

Association of Common *JAK2* Variants With Body Fat, Insulin Sensitivity and Lipid Profile

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The leptin signal is transduced via the JAK2-STAT3 (Janus kinase 2-signal transducer and activator of transcription-3) pathway at the leptin receptor. JAK2 also phosphorylates insulin receptor substrate, integral to insulin and leptin action and is required for optimum adenosine triphosphate-binding cassette transporter A1 (ABCA1)-dependent transport of lipids from cells to apolipoprotein A-1 (apoA-I). We hypothesized that common variation in the *JAK2* gene may be associated with body fat, insulin sensitivity, and modulation of the serum lipid profile in the general population. Ten tagging single-nucleotide polymorphisms (SNPs) spanning the gene were genotyped in 2,760 white female twin subjects (mean age 47.3 ± 12.6 years) from the St Thomas' UK Adult Twin Registry. Minor allele frequencies were between 0.170 and 0.464. The major allele of rs7849191 was associated with higher central fat ($P = 0.030$), percentage of central fat ($P = 0.014$) and waist circumference ($P = 0.027$) the major allele of rs3780378 with higher serum apoA ($P = 0.026$), total cholesterol ($P = 0.014$), low-density lipoprotein (LDL) cholesterol ($P = 0.012$) and lower triglyceride ($P = 0.023$). However, no associations were significant at a level which took account of multiple testing. Although JAK2 is a critical element in leptin and insulin signaling and has a role in cellular cholesterol transport, we failed to establish associations of common SNPs with relevant phenotypes in this human study.

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Janus kinase 2 (JAK2) is a cytoplasmic protein-tyrosine kinase recruited by receptors that lack intrinsic kinase activity, to initiate diverse signaling pathways (1). Leptin signals adiposity levels to centers in the hypothalamus and is an important regulator of energy homeostasis through its inhibition of appetite and enhancement of energy expenditure (2). On binding to receptors, leptin induces activation and tyrosine phosphorylation of JAK2, and subsequent tyrosine phosphorylation of specific residues on the receptor (3), which form high-affinity binding sites for signaling proteins containing Src homology 2 and other phosphotyrosine-binding domains. These include signal transducer and activator of transcription-3 (STAT3), which on tyrosine phosphorylation translocates to the nucleus to activate gene transcription (4), including that of suppressor of cytokine signaling-3 (SOCS3) (3). A different leptin receptor tyrosine residue phosphorylated by JAK2 recruits the Shc adaptor protein SHP2, which activates the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway involved in the mitogenic response (3). JAK2 is also involved in activating insulin receptor substrates 1 and 2, receptor docking proteins

that mediate signaling of both insulin and leptin (5). One of the targets of insulin receptor substrate is phosphatidylinositol 3-kinase, involved in a range of metabolic functions initiated by leptin and insulin (6,7), with variation in the *PIK3R1* gene shown by us to be associated with leptin and body fat (8). SOCS3 mediates feedback inhibition of signaling at both receptors by interfering with activation of the insulin receptor substrate proteins by JAK2 (9). The adenosine triphosphate-binding cassette transporter A1 (ABCA1), located in the cell membrane, mediates transport of excess cholesterol and phospholipids out of cells to high-density lipoprotein (HDL) apolipoproteins, such as apolipoprotein A-1 (apoA-I) (10). Interaction of apoA-I with ABCA1 acutely stimulates the autophosphorylation of JAK2, which is required for binding of apoA-I to ABCA1 and removal of cellular lipids (11).

As JAK2 is involved in leptin-, insulin-, and ABCA1-signaling pathways, we hypothesized that common variants in the *JAK2* gene may influence body fat mass, insulin sensitivity, or serum lipid profile in humans. In this first gene-wide association study of *JAK2* variation in relation to metabolic

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variables, we have used tagging single-nucleotide polymorphisms (tSNPs) to test associations with nine relevant phenotypes in 2,759 white female twin subjects (mean age 47.3 ± 12.6 years). Characteristics of subjects in the St Thomas' UK Adult Twin Registry study sample are shown in **Table 1**. Seventy-five polymorphic SNPs with minor allele frequency >0.05 in the selected 146.8-kb region, (which includes 2 kb upstream and downstream of the 142.75-kb *JAK2* gene) are listed on the HapMap database (HapMap Data Release no. 20/phase 2 January 2006; <http://www.hapmap.org>).

tSNPs effectively capture information of most common variants by taking into account patterns of linkage disequilibrium across a gene (12). *JAK2* lies in a region of strong linkage disequilibrium on chromosome 9 and is therefore amenable to tagging. We used the *htSNP2* program developed by

Chapman *et al.* (12) to select 10 tSNPs: rs3808850, rs1887429, rs2274471, rs7849191, rs1536800, rs10974947, rs7857730, rs3780373, rs3780378, and rs3780379, all of which are in non-coding regions (**Figure 1**). **Supplementary Table S1** online shows pairwise linkage disequilibrium between selected tSNPs in the study sample. **Supplementary Table S2** online shows the genotype and allele frequencies of the tSNPs, based on one monozygous (MZ) and both dizygous (DZ) twins genotyped for each pair.

We found no associations between any of the 10 tSNPs and general obesity scores (see Methods) or insulin sensitivity indices homeostasis model assessment or sensitivity to insulin measurement, so individual variables were not tested further. An association for a 2 degrees of freedom overall genotypic test (i.e., a codominant model) showing borderline nominal significance ($P = 0.03$) was found between tSNP rs7849191 and central obesity. We performed follow-up analyses to determine the best model for all central obesity-related phenotypes and found dominant associations with central fat ($P = 0.030$), percentage of central fat ($P = 0.014$), and waist circumference ($P = 0.027$) in subjects with available data ($n = 2,660$) (**Table 2**). Subjects homozygous for the major allele had significantly higher waist or central fat measurements, in comparison to subjects homozygous or heterozygous for the minor allele, explaining 0.1–0.2% of variance. The only other associations for a codominant model showing borderline nominal significance ($P = 0.03$ – 0.05) were found between tSNP rs3780378 and a number of serum lipid variables (**Table 2**). The major allele was associated with higher serum apoA ($P = 0.026$), lower total cholesterol ($P = 0.014$), and lower low-density lipoprotein (LDL) cholesterol ($P = 0.012$) based on an additive model, but there was no significant association with HDL cholesterol ($P = 0.24$). Subjects homozygous for the major allele had lower levels of triglyceride ($P = 0.023$) in comparison to subjects homozygous or heterozygous for the minor allele. However, no associations with either tSNP were significant at levels which took account

Table 1 Characteristics of subjects

Variable	n	Mean (s.d.)
Age (years) ^a	2,760	47.3 (12.6)
Postmenopausal (%)	2,439	47.5
Obesity-related variables		
Leptin (ng/ml)	2,760	16.5 (12.0)
BMI (kg/m ²)	2,743	24.8 (4.4)
Weight (kg)	2,744	65.4 (11.8)
Waist (cm)	2,692	78.4 (10.2)
Total fat (kg)	2,703	23.4 (8.8)
Total fat (%)	2,662	35.6 (8.0)
Central fat (kg)	2,660	1.33 (0.73)
Central fat (%)	2,660	31.1 (11.5)
Lipid profile		
ApoA (g/l)	2,421	1.70 (0.34)
ApoB (g/l)	2,439	1.17 (0.36)
Triglyceride (mmol/l)	2,473	1.27 (0.80)
Total cholesterol (mmol/l)	2,585	5.56 (1.25)
LDL cholesterol (mmol/l)	2,443	3.46 (1.15)
HDL cholesterol (mmol/l)	2,594	1.55 (0.39)
Insulin sensitivity ^b		
Fasting glucose (mmol/l)	1,002	4.39 (0.45)
Fasting insulin (μIU/ml)	1,002	6.27 (4.41)
2-h glucose (mmol/l)	735	5.19 (1.10)
2-h insulin (μIU/ml)	735	34.3 (25.5)
HOMA	1,002	1.24 (0.89)
SiM ^c , 10 ³ μU ⁻¹ mmol ⁻¹ l	735	88.5 (69.0)

HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; SiM, sensitivity to insulin measurement.

^aNumber of subjects (836 MZ, 1924 DZ) with leptin data and genotype data on at least 1 single-nucleotide polymorphism. ^bNon-fasting subjects, patients with either type 1 or type 2 diabetes, patients on any antidiabetic drugs, and subjects with fasting glucose >7.8 mmol/l or 2-h glucose >11.1 mmol/l were all excluded. ^cSiM was calculated according to the following formulae: $SiM = (0.137 \times SiB + SiH2)/2$, where $SiB = 10^3 / (\text{fasting insulin} \times \text{fasting glucose} \times VD)$; $SiH2 = 10^3 / (2\text{-h insulin} \times 2\text{-h glucose} \times VD)$ and $VD = 150 \text{ ml/kg} \times \text{body weight}$. SiB, insulin sensitivity at baseline; SiH2, insulin sensitivity 2 h after oral glucose; VD, glucose distribution volume.

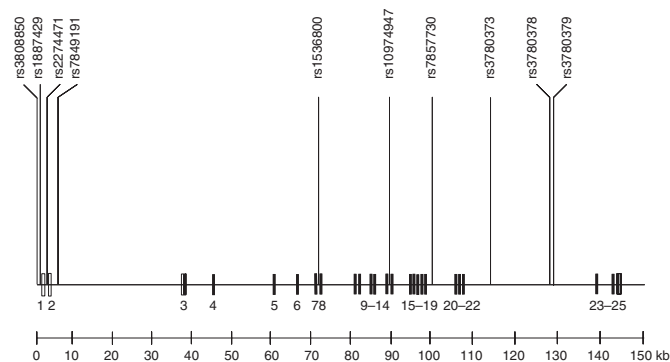


Figure 1 *JAK2* gene map showing selected tagging single-nucleotide polymorphisms (tSNPs). Genotype data for SNPs of minor allele frequency >0.05 in the region Chr 9: 4,975,242–5,117,996 in 90 CEU subjects were downloaded from <http://www.hapmap.org> (phase 2 HapMap Release January 2006). The 10 tSNPs selected using the method of Chapman *et al.* (12) are shown. Numbers refer to exons, with coding exons shown as solid boxes and untranslated exons as open boxes.

Table 2 Association of JAK2 tSNPs with obesity-related phenotypes and lipids profile

tSNP	Phenotypes	No.		Mean (s.d.)			Genetic model	Variance (%)	P GEE
		11/12/22	11	12	22				
rs7849191	Central fat (kg)	851/1148/349	1.34 (0.75)	1.30 (0.70)	1.35 (0.73)	Dominant	0.2	0.030	
	Central fat (%)	851/1148/349	31.4 (11.3)	30.5 (11.6)	31.8 (11.7)	Dominant	0.1	0.014	
	Waist (cm)	846/1166/352	78.7 (10.9)	77.9 (9.6)	78.4 (10.4)	Dominant	0.2	0.027	
rs3780378	ApoA (g/l)	582/1077/459	1.71 (0.36)	1.70 (0.35)	1.66 (0.32)	Additive	0.2	0.026	
	Total cholesterol (mmol/l)	623/1141/489	5.47 (1.21)	5.59 (1.27)	5.64 (1.27)	Additive	0.2	0.014	
	LDL cholesterol (mmol/l)	583/1084/464	3.39 (1.11)	3.48 (1.17)	3.55 (1.16)	Additive	0.2	0.012	
	Triglyceride (mmol/l)	590/1094/472	1.21 (0.70)	1.31 (0.79)	1.28 (0.85)	Dominant	0.3	0.023	

ApoA, apolipoprotein A; GEE, generalized estimating equations; JAK2, janus kinase 2; LDL, low-density lipoprotein; tSNP, tagged single-nucleotide polymorphism.

Table 3 Association of JAK2 haplotypes (frequency > 5%) with phenotypes

Haplotype	Frequency (s.e.) (%)	ApoA			Waist		
		β (s.e.)	P	Variance explained (%)	β (s.e.)	P	Variance explained (%)
1. 2112112121 ^a	18.2 (0.3)	—	—	—	—	—	—
2. 1111211211	15.1 (0.3)	0.08 (.04)	0.042	0.19	-0.48 (.66)	NS	0.1
3. 1221121112	7.9 (0.3)	0.09 (.05)	NS	—	-0.36 (.84)	NS	—
4. 2112112111	7.0 (0.3)	0.05 (.06)	NS	—	-2.11 (.88)	NS	—
5. 1111211221	5.3 (0.3)	0.09 (.06)	NS	—	-1.56 (.97)	0.026	—
			NS*			NS*	

ApoA, apolipoprotein A; JAK2, janus kinase 2; NS, not significant.

^aThe most common haplotype with which the others were compared. *The P value for the overall haplotypic effects.

of multiple testing of the 10 tSNP genotypes versus 19 phenotypes, pragmatically taken as $P < 0.01$ (see Methods) (13).

Supplementary Table S3 online shows the distribution of the five tSNP haplotypes at frequencies >5.0% in the study sample, which cover 53.5% of the haplotype diversity. All phenotypes listed in **Table 2** were tested in haplotypic analysis. Remaining haplotypes (**Supplementary Table S3** online) were not analyzed, as based on simulations; Lake *et al.* (14) suggest that haplotype frequencies of at least 5% are required to avoid biased regression parameters. Haplotype 2 was associated with an increase in serum apoA of 0.08 g/l compared with carriers of the common haplotype 1. Another, haplotype 5, was associated with 1.56-cm smaller waist measurement ($P = 0.026$) compared with the most common haplotype. However, combined haplotypes accounted for <0.2% of the variance in either parameter (**Table 3**).

The main strengths of this study lie in the large number of subjects with measures of body fat, regional fat distribution, serum lipids, and insulin sensitivity and the comprehensive coverage of variation in this 142.75-kb gene using tSNPs. The test of association with obesity variables involving 2,760 subjects (962 DZ pairs and 418 MZ pairs) provided power in excess of 80% (and $\alpha = 0.01$) to detect a locus effect of 0.75%. There were only slightly fewer subjects (2,421–2,594) available with serum lipid data and although there were fewer subjects than this available with insulin-resistance measures, the current study had 80% ($\alpha = 0.01$) power to detect a locus effect explaining 1.25% of the variance in homeostasis model

assessment index ($n = 1,002$) and 1.7% of the variance in sensitivity to insulin measurement ($n = 735$). However, in tagging only common SNPs, we excluded the possibility of discovering any substantial effect associated with low frequency SNPs (minor allele frequencies <0.05), which could be functional.

We have found only suggestive associations between two tSNPs and phenotypes that could reflect the known involvement of JAK2 in the leptin-signaling pathway (accumulation of central fat) and activation of the apoA1 transporter ABCA1 (level of serum apoA and total cholesterol). We did not, however, establish any association with serum leptin, so the association with central fat could originate elsewhere. Neither did we find any association with HDL cholesterol, which would be expected if the association with apoA reflected an effect of JAK2 on ABCA1 activity. In conclusion, common JAK2 variants were not strongly associated with body fat, insulin sensitivity, or lipid profile in our sample of normal female twins.

METHODS

Study design

The St Thomas' UK Adult Twin Registry comprises unselected, white mostly female volunteers ascertained from the general population through national media campaigns in the UK (15). The study sample comprised 2,759 subjects (836 MZ, 1924 DZ) with available leptin data. 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 St Thomas' UK Adult Twin Registry are similar to an age-matched sample of the UK female population (16). Informed consent was obtained from all participants before they participated in the studies, which were approved by the local research ethics committee.

Zygoty, body composition, and biochemical analyses

Zygoty was determined by standardized 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 (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). Serum leptin concentration was determined after an overnight fast using a radioimmunoassay (Linco Research, St Louis, MO). Fasting insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, UK) and glucose was measured on an Ektachem 700 multichannel analyzer using an enzymatic colorimetric slide assay (Johnson and Johnson Clinical Diagnostic Systems, Amersham, UK). A random subsample of 738 subjects underwent an oral glucose tolerance test for which glucose and insulin levels were measured before and 2 h after a 75-g oral glucose load. Blood sample collection for determination of fasting lipids was drawn from most subjects after a minimum 8-h overnight fast. Serum was stored at -45°C until analyzed using a Cobas Fara machine (Roche Diagnostics, Lewes, UK). A colorimetric enzymatic method was used to determine total cholesterol, triglycerides, and HDL cholesterol levels. The latter was measured after precipitation from chylomicron, LDL, and very low-density lipoprotein particles by magnesium and dextran sulfate. Apolipoproteins A1 and B were assayed by an immunoturbidometric method. The Friedewald equation was used to calculate LDL cholesterol levels in subjects with triglycerides $\leq 4.52 \text{ mmol l}^{-1}$.

Selection of tSNPs

Seventy-five polymorphic SNPs with minor allele frequency < 0.05 in the region Chr 9: 4975242..5117996 are listed on the HapMap database (HapMap Data Release no. 20/phase 2 January 2006; <http://www.hapmap.org>). Genotypes of 90 CEU (Centre d'Etude du Polymorphisme Humain Utah) parent-offspring trio subjects were downloaded from HapMap and the package *htSNP2* was used to select a tSNP set that predicts remaining SNPs with a minimum R_L^2 of 0.8. This approach selects an optimal set of tSNPs in such a way that the allele frequencies of the remaining (non-tSNPs) can be predicted well. A series of regression equations are calculated for which the predictive efficiency is 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).

tSNP genotyping

The tSNPs were genotyped by Pyrosequencing, (Biotage, Uppsala, Sweden). Genotyping accuracy as assessed by inclusion of duplicates (50 pairs of MZ twins) in the arrays was $\sim 98\%$ and negative controls (water blanks) were included on each plate. Two SNPs refractory to genotyping by pyrosequencing, rs1536800 and rs3780378, were genotyped by KBiosciences, Hoddesdon, Hertfordshire, UK, using the KASPar system. This is a fluorescence-based allele-specific polymerase chain reaction with improved robustness and discriminating power over conventional ARMS (amplification refractory mutation system), (<http://www.kbioscience.co.uk/chemistry/chemistry-intro.htm>). Genotyping success rates varied between 79.43 and 91.94%. Primers and polymerase chain reaction conditions for tSNP genotyping by pyrosequencing are given in **Supplementary Table S4** online.

Statistical analyses

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 percentage of total fat) and one for central obesity (waist circumference, central fat mass, and percentage of central fat). Insulin-resistance measures homeostasis model assessment and sensitivity to insulin measurement described previously (17) were available for subsets of subjects with leptin data. Serum lipids were

total, LDL and HDL cholesterol, triglycerides, and apolipoproteins A1 and B. Phenotypes significantly ($P < 0.05$) deviating from normal were log transformed to obtain normal distributions prior to analysis.

Preliminary association analyses were performed using STATA 8 (StataCorp, College Station, TX). To reduce the likelihood of generating false positive associations through multiple testing, single variables characterizing obesity were analyzed only if initial tests with the general and central obesity scores yielded a positive association for at least one of these combined variables. This strategy was also used for homeostasis model assessment and sensitivity to insulin measurement.

For related individuals, conventional statistical analyses lead to inflated significance. Dependency of the observations within pairs was accounted for by use of the generalized estimating equations procedure (18) in which both MZ and DZ twins can be used in tests of association. The approach accounts for dependency of the observations within pairs and yields unbiased s.e. and P values. Association analyses in the full cohort included both twin subjects from each pair.

Analyses were done separately for each of the SNPs and followed up by haplotype analyses. For individual SNP association analyses, we first performed a 2 degrees of freedom overall test of genotypic association. Additive, dominant, and recessive models (all 1 degrees of freedom) were further tested to find the best mode of inheritance. In adjusting the P value to account for multiple testing we follow the recommendations of van den Oord and Sullivan (13). The adjustment depends on p_0 , the number of markers for which there is no true effect (i.e., the null hypothesis is true), which is generally unknown in candidate gene studies. For a range of plausible p_0 values for candidate gene studies, a significance level of $P = 0.01$ will, on average, control the false discovery rate at 0.10. Lower false discovery rates generally resulted in sharp increases in sample size, i.e., loss of power. Thus, the significance level of this study was pragmatically taken as $P < 0.01$.

Age and menopausal status were included as covariates in all models. BMI was included as an additional covariate in models testing lipids and insulin sensitivity variables. Details of our approach to test the association of statistically inferred haplotypes with continuous traits have been described previously (19). The probabilities of haplotype pairs were estimated by PHASE 2.0 software (20). Individual SNP and haplotype association analyses were performed using STATA 8 (StataCorp, College Station, TX). Where needed, phenotypic variables were log transformed to obtain better approximations of the normal distribution prior to analysis. Hardy-Weinberg equilibrium was tested by a χ^2 test with 1 degree of freedom in one twin of each pair chosen at random to prevent inflated significance. Assuming a sibling correlation of 0.3, a sample of 840 DZ pairs is adequate to detect a locus effect of 0.75 with 80% power (and $\alpha = 0.01$). This study of 962 DZ pairs with an additional 418 MZ pairs provided even greater power.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

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DISCLOSURE

The authors declared no conflict of interest.

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REFERENCES

1. Rane SG, Reddy EP. Janus kinases: components of multiple signalling pathways. *Oncogene* 2000;19:5662–5679.
2. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763–770.

3. Banks AS, Davis SM, Bates SH, Myers MG Jr. Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem* 2000;275:14563–14572.
4. Bjorbaek C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem* 1997;272:32686–32695.
5. Carvalheira JB, Ribeiro EB, Folli F, Velloso LA, Saad MJ. Interaction between leptin and insulin signaling pathways differentially affects JAK-STAT and PI 3-kinase-mediated signaling in rat liver. *Biol Chem* 2003;384:151–159.
6. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG, Schwartz MW. Intracellular signalling. Key enzyme in leptin-induced anorexia. *Nature* 2001;413:794–795.
7. Niswender KD, Morrison CD, Clegg DJ *et al*. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes* 2003;52:227–231.
8. Jamshidi Y, Snieder H, Wang X, Pavitt M, Spector TD, Carter ND, O'Dell SD. Phosphatidylinositol 3-kinase p85 alpha regulatory subunit gene *PIK3R1* haplotype is associated with body fat and serum leptin in a female twin population. *Diabetologia* 2006;49:2659–2667.
9. Howard JK, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. *Trends Endocrinol Metab* 2006;17:365–371.
10. Oram JF. HDL apolipoproteins and ABCA1: partners in the removal of excess cellular cholesterol. *Arterioscler Thromb Vasc Biol* 2003;23:720–727.
11. Tang C, Vaughan AM, Oram JF. Janus kinase 2 modulates the apolipoprotein interactions with ABCA1 required for removing cellular cholesterol. *J Biol Chem* 2004;279:7622–7628.
12. Chapman, JM, Cooper JD, Todd JA, Clayton DG. Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power. *Hum Hered* 2003;56:18–31.
13. van den Oord EJ, Sullivan PF. False discoveries and models for gene discovery. *Trends Genet* 2003;19:537–542.
14. Lake SL, Lyon H, Tantisira K *et al*. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. *Hum Hered* 2003;55:56–65.
15. Spector TD, Williams FM. The UK Adult Twin Registry (TwinsUK). *Twin Res Hum Genet* 2006;9:899–906.
16. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res* 2001;4:464–477.
17. Spencer-Jones NJ, Wang X, Snieder H *et al*. Protein tyrosine phosphatase-1B gene *PTPN1*: selection of tagging SNPs and association with body fat, insulin sensitivity and the metabolic syndrome in a normal female population. *Diabetes* 2005;54:3296–3304.
18. Trégouët D-A, Ducimetère P, Tiret L. Testing association between candidate-gene markers and phenotype in related individuals, by use of estimating equations. *Am J Hum Genet* 1997;61:189–199.
19. Dong Y, Zhu H, Wang X *et al*. Obesity reveals an association between blood pressure and the G-protein beta3-subunit gene: a study of female dizygotic twins. *Pharmacogenetics* 2004;14:419–427.
20. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162–1169.