genotype result was identical to original. Lipids were measured by standard procedures as described previously.<sup>11</sup>

### Statistical analyses

Total alleles on all chromosomes were calculated from genotype data, and  $\chi^2$  analysis with 1 or 2 degrees of freedom, as appropriate, was performed with StatView<sup>®</sup> (Abacus Concepts, Berkeley, CA). Comparison of different parameters across genotypes was by one-way ANOVA.

**Table 1** Demographic parameters for each set of case-control groups studied

Parameter	Set (i)		Set (ii)		Set (iii)	
	Non-obese (BMI ≤ 30)	Obese (BMI > 30)	NT (BMI ≤ 25)	NT (BMI > 25)	HT (BMI ≤ 25)	HT (BMI > 25)
Male:female	109:80	22:70	55:50	54:30	28:28	25:30
Age (y)	48 ± 10	43 ± 13	48 ± 10	48 ± 9	52 ± 13	52 ± 12
		(0.001)		(0.71)		(0.99)
BMI (kg/m <sup>2</sup> )	26 ± 4	43 ± 8	23 ± 1	29 ± 5	23 ± 2	30 ± 4
		(< 0.0001)		(< 0.0001)		(< 0.0001)
Systolic blood pressure (mmHg)	120 ± 11	126 ± 18	118 ± 10	122 ± 11	173 ± 24	174 ± 26
		(0.0004)		(0.038)		(< 0.0001)
Diastolic blood pressure (mmHg)	73 ± 8	81 ± 15	73 ± 8	74 ± 8	107 ± 12	114 ± 20
		(< 0.0001)		(0.0025)		(< 0.0001)
Cholesterol (mmol/l)	5.2 ± .1	5.3 ± .1	5.1 ± .1	5.4 ± .1	5.6 ± .2	5.8 ± .1
		(0.30)		(< 0.0001)		(< 0.0001)
Triglycerides (mmol/l)	1.5 ± .1	1.6 ± .1	1.3 ± .1	1.8 ± .1	2.5 ± .2	2.5 ± .2
		(0.68)		(0.0052)		(0.95)
HDL (mmol/l)	1.3 ± .04	1.2 ± .03	1.5 ± .1	1.2 ± .1	1.1 ± .1	1.1 ± .1
		(0.072)		(< 0.0001)		(0.99)
LDL (mmol/l)	3.2 ± .1	4.3 ± .1	3.0 ± .1	3.4 ± .1	3.4 ± .2	3.6 ± .1
		(0.066)		(< 0.0001)		(< 0.0001)

Values are mean ± s.d., or, for lipid measurements, mean ± s.e.

Shown in brackets are *P*-values for *t*-tests comparing values for each case vs control group. NT, normotensive; HT, hypertensive; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol.

**Table 2** Association analyses of C825T variant of *GNB3* in (i) obesity clinic subjects (BMI = 43 ± 8 kg/m<sup>2</sup>) and controls, (ii) overweight/obese normotensive (NT) volunteers (BMI = 29 ± 5 kg/m<sup>2</sup>) and controls, and (iii) overweight/obese hypertensive (HT) volunteers (BMI = 30 ± 4 kg/m<sup>2</sup>) and controls

Group	Genotypes (frequency)					Total alleles on all chromosomes (frequency)			
	CC	CT	TT	$\chi^2$	P	C	T	$\chi^2$	P
Set (i)									
Obese (BMI > 30)	48 (0.52)	37 (0.40)	7 (0.08)	2.8	0.25	133 (0.72)	51 (0.28)	0.52	0.47
Non-obese (BMI ≤ 30)	101 (0.53)	82 (0.43)	5 (0.03)			284 (0.75)	94 (0.25)		
Set (ii)									
NT (BMI > 25)	42 (0.56)	38 (0.42)	4 (0.02)	1.7	0.44	122 (0.73)	46 (0.27)	1.0	0.31
NT (BMI ≤ 25)	59 (0.50)	44 (0.45)	2 (0.05)			162 (0.77)	48 (0.23)		
Set (iii)									
HT (BMI > 25)	9 (0.16)	38 (0.69)	8 (0.15)	3.9	0.14	56 (0.51)	54 (0.49)	2.6	0.11
HT (BMI ≤ 25)	17 (0.16)	35 (0.63)	4 (0.07)			69 (0.62)	43 (0.38)		

**Table 3** Comparison of BMI across genotypes of the *GNB3* C825T variant in the obesity clinic group and each overweight/obese community volunteer group

Group	CC	CT	TT	F	P
(i) Obesity clinic subjects	40 ± 6	45 ± 10	46 ± 4	4.2	0.018
(ii) Overweight/obese NT	26 ± 0	29 ± 4	30 ± 5	1.2	0.30
(iii) Overweight/obese HT	29 ± 4	30 ± 4	29 ± 2	0.11	0.90

## Results

The characteristics of each group are shown in Table 1. Genotype and derived allele frequencies for the C825T polymorphism in case and control groups for study group sets (i), (ii) and (iii) are shown in Table 2. Genotype frequencies did not deviate from Hardy–Weinberg expectations.<sup>12</sup> No significant association with obesity was observed. For a calculated relative risk of 1.12,<sup>13</sup> in study group set (i) our investigation had 67% power to detect significant association with obesity, given the *P*-value we obtained. Power in the study groups for set (ii) was 99% and for set (iii) was 93%.

The BMI for each genotype is shown in Table 3. In the obesity clinic group (i) the *T* allele tracked with elevation in BMI (*P* = 0.018), but no significant association was seen in the overweight/obese groups of sets (ii) and (iii). In addition, plasma lipid parameters listed in Table 1 were examined across genotypes, but showed no significant differences (data not shown).

## Discussion

We found no association of the C825T polymorphism of *GNB3* with obesity or overweight. However, a small, but

significant effect on BMI was observed in an obesity clinic group with severely affected individuals. The level of significance we obtained for the latter (*P* = 0.018) was similar to that reported by Siffert *et al*<sup>7</sup> (*P* = 0.02). However, whereas they saw *T* allele frequencies of 0.40, 0.31 and 0.24 in their obese, overweight and normal weight groups, respectively (of hypertensive German Caucasians), the values we saw were 0.28, 0.27 and 0.23 for our severely obese, overweight/obese, and non-overweight groups. In healthy young adult male Germans Siffert *et al* reported values for obese, overweight and normal-weight subjects of 0.48, 0.39 and 0.29.<sup>6</sup> Only in our hypertensive subjects from study group (iii) did *T* allele frequency reach such high values as reported in obese German subjects, being greater, although not significantly so, in our overweight/obese (0.49) compared with our non-overweight (0.38) hypertensives. However, this was contributed by the much higher frequency we see for the *T* allele in hypertension *per se*, irrespective of overweight status.

Mechanisms leading to hypertension or obesity remain speculative. Initially it was thought that Gβ3-s might affect Na<sup>+</sup>/H<sup>+</sup> exchanger activity, which is elevated in obese subjects.<sup>14</sup> Although anti-sense studies in mice and stimulation

experiments in embryogenesis support a role for pertussis toxin-sensitive G proteins in adipogenesis,<sup>4,15</sup> such mechanisms were stated by Siffert *et al* not to explain the association with obesity they noted.<sup>6</sup> Instead they proposed that the effect of G $\beta$ 3-s had a slow time-course, although a possible mechanism was not specified. They postulated that the supposed elevation in body weight that ensued then increased blood pressure secondarily.<sup>6</sup> Our data is consistent with such an effect only in a clinic group with severe obesity.

In contrast, in our hypertensive subjects the association we saw previously between the *T* allele and hypertension, as well as the tracking of the *T* allele with systolic and diastolic blood pressure, was very strong.<sup>3</sup> Although both positive and negative findings have appeared in the literature in studies that have tested the *GNB3* C825T variant for association with hypertension, in our patients the level of significance we found for association with hypertension ( $P=0.00002$ )<sup>3</sup> was stronger than that for any of the many other genetic variants we have tested in relation to hypertension in our population previously. In contrast, considering the extreme phenotype of our obesity clinic subjects (mean BMI = 43 kg/m<sup>2</sup>), if an association existed between *GNB3* genotype and obesity it should have been apparent in this study group, particularly as the number of obese subjects we included in our analyses was very much greater than in the previous studies by Siffert *et al*. The disparity between our findings and those of Siffert *et al* could be due to selection bias and inappropriate matching of case and control groups, admixture, or a result of geographical variation in these different Caucasian populations. Such factors have the potential to lead to false positive findings in case-control studies. Moreover, in case-control studies in which many polymorphisms are tested one might expect one in 20 to be significant at the  $P < 0.05$  level by chance. Thus the absence of an association would indicate that the C825T variant is not a major determinant in the onset of obesity, at least in the present study groups.

In conclusion, the *T* allele of the *GNB3* C825T variant may contribute little if anything to obesity or overweight in the Australian Anglo-Celtic Caucasians studied. This contrasts sharply with the strong effect on blood pressure that we have described previously.<sup>3</sup>

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