Genetic Variation in Female BMI Increases with Number of Children Born but Failure to Replicate Association between *GNβ3* Variants and Increased BMI in Parous Females

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Objective: As post-pregnancy weight retention in women is a risk factor for obesity later in life, we assessed the changes in magnitude of genetic and environmental variation in BMI due to parity in an Australian female sample, comprising 11,915 female twins and their sisters. *Results:* Total variance of BMI increased with parity, primarily driven by an increase in nonadditive genetic variance. This finding was of particular interest given Gutersohn et al's (2000) report of recessive association between post-partum weight retention and the 825T allele of the *GNβ3* gene (rs5443) at 12p13.31. Hence, we attempted to replicate this association and test an additional three SNPs also located near or on *GNβ3* (rs10744716 (upstream), rs5442 (exon 10), rs5446 (exon 11)) in a sample of 3131 females and 1693 males from 2585 twin families. No association was found between these SNPs among females, even when allowing for a genotype by parity effect. However, a significant association was observed among males for rs10744716 ($\chi^2_{2} = 10.22$, $p = 0.006$; effect size = 0.47kg/m^2), representing a novel association between a region upstream of *GNβ3* and male BMI.

Keywords: body mass index (BMI), parity, association, guanine nucleotide binding protein 3 (*GNβ3*)

The prevalence of being overweight or obese has doubled in the past 30 years and has now reached epidemic proportions in western countries. Obesity is the second leading cause of preventable death in the United States, resulting in more than 300,000 deaths per year. However, the rate with which the prevalence of obesity is increasing reflect changes in exposures to environmental risks rather than changes in genetic background, which explain around 70% of variation in body mass index (BMI; Maes et al., 1997). While recent genome-wide association analyses have led to notable successes in identifying risk loci (Frayling et al., 2007), the genetic influences on obesity are complex and poorly understood. There is a growing body of literature suggesting genetic effects are moderated by a range of intrinsic factors, including sex (Cornes et al., 2005; Cornes et al., 2007; Schousboe et al., 2003) and age (Cornes et al., 2007; Franz et al., 2007).

The major determinant of obesity is the balance between food intake, metabolism, and energy expenditure (Newcombe, 1982). However, physiological factors associated with pregnancy may also cause long-term excess weight among women (Parker & Abrams, 1993). Many studies have suggested that childbirth may be an environmental trigger that may affect genetic expression of BMI in women (Korkeila et al., 1991, 1995; Parker & Abrams, 1993) in terms of gained fat stores and increased metabolism necessary for fetal development and preparation for prolonged lactation. In addition, an association study by Gutersohn et al. (2000) found that parity (the number of children borne by one woman) appeared to moderate the effects of guanine nucleotide binding protein 3 (*GNβ3*) gene on female BMI. The study examined the association of the 825T/C (rs5443) polymorphism in exon 10 of *GNβ3* with BMI in nulliparous (have never given birth) and primiparous (have given birth one or more times) women (within 12 months of giving birth to their first child). They found that the 825T allele had significant effects on postpartum weight retention

Table 1

Composition of Families in the Analyzed Sample

Zygosity	Family structure								Total
	●●	●●♀	●●♀♀	● ○	●○♀	●○♀♀	○○♀	○○♀♀	
MZ Female	1680	369	117	238	6	—	—	—	2410
DZ Female	1207	207	72	255	7	2	—	—	1750
DZOS Female	—	—	—	1772	276	91	—	—	2139
Non-twin siblings	—	—	—	—	—	—	347[†]	94[‡]	441

Note: Family structures are shown for monozygotic (MZ) and dizygotic (DZ) families with same-sex and opposite-sex (OS) twins. A solid circle (●) indicates the twin was included in the sample, an open circle (○) indicates the twin was not included or did not participate. Female (♀) symbols indicate the presence of included female siblings.

† the inclusion of these singleton siblings (similar to singleton twins) helps to improve the accuracy of estimates of means and variances (Neale & Cardon, 1992; Posthuma & Boomsma, 2000)

‡ the inclusion of these non-twin sibling pairs increases the power as they provide similar information as DZ twins twins but they are of different ages.

and primiparous women with the TT genotype were significantly more likely to be overweight. However, this effect was completely abolished by the engaging in more than 1 hour of physical activity per week.

Nevertheless, it remains unclear whether increases in obesity postpartum are simply the result of changes in genetic expression of energy metabolism during pregnancy and lactation, or whether they are influenced by inherent changes in lifestyle that accompany pregnancy and motherhood. Furthermore, any effect of parity on body weight is complicated by the tendency for weight to increase with age (Harris & Ellison, 1997). Here we report a series of biometrical and association analyses examining the way in which these risk factors may affect the genetic and environmental influences on female body weight using data on 11,915 female twins and their sisters from 6740 Australian twin families.

Materials and Methods

Subjects and Measures

Clinical and self-reported height and weight data were derived from several studies of adult twins and their families enrolled on the Australian Twin Registry. Self-reported measures were taken from questionnaires mailed to twins, parents, siblings, spouses and children of the twins between 1980 and 1996, whilst clinical measurements were taken in the context of studies conducted in the years 1992 to 1996. The majority of participants were involved in more than one study and therefore most individuals had several measurements of BMI, calculated as weight (in kilograms)/height (in metres)². As a result, rules were implemented to choose the optimum BMI measurement which have been explained elsewhere (Cornes et al., 2005).

Data were screened for family outliers using SPSS 15.0 for Windows (SPSS Inc., 1989–2006). Those data with z-score values greater than ± 3.00 standard deviations from the mean (74 families) were excluded from all reported analyses. In addition, a natural logarithmic transformation was performed on BMI to reduce the skewness of the distribution (used in all

further analyses). The final sample includes BMI data for 11,915 females (2166 monozygotic [MZ] twin pairs, 1486 dizygotic [DZ] twin pairs, 244 MZ singleton twins, 2403 DZ singleton twins and 1964 siblings) from 6740 families. The composition of this sample is described in more detail in Table 1.

To test the effect of childbirth on BMI, the number of births was tallied until the time of when the BMI measurement was taken. Where available, we used the number of full-term pregnancies. If this was not available, we used the number of total pregnancies minus any terminations or miscarriages. In most studies, women were asked 'How many times have you been pregnant, including pregnancies that ended in miscarriage, termination or stillbirth?' without further information about the number of miscarriages, terminations or stillbirths. If this was the case, the number of children was used, excluding any step or adopted children. If information about adopted or stepchildren was not available, the number of children as reported was used.

Approximately 58% of women (N = 6874) had given birth at least once and the range of number of births was between 1 and 10 (mean number of births was 1.46). All participants were aged between 18 and 94 years of age (mean, 38.14, SD 14.95 years) and born between 1863 and 1979 (median year of birth 1957). For all analyses, age variables were converted to a z-score.

Zygosity

The zygosity of twin pairs was initially determined by twins' responses to standard items about physical similarity and the degree to which the others confused them with one another. For some of the twins (21.2% ≈ 2567 females), zygosity was subsequently confirmed through participation in studies in which they were typed for large numbers of genetic markers (Cornes et al., 2005).

Statistics

Genetic Analysis

The biometric modeling of twin data uses information from identical (MZ) and non-identical (DZ) twins to

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estimate components of variance in liability to a trait. Variance in liability to a particular trait may be decomposed into: additive genetic variance (A), nonadditive (dominant or epistatic) genetic effects (D), environmental influences shared by members of a family (C), and environmental influences unique to each family member (E). However, C and D are confounded in analyses consisting of twins reared together, and thus only one of these parameters can be estimated in the model at a time (Grayson, 1989; Hewitt, 1989). MZ twins are perfectly correlated for additive and nonadditive genetic effects, and thus A and D are both correlated as 1.0 in these twin pairs. Since DZ twins share on average half their segregating genes in common, the expected correlation for additive genetic effects is 0.5 and 0.25 for nonadditive genetic effects. Shared environmental effects are perfectly correlated for members of both MZ and DZ pairs and by definition, twins are not correlated for unique environmental effects. Biometrical modeling is discussed in more detail elsewhere (Neale & Cardon, 1992).

The standard twin design can be extended to include information from additional non-twin siblings and their expected genetic and environmental correlations with twins and other siblings are as for DZ twins above. Thus the variance–covariance matrix may be extended to incorporate as many siblings as desired.

Gene by Environment Interaction (G × E)

We examined the way in which parity and age affects the genetic and environmental influences on BMI in females by using a moderator biometrical model which allows for the quantification of genotype by environment interactions (Purcell, 2002). Figure 1 shows a classic twin model (for one twin only) that has been extended to include a moderation component. In this extension of the standard twin design the magnitudes of genetic and environmental influences on a trait were modeled as functions of the moderating variables, allowing an exploration of interactions between the effects of the latent genetic and measured environmental factors. In the case of a continuous moderating variable this model avoids stratification of the sample, and consequent loss of power. More than one moderating variable, and interactions between moderating variables, can also be modeled.

For this analysis, several moderating influences on BMI were modeled: age, including a quadratic age term (as age does not have a linear relationship with BMI) as well as a variable for number of births (a quadratic term for number of births was not included as number of births had only a linear relationship with BMI). In addition, age*birth and age²*birth interaction terms were included as parity and age are highly correlated (0.53). In our saturated model we allowed for the influence of these moderators on the magnitude of additive genetic, nonadditive genetic or shared environmental, and unique environmental variance components. Thus, the path coefficients for each variance component are represented by the equation:

$$x + \beta_{age}M_{age} + \beta_{age^2}M_{age^2} + \beta_{birth}M_{birth} + \beta_{age.birth}M_{age}M_{birth}$$
$$+ \beta_{age^2.birth}M_{age^2}M_{birth}$$

where x represents the unmoderated component, M_{age} represents the moderator for age, M_{age^2} represents the moderator for age² and M_{birth} represents the moderator for birth, and β_{age}, β_{age^2}, β_{birth}, $\beta_{age.birth}$ and $\beta_{age^2.birth}$ are all unknown regression parameters to be estimated. Thus, the expected trait variance for an individual (under an ADE model) is:

$$(a + \beta A_{age}M_{age} + \beta A_{age^2}M_{age^2} + \beta A_{birth}M_{birth} + \beta A_{age.birth}$$
$$M_{age}M_{birth} + \beta A_{age^2birth}M_{age^2}M_{birth})^2 + (d + \beta D_{age}M_{age}$$
$$+ \beta D_{age^2}M_{age^2} + \beta D_{birth}M_{birth} + \beta D_{age.birth}M_{age}M_{birth}$$
$$+ \beta D_{age^2birth}M_{age^2}M_{birth})^2 + (e + \beta E_{age}M_{age} + \beta E_{age^2}M_{age^2}$$
$$+ \beta E_{birth}M_{birth} + \beta E_{age.birth}M_{age}M_{birth} + \beta E_{age^2birth}M_{age^2}$$
$$M_{birth})^2$$

In addition, the expected covariance for MZ twin pairs can be represented as:

$$(a + \beta A_{age}M_{age} + \beta A_{age^2}M_{age^2} + \beta A_{birth}M_{birth} + \beta A_{age.birth}$$
$$M_{age}M_{birth} + \beta A_{age^2birth}M_{age^2}M_{birth})^2 + (d + \beta D_{age}M_{age}$$
$$+ \beta D_{age^2}M_{age^2} + \beta D_{birth}M_{birth} + \beta D_{age.birth}M_{age}M_{birth}$$
$$+ \beta D_{age^2birth}M_{age^2}M_{birth})^2$$

and for DZ twin pairs or sibling pairs:

$$0.5(a + \beta A_{age}M_{age} + \beta A_{age^2}M_{age^2} + \beta A_{birth}M_{birth} + \beta A_{age.birth}$$
$$M_{age}M_{birth} + \beta A_{age^2birth}M_{age^2}M_{birth})^2 + 0.25(d + \beta D_{age}$$
$$M_{age} + \beta D_{age^2}M_{age^2} + \beta D_{birth}M_{birth} + \beta D_{age.birth}M_{age}M_{birth}$$
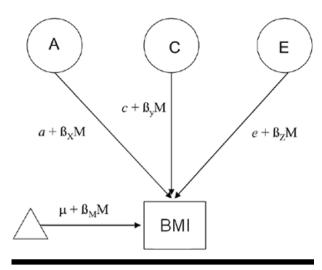$$+ \beta D_{age^2birth}M_{age^2}M_{birth})^2$$

Any variable which has a moderating, or interactive, effect on a trait may also have a mediating, or main effect (Purcell, 2002). Therefore, we allowed for the main effect of each of these moderating variables and their interaction terms on BMI by incorporating the moderating variables in the standard means model:

$$\mu_i = \mu + \beta M_{age}M_{age_i} + \beta M_{age^2}M_{age^2_i} + \beta M_{birth}M_{birth_i}$$
$$+ \beta M_{age.birth}M_{age}M_{birth_i} + \beta M_{age^2birth}M_{age^2}M_{birth_i}$$

where μ_i is the mean for the ith individual, μ represents the un-moderated mean, and β_{age}, β_{age^2}, β_{birth}, $\beta_{age.birth}$ and $\beta_{age^2.birth}$ represent the phenotypic regression coefficients, and M_{age}, M_{age^2} and M_{birth} are the values of moderator variables for that individual (see Figure 1). Note that different set of βs are used for the means model and for each of the different variance components A, D (or C) and E (as indicated by β_M, β_A, β_D (or β_C) and β_E, respectively).

Models were fitted to data from twins and up to two additional siblings by the method of maximum likelihood as implemented in Mx (Neale et al., 2003). In the current study, the significance of the β terms was assessed by the change in log-likelihood when

Figure 1

Gene–environment interaction model. The latent variable A, represented in a circle, indicates additive genetic influences on the trait of interest (BMI). Latent C represents common (shared) environmental influences on a trait, and latent E represents unique environmental influences which are uncorrelated between the twins. The triangle indicates the means for BMI and is necessary when modeling raw data. The standard paths *a*, *c* and *e*, indicating the magnitude of effect of each latent variable on the trait, each include a β term, which indicates the significance of a potential environmental moderator *M* on each of these genetic and environmental influences. Path diagram shown is for one twin only. Latent variables have unit variance (Purcell, 2002).

each term was dropped from the model. Twice the difference in log-likelihood is distributed as $\Delta\chi^2$ with degrees of freedom (*df*) for the test equal to the difference in *df* between the full and sub model. A significant change in fit between the models is indicated by a p-value *(p)* < .05 and indicates that the dropped parameter significantly contributes to the outcome trait and should be retained in the model.

Association Analysis

Following from these results, we sought to replicate and extend the association analysis of Gutersohn et al. (2000), by testing for association between BMI and the 825T allele of the *GNβ3* gene. We also typed two other polymorphisms (rs5442, rs5446) within *GNβ3*, as well as a novel SNP, rs10744716, located upstream of *GNβ3*. SNPs were selected across the *GNβ3* gene as part of a candidate gene association study and were chosen based on previous studies spanning the last 10 years, as well as searching databases such as NCBI dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and SNPper (http://snpper.chip.org/). Although dbSNP (build 129) reports rs10744716 to be located in exon 4 of the *LEPREL2* gene, according to SNPper (http://snpper.chip.org/bio/view-snp/10744716), this SNP also lies within the promoter region of *GNβ3*.

Since samples were already plated, these SNPs were genotyped in 3,131 females and 1,693 males from 2,585 twin families (SNP characteristics are described in Table 2). DNA was collected from some

of these twins who participated in a 1992–1996 twin study based upon an Australian modified version (Mini-SSAGA-OZ) of the Semi-Structured Assessment for the Genetics of Alcoholism instrument (Bucholz et al., 1994; Heath et al., 1997). Subjects gave written informed consent and provided blood samples from which DNA was isolated using standard protocols. Genetic studies were approved by the QIMR Human Research Ethics committee.

Forward and reverse polymerase chain reaction (PCR) primers and an extension primer were designed using Sequenom MassARRAY Assay Design (version 3.0) software (Sequenom Inc., San Diego, CA) and purchased from Bioneer Corporation (Daejeon, Korea). Genotyping was carried out in standard 384-well plates with 12.5ng genomic DNA used per sample. We used a modified iPLEX Gold Sequenom protocol where half reaction volumes were used in each of the PCR, SAP and iPLEX Gold stages giving a total reaction volume of 5.5µL. The reaction products were desalted by diluting samples with 15µL of water and 3µL SpectroCLEAN resin (Sequenom) and then were spotted on a SpectroChip (Sequenom), processed and analyzed on a Compact MALDI-TOF Mass Spectrometer by MassARRAY Workstation software (version 3.3) (Sequenom). Allele calls for each 384-well plate were reviewed using the cluster tool in the SpectroTyper software (Sequenom) to evaluate assay quality. Genotype error checking, including Mendelian inconsistencies and Hardy-Weinberg equilibrium analyses were using MERLIN (Abecasis et al., 2002) and PEDSTATS (Wigginton & Abecasis, 2005). One of these SNPs, rs5442 was excluded from analysis as it was not in Hardy-Weinberg equilibrium ($p = 8.9 \times 10^{-51}$). In addition, 56 individuals (13 males) were excluded from all reported analyses due to low DNA concentration.

All association analyses were conducted using QTDT as described at http://www.sph.umich.edu/csg/abecasis/QTDT/ (Abecasis, Cardon et al., 2000; Abecasis, Cookson et al., 2000). Each SNP was tested for population stratification before testing for association. As in previous analyses on the current data, several covariates were used including sex, age and number of births for women as well as several interaction terms (sex*age, sex*age², age*births, age²*births). Given the recessive nature of the 825T allelic effect reported by Gutersohn et al. (2000) we tested for both additive and nonadditive allelic effects using the -g option in QTDT.

Results

Preliminary Analysis

Means and standard deviations of BMI are given for the MZ twins, DZ twins and non-twin siblings in Table 3. After correcting for mean effects of age, age² and parity (i.e., β terms in the means model) and their interaction terms as well as excluding outliers, means were found not to be significantly different between

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Table 2

Descriptive Statistics For $GN\beta3$ SNPs

SNP	Position(bp)[†]	Gene region	Alleles	Minor allele frequency in sample	Minor allele frequency in dbSNP[‡]
rs10744716	6810111	Upstream [a]	A/G	A:0.511 G:0.489	A:0.510 G:0.490
rs5442	6825125	Exon 10	A/G	A:0.131 G:0.869	A:0.100 G:0.900
rs5443 (825C/T)	6825136	Exon 10	C/T	C:0.706 T:0.294	C:0.612 T:0.388
rs5446	6826723	Exon 11	C/T	C:0.717 T:0.283	C:0.629 T:0.371

Note: † based on dbSNP (build 129)
‡ based on dbSNP (build 129) HapMap CEU or EUR
a. dbSNP (build 129) reports rs10744716 to be located in exon 4 of the *LEPREL2* gene, though according to SNPper, it also lies within the promoter region of $GN\beta3$.

Table 3

Statistical Descriptions of BMI for the MZ and DZ Twins and Their Non-Twin Sisters

	MZ twins	DZ twins[†]	Non-twin sisters
N	4576	5375	1964
Mean (SD)	23.76 (3.74)	23.84 (3.76)	23.93 (4.01)
Mean Age (SD, range)	39.68 (15.70, 18–91)	37.55 (15.20, 18–94)	36.19 (11.87, 18–84)
Mean number of births (SD, range)	1.50 (1.60, 0–10)	1.36 (1.56, 0–10)	1.64 (1.55, 0–8)

Note: † The DZ sample contains both female–female DZ pairs and female twins from DZ opposite sex twin pairs.

twins and their non-twin siblings ($\chi^2_1 = 1.42$, $p = .234$; mean = 23.87 kg/m², SD = 3.80).

Similarly, there were no differences between the dizygotic twin, twin–sibling, or sibling–sibling covariance ($\chi^2_2 = 4.51$, $p = .105$). The within-pair correlations for MZ/MZ, DZ/DZ, sibling/sibling and twin/sibling pairs are given in Table 4. The pattern of covariation between relatives was best explained by a combination of additive and nonadditive genetic effects (A = 0.41 [95%CI = 0.31–0.47]; D = 0.16 [95%CI =0.15–0.26]) with the remaining variation accounted for by non-shared environmental influences (E = 0.25 [95%CI = 0.23–0.26]). A model in which the covariation between relatives was partially explained by shared environmental influences provided a worse fit of the data (ACE: AIC = 5875.722 vs. ADE: AIC = 5865.629)

Moderator Model

Allowing age, parity and their interactions to moderate the amount of variance explained by genetic and

Table 4

Twin–Twin Correlations for lnBMI in MZ pairs, DZ pairs, Sibling Pairs and Twin/Non-Twin Pairs After the Adjustment of Covariates and the Exclusion of Outliers
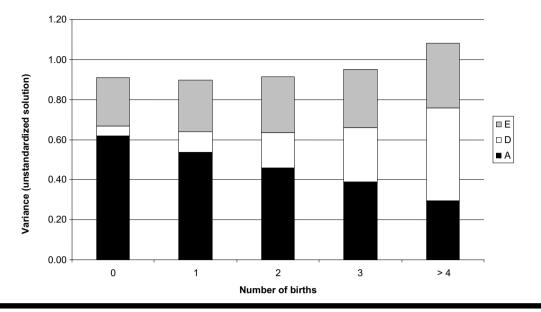
	N	r (95% CI)
MZ/MZ pairs	2166	0.69 (0.67–0.71)
DZ/DZ pairs	1486	0.34 (0.30–0.38)
Twin/Sib pairs	2383	0.28 (0.24–0.33)
Sib/Sib pairs	376	0.25 (0.16–0.34)

non-shared environmental influences significantly increased the fit of the model ($\Delta\chi^2 = 1.24$) and thus an ADE moderator model was chosen. The moderating and main effect parameters (i.e., the β terms in the variance components and means model respectively) for the saturated and best fitting moderator models are displayed in Table 5. For the nonadditive genetic variance, the β term for the number of births (β_{birth}) and age² ($\beta_{age}{}^2$) moderated components were the only moderator variables that could not be dropped without a significant loss of fit ($\chi^2_2 = 15.94$, $p < .001$). However, none of the moderated components of the additive genetic or unique environmental variance could be removed from the model without significantly worsening the fit of the model. Two-dimensional graphs of the variance components across parity and across age are shown in Figures 2 and 3, respectively.

Association Analysis

In an attempt to replicate Gutersohn et al. (2000), we stratified the sample by sex and then further stratified the females into parous (N = 3211) and nulliparous (N = 101) women. Given the recessive nature of the 825T allelic effect we adopted the more conservative two degree of freedom dominance test. As shown in Table 6, there was no association with any of the three SNPs among the females. However, a significant association was observed among the males for rs10744716 at the 5% significance level ($\chi^2_2 = 10.22$, $p = 0.006$; effect size = 0.47kg/m²). The effect was nonadditive genetic in nature given that the nonadditive genetic beta from QTDT was equal to –0.019 compared to the additive genetic beta of –0.008. In contrast, the effect sizes in females and in the sexes combined were
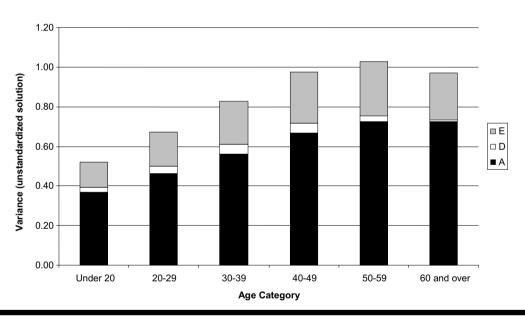
Figure 2

Unstandardized variance components† for female BMI after applying childbirth moderating effects.

Note: † after correction for fixed effects.

Figure 3

Unstandardized variance components† for female BMI after applying age moderating effects.

Note: † after correction for fixed effects.

0.11kg/m² and 0.19kg/m², respectively. Furthermore, the specified covariates did not alter results for all SNPs. Mean BMIs for all three *GNβ3* SNPs by genotype and sex are shown in Table 7.

Discussion

In the current analyses, the increase in variance of BMI due to age (see Figure 3) was predominantly due to increasing additive genetic effects and was quadratic in nature beginning to taper off among the older participants. In contrast, the increase in variance associated with parity was predominantly driven by increases in the nonadditive genetic variance (see Figure 2). This finding was of particular interest given Gutersohn et al's (2000) report of a recessive association between post-partum weight retention and the 825T allele (rs5443) of the *GNβ3* gene. These findings suggest age, parity, and their interactions, may significantly influence the variance of BMI in females.

In modern societies, the prevalence of obesity is likely to be explained by previously 'silent' genetic variants, which may now play important permissive

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Table 5

Regression β Terms for the Means and for Moderation of Residual Components (With 95% Confidence Intervals)

Moderator (β)	Means		Variance components					
			A		D		E	
	Saturated	Best fitting	Saturated	Best fitting	Saturated	Best fitting	Saturated	Best fitting
no modifier	—	—	0.79 (0.69 – 0.86)	0.79 (0.74 – 0.84)	0.23 (0.15 – 0.45)	0.22 (0.20 – 0.39)	0.49 (0.45 – 0.53)	0.49 (0.45 – 0.53)
β_{age}	0.31 (0.28 – 0.34)	0.31 (0.28 – 0.34)	0.07 (0.07 – 0.15)	0.10 (0.07 – 0.14)	0.16 (−0.02 – 0.28)	—	0.06 (0.03 – 0.09)	0.06 (0.03 – 0.08)
β_{age2}	−0.14 (−0.16 – −0.12)	−0.14 (−0.16 – −0.12)	−0.04 (−0.09 – −0.02)	−0.03 (−0.04 – −0.02)	−0.05 (−0.12 – −0.03)	−0.05 (−0.05 – −0.01)	−0.03 (−0.04 – −0.01)	−0.03 (−0.05 – −0.03)
β_{birth}	0.03 (0.01 – 0.05)	0.03 (0.01 – 0.05)	−0.06 (−0.10 – −0.01)	0.05 (0.02 – 0.09)	0.10 (0.04 – 0.15)	0.10 (0.09 – 0.13)	0.02 (0.01 – 0.04)	0.02 (0.01 – 0.04)
$\beta_{age \leftrightarrow birth}$	0.01 (0.00 – 0.03)	0.01 (0.01 – 0.03)	−0.07 (−0.11 – −0.03)	−0.04 (−0.06 – −0.02)	0.00 (−0.05 – 0.07)	–	−0.04 (−0.06 – −0.02)	−0.04 (−0.06 – −0.02)
$\beta_{birth \leftrightarrow age2}$	0.01 (0.00 – 0.02)	0.01 (0.01 – 0.02)	0.03 (0.01 – 0.06)	0.02 (0.00 – 0.03)	−0.01 (−0.05 – 0.01)	–	0.02 (0.01 – 0.03)	0.02 (0.01 – 0.03)

Note: In best fitting model, dashes indicate the path coefficient was nonsignificant and set to zero.

Table 6

Association Results for SNPs in *GNβ3* (Significant Results in Bold)
Note: as no evidence of population stratification was found all association analyses used the QTDT total test for association

	Population stratification		Association	
	χ^2_2	*p* value[†]	χ^2_2	*p* value[†]
Females				
Parous				
rs10744716	1.32	0.517	0.00	1.000
825T (rs5443)	1.81	0.405	0.75	0.727
rs5446	1.97	0.373	0.47	0.791
Nulliparous				
rs10744716	Test not available as there		0.30	0.861
825T (rs5443)	were no nulliparous pairs		1.08	0.583
rs5446			0.89	0.641
Males				
rs10744716	4.68	0.096	**10.22**	**0.006**
825T (rs5443)	4.55	0.103	0.71	0.701
rs5446	4.98	0.083	0.18	0.914

Note: [†] uncorrected for multiple testing.

roles in the current environment, rather than changes in the current gene pool. For instance, a high prevalence of 825T allele of the *GNβ3* gene has been found across different ancestral ethnicities including Caucasian, Chinese and Black African (Gutersohn et al., 2000; Siffert et al., 1999), suggesting that it might have been advantageous in hunter gatherer societies, in that, prevention of post-pregnancy weight loss increased the breast feeding capability of the mother (Gutersohn et al., 2000). That is, the accumulation of additional energy stores during pregnancy had distinct advantages when the availability of food to support fetal growth (Hytten, 1991) and lactation (Whitehead, Lawrence, & Prentice, 1986) was unpredictable. But, this ability to store fat, which can be represented as a 'thrifty genotype', may be exacerbated by the current environment leading to the increase of maternal body

weight over and above what is required for growth of the foetus, placenta and other products of conception (Harris & Ellison, 1997).

In an attempt to explain the increasing nonadditive genetic variance across parity, we tested for association between the 825C/T *GNβ3* polymorphism (rs5443) in addition to three other SNPs located near (rs10744716, upstream) or within *GNβ3* (rs5442 in exon 10 and rs5446 in exon 11) and BMI in nulliparous and parous females as well as in males. Although, we found no evidence for association between any of the four SNPs in either nulliparous or parous females, a significant association was found between male BMI and rs10744716 ($\chi^2_2 = 10.22$, *p* = .006).

The negative finding between rs5443 and BMI has been found across several populations including Brazilian, Danish, Swiss, Japanese, British and

Table 7

Maximum Likelihood Adjusted BMI Means† by Sex and Genotype for Each of the Three SNPs (rs5443 (C/T), rs5446 (C/T), rs10744716 (G/A) With 95% Confidence Intervals for Means in Brackets

	N	TT	N	TC	N	CC
SNP – rs5443						
Males	152	24.73 (24.13–25.32)	692	24.56 (24.57–25.15)	828	25.06 (24.80–25.32)
Females	274	24.54 (23.98–25.11)	1266	24.19 (23.92–24.47)	1558	24.31 (24.05–24.57)
Males and females	426	24.63 (24.21–25.05)	1958	24.43 (24.22–24.63)	2386	24.58 (24.38–24.78)
	N	TT	N	TC	N	CC
SNP – rs5446						
Males	138	24.80 (24.17–25.43)	671	24.91 (24.62–25.21)	859	25.00 (24.74–25.26)
Females	258	24.51 (23.91–25.10)	1234	24.21 (23.93–24.48)	1604	24.32 (24.07–24.58)
Males and females	396	24.62 (24.17–25.07)	1905	24.46 (24.25–24.67)	2463	24.56 (24.37–24.76)
	N	GG	N	GA	N	AA
SNP – rs10744716						
Males	403	24.53 (24.16–24.91)	804	25.18 (24.91–25.45)	468	24.89 (24.55–25.24)
Females	768	24.24 (23.89–24.60)	1525	24.34 (24.10–24.62)	806	24.22 (23.88–24.57)
Males and females	1171	24.36 (24.09–24.63)	2329	24.62 (24.43–24.82)	1274	24.51 (24.26–24.76)

Note: † corrected for age, age^2, birth, age*birth, age^2*birth as well as sex for males and females combined group only.

European Caucasian (Andersen et al., 2006; Benjafield et al., 2001; Danoviz et al., 2006; Hinney et al., 2001; Mattevi et al., 2002; Ohshiro et al., 2001; Potoczna et al., 2004; Suwazono et al., 2004). In addition, rs5443 was not found to be associated with BMI in a specific female Caucasian sample (Terra et al., 2005). The lack of association found in this study could possibly be due to measurement error given the parity information was self reported and little information was available on when BMI measurements were recorded since last birth. Furthermore, the original report was conducted in a German population, which may suggest that there may be a rarer, population specific variant more strongly associated with BMI in parous women in an Australian population.

The *GNβ3* SNP rs10744716 has not previously been associated with any other trait. It is, however, in moderate linkage disequilibrium (LD) with rs5443 (r^2 = 0.6) and rs5446 (r^2 = 0.5), which may explain the presence of the current association. That is, SNP rs5446 has been associated with systolic and diastolic blood pressure in obese Caucasian female dizygotic twins (Dong et al., 2004) and hypercholesterolemia in Japanese males (Suwazono et al., 2006) but not hypertension in a northern Chinese Han population (Li et al., 2005). Therefore, suggesting differences in population LD may explain the detection of association

with different SNPs in different populations. Taken together, these results suggest that if variation with *GNβ3* influences BMI, the SNPs tested thus far are not the causative effects, rather they are in LD with other, as yet ungenotyped, causative mutation(s).

Nonetheless, little information is available about rs10744716 and its possible association with BMI. Indeed, it is possible that our analyses lacked power and the association of rs10744716 with male BMI may have been the result of a type I error. However, we consider this unlikely given our large sample size. Therefore, rs10744716 might be, or be in LD with, a novel variant within *GNβ3* that is involved in the regulation of body weight in males. Whether other variants with *GNβ3* influence BMI in females remains to be seen.

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