

Phosphatidylinositol 3-kinase p85 α regulatory subunit gene *PIK3R1* haplotype is associated with body fat and serum leptin in a female twin population

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Abstract

Aims/hypothesis Phosphatidylinositol 3-kinase (PI3K) couples the leptin and insulin signalling pathways via the insulin receptor substrates IRS1 and IRS2. Hence, defective activation of PI3K could be a novel mechanism of peripheral leptin or insulin resistance. We investigated associations of tagging single-nucleotide polymorphisms (tSNPs) in the PI3K p85 α regulatory subunit gene *PIK3R1* with anthropometry, leptin, body fat and insulin sensitivity in a female twin population of European extraction.

Materials and methods Eight tSNPs were genotyped in 2,778 women (mean age 47.4 \pm 12.5 years) from the St Thomas' UK Adult Twin Registry (Twins UK).

Results SNP rs1550805 was associated with serum leptin ($p=0.028$), BMI ($p=0.025$), weight ($p=0.019$), total fat

($p=0.004$), total fat percentage ($p=0.002$), waist circumference ($p=0.025$), central fat ($p=0.005$) and central fat percentage ($p=0.005$). SNPs rs7713645 and rs7709243 were associated with BMI ($p=0.020$ and $p=0.029$, respectively), rs7709243 with weight, total and central fat ($p=0.026$, $p=0.031$ and $p=0.023$, respectively) and both SNPs with fasting glucose ($p=0.003$ and $p=0.001$, respectively) and glucose 2-h post OGTT ($p=0.023$ and $p=0.007$, respectively). Subjects with haplotype 222 (frequency 7.2%) showed higher serum leptin concentration ($p=0.007$) and body fat measures ($p\leq 0.001$ for all), and those with haplotype 221 (frequency 38.7%) showed higher fasting and 2-h glucose ($p=0.035$ and $p=0.021$, respectively) compared with subjects with the most common haplotype, 111 (frequency 45.5%).

Conclusions/interpretation Association of the *PIK3R1* SNP rs1550805 with serum leptin and body fat may reflect a diminished ability of PI3K to signal via IRS1 or IRS2 in response to leptin.

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Abbreviations

D'	pairwise linkage disequilibrium statistic
ESM	Electronic Supplementary Material
GEE	generalised estimating equations
JAK2	Janus activating kinase 2
LD	linkage disequilibrium
MAF	minor allele frequency
NCBI	National Center for Biotechnology Information (USA)
PI3K	phosphatidylinositol 3-kinase
<i>PIK3R1</i>	PI3K p85 α regulatory subunit gene
PKB	protein kinase B
SiM	insulin sensitivity measure

SNP single-nucleotide polymorphism
 tSNP tagging single-nucleotide polymorphism

Introduction

In the hypothalamus, insulin and leptin function as afferent adiposity signals, which are important for the regulation of body fat stores and glucose metabolism [1]. Insulin induces tyrosine phosphorylation of the insulin receptor, whose intrinsic kinase activity phosphorylates insulin receptor substrates IRS1 and IRS2. Binding of activated IRS1 and IRS2 to the p85 α regulatory subunit of phosphatidylinositol 3-kinase (PI3K) then leads to association with the p110 catalytic subunit and activation of downstream targets via protein kinase B (PKB) [2]. Binding of leptin to hypothalamic receptors, inducing tyrosine-phosphorylation of Janus activating kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3), is paralleled by a comparable increase in PI3K activity associated with IRS2 [3] and to a lesser extent with IRS1 [4–6]. Hence IRS1 and IRS2 represent convergence points in the two hypothalamic signalling pathways and cross-talk could explain the overlapping activities of these two hormones [3].

Overweight individuals have high circulating levels of leptin, implying central resistance to its effect as an adiposity factor [7]. It has been established that pharmacological inhibition of PI3K activity in the hypothalamus inhibits the effects of leptin on food intake [3, 8], suggesting that defective activation of hypothalamic PI3K may play a role in central leptin resistance. Recently, leptin has been shown to increase PI3K activity in the liver, leading to lowering of hepatic triglyceride levels in lean rats, but not in obese animals on a high-fat diet [9]. Hence, defective activation of PI3K could also be a novel mechanism of peripheral leptin resistance. Direct interaction between PI3K and membrane vesicles leads to mobilisation of GLUT4 glucose transporters in response to insulin stimulation in adipose, muscle and liver cells [10], so it is possible that defective PI3K activity may also contribute to peripheral insulin resistance.

The PI3K regulatory subunit isoforms p85 α , p55 α and p50 α , which are generated from the same *PIK3R1* gene by alternative splicing [11], negatively modulate catalytic activity to different extents [12], the strongest effect being shown by p85 α [13]. The p85 α protein includes two SH2 domains, which bind to specific phosphotyrosines of IRS1 and IRS2 [14] and are preserved in the p55 α and p50 α variants [15]. Three amino acid substitutions have been identified in *PIK3R1*, Met326Ile (rs3730089) [16], Arg409Gln in one insulin-resistant subject [17], and an unvalidated single-nucleotide polymorphism (SNP),

Ala440Asp (rs3730091). The first was associated with alterations in glucose/insulin homeostasis in one study [16] but not in a larger sample [18]. One other study [19] found an association between intronic SNP IVS4+82 and type 2 diabetes.

Aside from Met326Ile and IVS4+82, there are no other studies of association of *PIK3R1* variants with variables characterising the action of leptin or insulin. Rather than focusing on one or two functional SNPs, which are often difficult to replicate, examination on a gene-wide level is preferable, common variants within a candidate gene being considered jointly through the selection of a minimal set of tagging SNPs (tSNPs) [20]. These effectively capture information about the common variants by taking into account patterns of linkage disequilibrium (LD) across the gene [21, 22]. We selected a set of eight tSNPs in *PIK3R1* and tested their association with a range of variables characterising leptin, body fat and insulin sensitivity in a large population of female twins ($n=2,778$; mean \pm SD age 47.4 \pm 12.5 years), approximately double the size of the larger study of Hansen et al. [18]. SNPs were tested individually and as haplotypes.

Subjects and methods

Study design

The Twins UK Registry comprises unselected, mostly female volunteers ascertained from the general population through national media campaigns in the UK [23]. The study cohort included 2,778 subjects (423 monozygotic pairs, 940 dizygotic pairs and 52 singletons) with available leptin data. No difference was observed in the distributions of age and menopausal status between subjects with and without leptin data ($n=362$). The number of individuals in the study cohort with data on other phenotypic variables is shown in Table 1.

Means and ranges of quantitative phenotypes in Twins UK are similar to an age-matched sample of the UK female population [24]. Informed consent was obtained from participants before they entered the study, and the study was approved by the local research ethics committee.

Zygoty, body composition and biochemical analyses

Zygoty was determined by a standardised questionnaire and confirmed by DNA fingerprinting. 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

Table 1 General characteristics of Twins UK subjects

Variable	<i>n</i>	Mean±SD
Age (years)	2,778 ^a	47.4±12.5
Postmenopausal (%)	2,455	47.7
Obesity-related variables		
Leptin (ng/ml)	2,778	16.5±12.0
BMI (kg/m ²)	2,762	24.8±4.4
Weight (kg)	2,763	65.3±11.8
Waist circumference (cm)	2,711	78.4±10.2
Total fat (kg)	2,722	23.4±8.8
Total fat (%)	2,681	35.6±8.0
Central fat (kg)	2,699	1.33±0.73
Central fat (%)	2,699	31.2±11.5
Insulin sensitivity ^b		
Fasting glucose (mmol/l)	2,318	4.49±0.55
Fasting insulin (μU/ml)	1,986	6.97±5.96
2-h glucose (mmol/l)	739	5.18±1.10
2-h insulin (μU/ml)	739	34.2±25.4
SiM (10 ⁸ μU ⁻¹ mmol ⁻¹ l ⁻¹)	739	88.5±68.8

^aNumber of subjects with leptin data and genotype data on at least one SNP; includes 423 monozygotic pairs, 940 dizygotic pairs and 52 singletons

^bNon-fasting subjects, patients with either type 1 or type 2 diabetes, patients on any glucose-lowering drugs, and subjects with fasting glucose >7.8 mmol/l or 2-h glucose >11.1 mmol/l were all excluded

(cm) was measured at the level midway between the lower rib margin and the iliac crest. Body composition was measured by dual emission X-ray absorptiometry (Hologic QDR-2000, Vertec, Waltham, MA, USA). Serum leptin concentration was determined after an overnight fast using a radioimmunoassay (Linco Research, St Louis, MO, USA). Fasting insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, UK) and glucose was measured on an Ektachem 700 multichannel analyser using an enzymatic colorimetric slide assay (Johnson and Johnson Clinical Diagnostic Systems, Amersham, UK). A subsample of approximately 739 subjects, representing unselected female twins from the general population, underwent an OGTT, for which glucose and insulin levels were measured before and 2 h after a 75-g oral glucose load.

Genotyping for SNP validation and tSNP selection

We used the National Center for Biotechnology Information (USA) (NCBI; <http://www.ncbi.nlm.nih.gov/SNP/>) and CHIP Bioinformatics (<http://www.snpperchip.org>) databases to choose SNPs for validation and tSNP selection in our cohort. Information from the HapMap (<http://www.hapmap.org>) for *PIK3R1* was not available at the time of SNP selection. Thirteen SNPs validated in the database populations were genotyped in 94 unrelated subjects from the Twins UK cohort for tSNP selection. PCR primers and

conditions are given in Electronic Supplementary Material (ESM) Table 1. This sample size is three times that suggested by one permutation study [25], which indicates that genotyping 25–32 unphased individuals is sufficient to select tSNPs. The rs numbers and relative positions of the 13 SNPs are shown in Fig. 1.

Genotyping in cohorts

Eight tSNPs (rs706713, rs7713645, rs7709243, rs251406, rs40318, rs1550805, rs831125 and rs3730089) were selected and genotyped in the complete Twins UK cohort by Pyrosequencing (Biotage, Uppsala, Sweden). Genotyping accuracy, as assessed by inclusion of duplicates (pairs of monozygotic twins) in the arrays, was 98% and negative controls (water blanks) were included on each plate. Genotyping success rate for each tSNP varied between 77.2 and 93.8%. Primers and PCR conditions for SNP genotyping in the full cohort by Pyrosequencing are given in ESM Table 2.

Statistical analysis

The main purposes of our analyses were to select a set of tSNPs representing the common variants in *PIK3R1* and to test the effects of these tSNPs, individually and/or as haplotypes, on a range of variables, including serum leptin, measures of body fat and insulin sensitivity.

We used the htSNP2 program developed by Chapman et al. [21] to identify the optimal subset of tSNPs, which were selected in such a way that the allele frequencies of the remaining (non-tagging SNPs) could be predicted well. A series of regression equations were calculated for which the predictive efficiency was assessed in terms of R_L^2 , which measures the proportion of variance of each remaining SNP explained by regression on the tSNP alleles (locus-based scoring). htSNP2 was used to select a tSNP set that predicted remaining SNPs with a minimum R_L^2 of 0.80, as recommended by Chapman et al. [21]. Pairwise LD coefficients in the tSNP test sample were calculated using GOLD (available from <http://www.sph.umich.edu/csg/abecasis/GOLD/>, last accessed in July 2006) and are reported as the pairwise linkage disequilibrium statistic (D') and r^2 [26].

Preliminary association analyses were performed using STATA 8 (StataCorp, College Station, TX, USA). Phenotypes significantly ($p < 0.05$) deviating from normal were log-transformed to obtain normal distributions prior to analysis. Hardy–Weinberg equilibrium was tested with a χ^2 test with one degree of freedom in one twin of each pair chosen at random to prevent inflated significance. Association analyses were performed using generalised estimating equations (GEE) [27], a method that allows for the

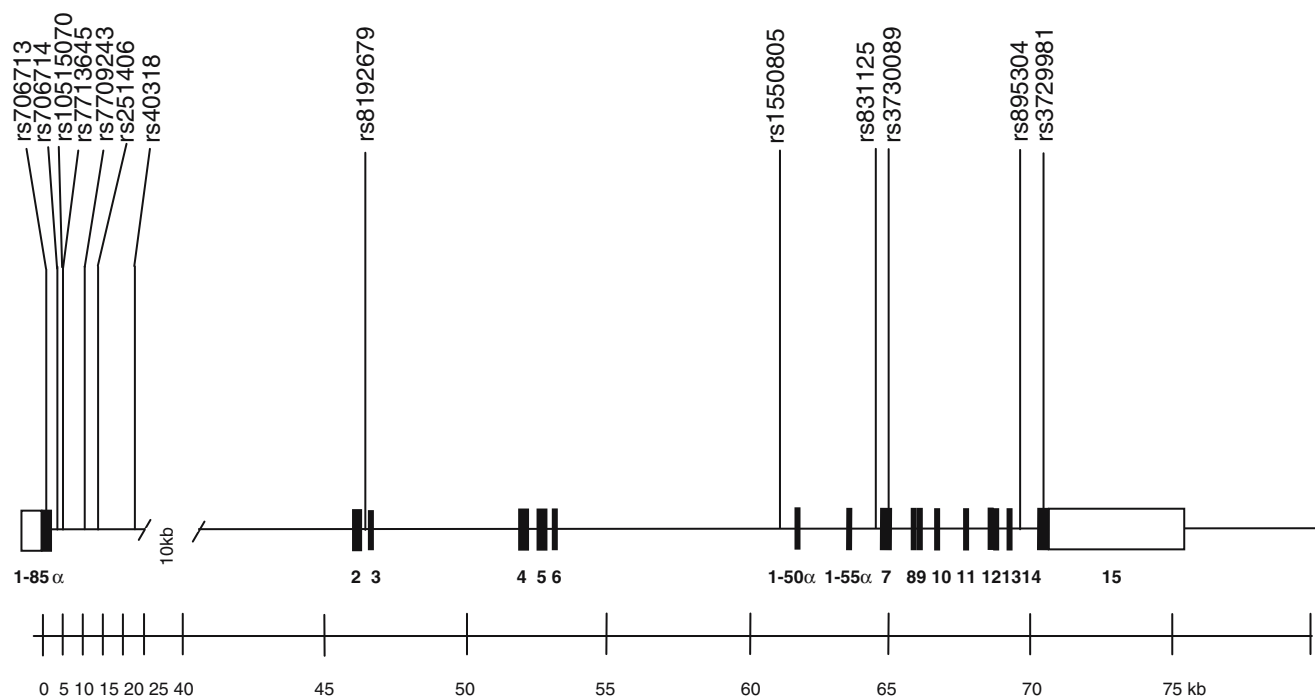


Fig. 1 Genomic map of *PIK3R1* gene with rs numbers and locations of the 13 validated SNPs. Boxes represent exons. Solid boxes represent translated regions and open boxes represent untranslated regions

relatedness between twins and yields unbiased standard errors and *p* values. Analyses were done separately for each of the SNPs and followed up by haplotype analyses. For individual SNP association analyses, we first performed an overall test of genotypic association with two degrees of freedom. In the presence of a significant association, additive, dominant and recessive models (all with one degree of freedom) were further tested to find the best mode of inheritance. Age and menopausal status were included as covariates in the models. Details of our method of testing the association of statistically inferred haplotypes with continuous traits have been described previously [28]. In short, we adapted the haplotype trend regression (HTR) of Zaykin et al. [29] for the analysis of related subjects such as twins by replacing the linear regression with the GEE procedure. Assuming additive effects of the haplotypes on the trait, the HTR tests for the contribution of individual haplotypes. The most frequent haplotype was used as the baseline with which the effects of the other haplotype were contrasted. Haplotypes with estimated frequencies below 5% in all the subjects were pooled and included in the model as one term. The probabilities of haplotype pairs were estimated with PHASE 2.0 software (available from http://www.depts.washington.edu/ventures/UW_Technology/Express_Licenses/PHASEv2.php, last accessed in July 2006) [30]. A diplotype analysis under a codominant model was also tested by using a weighted GEE, the weights being the probability of each possible haplotype pair combination for an individual as estimated by PHASE 2.0. Only the

diplotypes with frequency >5% were considered for the diplotype analyses. Obesity-related variables included leptin, weight, BMI, waist, total fat mass, total fat percentage, central fat mass and central fat percentage. Factor analysis was used to combine strongly correlated indices of obesity into two measures: one for general obesity (serum leptin, BMI, weight, total fat mass and total fat percentage) and one for central obesity (waist, central fat mass and central fat percentage). To reduce the likelihood of identifying false-positive associations, we performed analyses of individual obesity-related variables only for those SNPs showing significant results for at least one of the combined scores. We initially used two indices of insulin sensitivity, fasting insulin and the insulin sensitivity measure (SiM). SiM is derived from both fasting and 2-h insulin and glucose data [31] and was calculated according to the following formulae: $\text{SiM} = (0.137 \times \text{SiB} + \text{SiH2}) / 2$, where the insulin sensitivity baseline (fasting) = $10^8 / (\text{fasting insulin} \times \text{fasting glucose} \times \text{distribution volume [VD]})$; insulin sensitivity 2 h after OGTT (SiH2) = $10^8 / (2\text{-h insulin} \times 2\text{-h glucose} \times \text{VD})$, and $\text{VD} = 150 \text{ ml/kg} \times \text{body weight}$. Both fasting insulin ($r=0.68$) and SiM ($r=0.92$) are highly correlated with insulin sensitivity in the normal population. SiM is also a good predictor of diabetes, especially in Europeans [32]. We subsequently tested fasting and 2-h glucose based on the hypothesis that direct stimulation of the mobilisation of glucose transporter GLUT4 by PI3K [10] could occur independently of metabolic effects initiated via its activation of PKB. The program developed

by Purcell et al. [33] was used to calculate the genetic power of the study. It provides automated power analysis for quantitative trait loci association tests in sibships. Assuming a sibling correlation of 0.3, a sample of 840 dizygotic pairs is adequate to detect a locus effect of 0.5% with 80% power (and $\alpha=0.05$). The present study, involving 940 dizygotic pairs and an additional 423 monozygotic pairs and 52 singletons, provided even greater power.

Results

Characteristics of subjects in the Twins UK study sample are shown in Table 1. Among the 13 SNPs chosen from the NCBI database, rs8192679, rs10515070, rs895304 and rs3729981 were not polymorphic in our test sample of 94 subjects. Of the remaining nine polymorphic SNPs, all except rs3730089 (Met326Ile in exon 6) and rs706713 (Tyr73Tyr in exon 1) were located in non-coding regions and their genotype frequencies were consistent with Hardy–Weinberg proportions. The minor allele frequencies (MAFs) and the pairwise D' and r^2 of these nine SNPs in 94 subjects are shown in Table 2. All the SNPs had MAFs >0.05 and strong LD was observed among rs706713, rs706714, rs7713645 and rs7709243 ($D'>0.8$ and p values <0.05). However, except for rs706713 and rs706714 ($r^2=0.878$), low r^2 values were found throughout the gene, indicating that in this case tagging would not increase efficiency. This was confirmed by the result that a total of eight tSNPs were selected to represent the nine SNPs by the htSNP2 software with the criterion $R_L^2 \geq 0.80$ (Table 2). The eight tSNPs rs706713, rs7713645, rs7709243, rs251406, rs40318, rs1550805, rs831125 and rs3730089 were further genotyped in all 2,778 subjects of the Twins UK cohort. ESM Table 3 shows the genotype and allele frequencies of the eight tSNPs, based on one monozygotic twin and both dizygotic twins genotyped for each pair. None of the loci showed deviation from Hardy–Weinberg equilibrium.

To reduce the potential generation of false-positive results through multiple testing, we first analysed the combined central and general obesity scores. We observed significant associations for three tSNPs in at least one of these combined scores (rs7713645, $p=0.072/0.040$; rs7709243, $p=0.033/0.020$; rs1550805, $p=0.006/0.009$ for central/general obesity scores). These SNPs explained between 0.22 and 0.31% of the variance in central obesity and between 0.12 and 0.25% of the variance in general obesity. Since we did not observe any significant effects of rs251406, rs40318, rs706713, rs831125 and rs3730089 on these combined scores (or on any of the glucose or insulin variables), results for these five tSNPs are not further shown. Association results of the three significant tSNPs

with the individual obesity phenotypes are shown in Table 3. Carriers of the minor allele of rs1550805 had significantly higher serum leptin, BMI, weight, total fat, total fat percentage, waist circumference, central fat and central fat percentage, based on additive and dominant models. Carriers of the minor allele of rs7709243 had significantly higher BMI, weight, total fat and central fat. Carriers of the minor allele of rs7713645 had significantly higher BMI. Carriage of the minor allele of either rs7713645 or rs7709243 was also associated with significantly higher fasting glucose (0.29–0.42% of the variance) and glucose 2 h after the OGTT (0.6–0.75% of the variance). However, we did not observe any significant effects of these tSNPs on the two insulin sensitivity indices, fasting insulin and SiM. Association results for homeostasis model assessment, another index of insulin sensitivity, based on fasting levels of insulin and glucose, are not shown because they were identical to those for fasting insulin.

The estimated haplotype frequencies of the eight tSNPs are shown in ESM Table 4. The low LD across this gene resulted in 24 haplotypes with frequency $>1\%$ and only five of them had frequency $\geq 5\%$. Based on simulations, Lake et al. [34] suggest that haplotype frequencies need to be at least 5% to avoid biased regression parameters. The fact that these five haplotypes cover only 50% of the haplotype diversity made the haplotype analysis based on the eight tSNPs uninformative and difficult to interpret. We decided to focus on the three SNPs which were individually associated with phenotypes to clarify the combined effect of these SNPs. ESM Table 5 shows estimated frequencies of haplotypes constructed by the three SNPs rs7713645, rs7709243 and rs1550805. Table 4 shows tests of association including the three common ($>5\%$) haplotypes (111, 221 and 222). Haplotype analyses showed results similar to those for the individual SNP associations, with haplotype rs7713645–rs7709243–rs1550805 222 (frequency 7.2%) showing higher serum leptin ($p=0.007$) and higher levels of all body fat measures (p values ≤ 0.001) compared with the most common haplotype, 111 (frequency 45.5%). Haplotype 221 (frequency 38.7%) showed higher fasting glucose ($p=0.035$) and 2-h glucose ($p=0.021$) compared with the most common haplotype. SNP rs1550805 had a strong association with obesity variables, whilst the effects on glucose appeared to be associated with rs7713645 and rs7709243. Because rs7713645 and rs7709243 showed the same alleles among the three most common haplotypes, a more parsimonious haplotype analysis based on one of these two SNPs and rs1550805 was further tested. Results remained unchanged. The 2344 individuals used to reconstruct haplotypes included some subjects with incomplete genotype data. We repeated the haplotype analyses based on the subjects with data on all three SNPs and observed

Table 2 Pairwise linkage disequilibrium coefficients between the nine SNPs genotyped in 94 subjects

$ D' /r^2$	MAF	rs706713	rs706714	rs7713645	rs7709243	rs251406	rs40318	rs1550805	rs831125	rs3730089
rs706713	0.180		0.878 ^a	0.183 ^a	0.171 ^a	0.017	0.017	0.003	0.001	0.000
rs706714	0.197	1.000 ^a		0.213 ^a	0.186 ^a	0.015	0.040	0.008	0.003	0.000
rs7713645	0.445	1.000 ^a	1.000 ^a		0.452 ^a	0.000	0.053 ^a	0.014	0.028	0.016
rs7709243	0.483	0.808 ^a	0.828 ^a	0.808 ^a		0.001	0.042 ^a	0.026	0.006	0.019
rs251406	0.331	0.416	0.357	0.026	0.048		0.030 ^a	0.024	0.030	0.001
rs40318	0.065	0.214	0.374	1.000 ^a	0.717 ^a	1.000 ^a		0.004	0.032	0.022
rs1550805	0.093	0.073	0.140	0.378	0.572	0.325	1.000		0.002	0.002
rs831125	0.131	0.150	0.266	0.365	0.221	0.632	0.296	0.369		0.025
rs3730089	0.149	0.014	0.009	0.324	0.304	0.080	0.230	0.055	0.909	
R_L^2	–	–	0.903	–	–	–	–	–	–	–

$|D'|$ below diagonal and r^2 above diagonal

MAF Minor allele frequency; R_L^2 proportion of the variance of each non-tagging SNP explained by regression on the tSNPs [21]

^a $p < 0.05$

the same results as in the analyses based on subjects with data on at least one SNP (data not shown). Diplo-type analyses confirmed the results of haplotype analyses: haplotype 222 heterozygotes showed higher levels of body fat measures and haplotype 221 heterozygotes and homozygotes showed higher 2-h glucose (ESM Table 6).

The overall significance of haplotype associations with leptin, BMI, waist and all fat measurements was high and the effect size was relatively large. For example,

individuals homozygous for haplotype 222 on average were 5 kg heavier, had 4 cm larger waist circumference and 1.73 kg/m² greater BMI than those homozygous for 111. However, the percentages of variance in obesity phenotypes explained by *PIK3R1* haplotypes ranged from only 0.35% ($p=0.003$) for central fat percentage to 0.54% for BMI ($p=0.003$), which is a reflection of the low frequency of haplotype 222 (7.2%) but much as expected for a trait influenced by many genes.

Table 3 Association analyses of *PIK3R1* tSNPs rs7713645, rs7709243 and rs1550805

	Number	Mean±SD			p value ^a
		11/12/22	11	12	
rs7713645					
BMI	622/1,202/572	24.5±4.4	24.8±4.5	25.1±4.3	0.020
Fasting glucose	514/995/480	4.45±0.53	4.49±0.56	4.57±0.56	0.003 ^b
2-h glucose	273/447/268	5.04±1.08	5.27±1.13	5.28±1.12	0.023 ^c
rs7709243					
BMI	674/1,332/587	24.4±4.2	24.6±4.4	25.1±4.3	0.029
Weight	675/1,332/587	64.6±11.8	65.1±11.7	66.2±11.7	0.026 ^d
Total fat	661/1,310/582	22.7±8.7	23.4±8.8	23.9±8.6	0.031 ^d
Central fat	656/1,299/580	1.28±0.74	1.33±0.73	1.36±0.70	0.023 ^d
Fasting glucose	571/1,106/503	4.46±0.53	4.47±0.56	4.56±0.56	0.001 ^b
2-h glucose	293/508/257	5.04±1.09	5.28±1.11	5.31±1.19	0.007 ^c
rs1550805					
Leptin	1,972/423/11	16.3±11.9	17.7±12.6	19.6±12.3	0.028 ^c
BMI	1,957/423/11	24.7±4.4	25.1±4.6	27.4±7.0	0.025 ^d
Weight	1,958/423/11	65.1±11.8	66.3±12.2	72.8±21.3	0.019 ^d
Total fat	1,924/420/11	23.3±8.7	24.3±9.4	28.6±13.7	0.004 ^d
% Total fat	1,899/412/11	35.4±8.0	36.4±8.0	39.7±6.9	0.002 ^d
Waist circumference	1,916/420/11	78.2±10.2	79.1±10.3	82.9±13.9	0.025 ^d
Central fat	1,912/416/11	1.32±0.73	1.39±0.74	1.65±1.01	0.005 ^d
% Central fat	1,912/416/11	31.1±11.5	32.1±11.5	34.5±11.0	0.005 ^c

^a p value adjusted for age

^b p value under the recessive genetic model

^c p value under the dominant genetic model

^d p value under the additive genetic model

Table 4 Tests of association of tSNP haplotypes (frequency >5%) with phenotypes

Variable	Haplotype 221 ^a		Haplotype 222 ^a		Overall	
	β	p^b	β	p^b	Explained variance (%)	p^b
Leptin	0.46	NS	4.67 (1.68–7.65)	0.007	0.43	0.043
BMI	0.38	NS	1.78 (0.71–2.85)	0.001	0.54	0.003
Weight	0.83	NS	5.00 (2.07–7.93)	0.001	0.47	0.003
Total fat	0.43	NS	4.00 (1.90–6.11)	0.0004	0.43	0.002
% Total fat	0.24	NS	3.30 (1.47–5.13)	0.0004	0.44	0.003
Waist	0.02	NS	4.00 (1.69–6.62)	0.001	0.41	0.005
Central fat	0.01	NS	0.28 (0.11–0.45)	0.001	0.44	0.002
% Central fat	0.28	NS	4.62 (2.00–7.23)	0.001	0.35	0.003
Fasting glucose	0.08 (0.01–0.15)	0.035	0.03	NS	0.36	NS
2-h glucose	0.24 (0.03–0.45)	0.021	0.31	NS	1.01	0.02

^a Estimates are contrasts with the most common haplotype, 111, where 1 denotes the major allele and 2 denotes the minor allele of the SNPs rs7713645, rs7709243 and rs1550805

^b p value adjusted for age

Discussion

We have examined the association of a set of eight tSNPs spanning the *PIK3R1* gene with a range of phenotypes representing serum leptin, anthropometry, body fat and glucose/insulin homeostasis in a large population of European British female twins. We showed in single-tSNP analyses that one tSNP in particular, rs1550805, was significantly associated with serum leptin, BMI, weight, waist circumference and all the fat variables. tSNPs rs7713645 and rs7709243 were significantly associated with BMI, rs7709243 with weight, total and central fat, and both tSNPs with fasting glucose and glucose 2 h after an OGTT. Haplotype analyses of these three tSNPs confirmed the significance of the individual associations, haplotype 222 showing significantly higher levels of serum leptin and all body fat measures. Haplotype 221 was significantly associated with higher levels of fasting glucose and 2-h glucose, but the most significant finding is the association of rs1550805 with serum leptin and body fat measurements.

The main strength of our study lies in the large size of the study sample, the availability of detailed measurements of body fat by dual X-ray absorptiometry in all subjects, and comprehensive measurement of variation in the gene. This is the first gene-wide association study of *PIK3R1* and the first reported association of variants with leptin and body fat measurements, strengthening the candidature of the gene in susceptibility to obesity and insulin resistance. Assuming a sibling correlation of 0.3, the present study provided power in excess of 80% (and $\alpha=0.05$) to detect a locus effect of 0.5% [33]. It should be noted that we have established these associations only in a female twin population, but that our findings in the twin subjects can be considered as representative of the UK female popula-

tion as a whole [24]. We have previously found few differences between twins and singletons in the population generally, the only indication being that monozygotic twins have a slightly lower weight and a smaller variance for weight than dizygotic twins and singletons [24].

The comprehensiveness of coverage of the *PIK3R1* gene by our selected tSNPs was limited by the availability of SNPs on public databases at the time of the study. Information from the HapMap (<http://www.hapmap.org>) for *PIK3R1* has only recently become available. Seven of our eight tSNPs are within the 27 common SNPs (minor allele frequency $\geq 5\%$ in Centre d'Etude du Polymorphisme Humain [CEPH] subjects) posted on the current release of HapMap (Phase II, January 2006). Sixteen HapMap SNPs cannot be tagged by our selection. The gene lies in a region of low LD, shown by the low r^2 values found throughout the gene, so that eight tSNPs were needed to represent the nine validated SNPs available in public databases at the time. All of our associated tSNPs, with the exception of the Met326Ile variant rs3730089, are intronic and are most likely to be LD markers.

Previously reported investigations of variation in *PIK3R1* have centred on tests of association with type 2 diabetes and associated phenotypes [16, 18, 35, 36], on account of the well-known involvement of PI3K in insulin signalling in peripheral tissues [37] and the hypothalamus [2]. Relatively little attention has been paid to the influence of PI3K on fat deposition, which could occur through the antilipolytic effect of insulin and be influenced by leptin through cross-talk with the insulin-signalling path [2, 38]. Aside from the Met326Ile variant [16] and an IVS4+82 SNP [19], there are to our knowledge no other reported studies of association of other *PIK3R1* variants with variables characterising the action of leptin or insulin.

We found no significant associations between any single tSNPs or haplotypes with fasting insulin and the SiM measure of insulin sensitivity. Among our tested tSNPs, the Met326Ile variant rs3730089 was previously reported to be associated with insulin sensitivity in 380 young healthy Danish subjects [16]. However, this group failed to replicate the association in a later study of 1,190 individuals [18].

In summary, we have established an association between *PIK3R1* SNP rs1550805 with serum leptin and a range of body fat measures in a large European female twin population. Phosphorylation of IRS2 by JAK2 lies at a convergence point of the leptin and insulin signalling pathways [39]. Leptin potentiates insulin signalling through its activation of IRS2 by JAK2 phosphorylated at the leptin receptor [40] and activates PI3K in muscle via this IRS2-dependent pathway [41]. The importance of PI3K signalling via IRS1 in the lipid-lowering effect of leptin in liver has been shown by Huang et al. [9], and Niswender et al. [3] have shown that PI3K and IRS2 are involved in the anorexic response to leptin in the hypothalamus. We speculate that the site marked by rs1550805 may reflect diminished ability of PI3K to signal via IRS1 or IRS2 in response to leptin. This could lead to impaired leptin action, failure of the normal feedback regulation of leptin levels, and the accumulation of body fat.

PI3K regulatory subunit isoforms p85 α , p55 α and p50 α , generated from the same *PIK3R1* gene by alternative splicing [11], negatively modulate catalytic activity to different extents [12], the strongest effect being shown by p85 α [13]. Future investigations need to focus on searching for potentially functional SNPs in the isoform-specific exons as well as putative regulatory regions in *PIK3R1*, in view of evidence that the balance and activity of the isoforms affect the overall activity of the PI3K enzyme [12, 13, 42, 43].

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