

ARTICLE

# Linkage and potential association of obesity-related phenotypes with two genes on chromosome 12q24 in a female dizygous twin cohort

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Obesity is a multifactorial disorder with a complex phenotype. It is a significant risk factor for diabetes and hypertension. We assessed obesity-related traits in a large cohort of twins and performed a genome-wide linkage scan and positional candidate analysis to identify genes that play a role in regulating fat mass and distribution in women. Dizygous female twin pairs from 1094 pedigrees were studied (mean age  $47.0 \pm 11.5$  years (range 18–79 years)). Nonparametric multipoint linkage analyses showed linkage for central fat mass to 12q24 (141 cM) with LOD 2.2 and body mass index to 8q11 (67 cM) with LOD 1.3, supporting previously established linkage data. Novel areas of suggestive linkage were for total fat percentage at 6q12 (LOD 2.4) and for total lean mass at 2q37 (LOD 2.4). Data from follow-up fine mapping in an expanded cohort of 1243 twin pairs reinforced the linkage for central fat mass to 12q24 (LOD 2.6; 143 cM) and narrowed the –1 LOD support interval to 22 cM. In all, 45 single-nucleotide polymorphisms (SNPs) from 26 positional candidate genes within the 12q24 interval were then tested for association in a cohort of 1102 twins. Single-point Monks–Kaplan analysis provided evidence of association between central fat mass and SNPs in two genes – *PLA2G1B* ( $P=0.0067$ ) and *P2RX4* ( $P=0.017$ ). These data provide replication and refinement of the 12q24 obesity locus and suggest that genes involved in phospholipase and purinoreceptor pathways may regulate fat accumulation and distribution.

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## Introduction

Obesity is a major public health problem. Estimates suggest that 315 million people are obese<sup>1</sup> and the number is increasing. At the basic level, obesity is always due to greater energy intake than is expended, but the interaction of genetic and environmental factors that regulate both accumulation and distribution of body fat is complex. Recent studies of twins from our cohort,<sup>2</sup> and others,<sup>3,4</sup> have shown that total and central fat mass are responsible for most of the additional circulating inflammatory

markers and cytokines that lead to increased heart disease. Determinants of fat accumulation include basal and sleeping metabolic rate, thermogenesis and the thermic effect of food, physical activity, efficiency of nutrient absorption by the gut and hormonal status,<sup>5</sup> which are all regulated differently depending on genetic make-up. Studies of twins have examined obesity-related trait heritability in both sexes, and values generally range from 0.47 to 0.86,<sup>6–9</sup> with slightly lower estimates yielded by family studies.<sup>10–12</sup> A number of genome-wide linkage studies have been performed for obesity-related traits to define the location of quantitative trait loci (QTLs) that regulate obesity phenotypes.<sup>13</sup> Among these, a role for the *SLC6A14* gene in appetite control and obesity represents an important demonstration of the utility of this approach.<sup>14–16</sup> Replication of other established linkage data remains a priority. In addition, while obesity-related phenotypes such as body mass index (BMI) have been considered previously, central fat mass is a core component of the metabolic syndrome and a stronger predictor of insulin resistance than trunk, limb or total fat.<sup>17,18</sup> Cardiovascular disease in women is also known to be strongly associated with central obesity,<sup>8,17,19,20</sup> and therefore identification of genes that regulate central fat mass is particularly important.

This report describes genome-wide linkage scan data from a large cohort of female dizygous twins, and the use of a positional candidate approach to find genes influencing central obesity.

## Materials and methods

### Subjects and clinical assessment

**Initial twin cohort** The St Thomas' UK twin registry (TwinsUK) was used to identify Caucasian dizygous twin sister pairs between 18 and 80 years of age, unselected for any trait or disease. This was a volunteer sample and none of the participants was aware of the specific hypothesis to be studied. Subjects were assessed for an extensive range of phenotypes. Genome-wide linkage scans for bone<sup>21,22</sup> and eye disease<sup>23</sup> have been reported previously for this cohort. Here we report the data from genome-wide linkage scans for obesity-related traits ( $n = 2188$  individuals).

**Expanded twin cohort for fine mapping** In an attempt to improve the resolution of a linkage peak at 12q24 by fine mapping, we recruited and genotyped additional twins from the TwinsUK registry and the volunteer-based Sydney Longitudinal Study of Twins (Australia). These pairs were also Caucasian dizygous twin sisters between 18 and 80 years of age, and were subjected to the same data collection protocols described for the Initial Twin Cohort. This increased the number of pairs in the fine-mapping linkage analysis by approximately 14% to 1243 pairs.

**Association cohort** The analysis of single-nucleotide polymorphisms (SNPs) in positional candidate genes used dizygous twin pairs ( $n = 1102$  individuals) selected from the extended twin cohort. Twins with the following conditions were excluded from the association study – diabetes, thyroid disease, renal impairment, discordance for menopause status and those receiving lipid lowering medication, beta blockers, thiazide diuretics or exogenous estrogen. Pairs from the linkage studies were ranked for central fat mass concordance and discordance. Twins ( $n = 551$  pairs) were selected sequentially from opposite ends of the trait distribution, maintaining a ratio of twin pairs who were discordant to those who were concordant of 5:3.<sup>24</sup> Twins from the selected pedigrees were then genotyped for 45 SNPs in 26 candidate genes.

### Phenotypes Measurement of obesity-related phenotypes (g):

Total fat mass, total lean mass and total fat percentage were measured using a QDR-4500/w dual X-ray absorptiometer (Hologic Inc., Bedford MA, USA).<sup>25</sup> Central fat mass (g) and central fat mass percentage were also derived from this analysis.<sup>8</sup> Height was measured without shoes using a Holtain stadiometer. Weight was measured when wearing light clothing and after removal of shoes, using a mechanical balance. Waist circumference was measured using a steel tape, halfway between the lower border of the ribs, and the iliac crest in a horizontal plane. Hip circumference was measured at the widest point over the buttocks. BMI and waist-to-hip ratio (WHR) were derived by computation.<sup>18</sup>

**Ethics approval** All subjects provided written informed consent and the institutional ethics committee of St Thomas' Hospital NHS Trust approved the experimental protocols.

### Genotyping

Microsatellite marker-based genotyping<sup>26</sup> was undertaken using standard ABI Prism™ methodologies (Applied Biosystems, Foster City, CA, USA) on DNA extracted from venous blood. Genome scans included the analysis of up to 737 markers on each individual as described previously.<sup>21</sup> Zygosity was tested using AmpFLSTR DNA profiling kit (Applied Biosystem), with twins showing less than 100% identity defined as dizygous.

Candidate genes were selected by prioritizing genes within the region based on biological properties that were consistent with a role in central adiposity. Considerations included: cDNA and EST sequences with tissue-specific expression (eg adipose, liver, brain and pancreas), potential contribution to obesity-related pathways and position. SNPs were selected based on multiple submissions to dbSNP, a unique validated position, availability of frequency data for Caucasian populations and minor allele frequency >5%, with a preference for coding SNPs.

**Table 1** Morphometric data for female dizygous twins

	Initial twin cohort	Expanded twin cohort	Association cohort
Age (years)	47.0 ± 11.5 (2188)	47.1 ± 11.8 (2486)	45.5 ± 12.5 (1102)
Height (m)	1.63 ± 0.06 (2146)	1.63 ± 0.06 (2444)	1.63 ± 0.06 (1098)
Weight (kg)	66.2 ± 12.5 (2184)	66.8 ± 12.8 (2482)	66.8 ± 13.4 (1099)
Central fat mass (g)	1348 ± 733 (2048)	1318 ± 733 (2486)	1360 ± 849 (1102)
BMI (kg/m <sup>2</sup> )	25.1 ± 4.6 (2146)	25.2 ± 4.7 (2482)	25.2 ± 4.9 (1098)
WHR	0.77 ± 0.06 (2116)	0.77 ± 0.06 (2420)	0.77 ± 0.06 (1075)

Data are mean ± SD (number of individuals).

Analysis of SNPs was analysed by nucleotide extension reaction on a MassArray MALDI-TOF mass spectrometer (Sequenom Inc., San Diego, CA, USA) or by Pyrosequencing on a PSQ96 (Pyrosequencing, Uppsala, Sweden), with reactions performed according to the manufacturer's instructions. Random duplicate genotyping was routinely undertaken throughout the study and indicated an error rate of <1%.

### Statistical analysis

Nonparametric multipoint linkage (NPL) analyses were performed using the MAPMAKER/SIBS Ver 2.0 program.<sup>27</sup> This method, based on the Wilcoxon rank-sum test, makes no assumptions about the distribution of phenotypic differences. The program also provides an estimate of the information content (IC; range 0–1), or the proportion of maximum identity-by-descent (IBD) information that is extracted using the selected markers.<sup>27</sup> Data from these NPL analyses were expressed as Z-scores and equivalent LOD scores. Marker loci map positions (NCBI Build 35) were determined from the Genethon linkage map as described previously.<sup>21</sup> All phenotypes studied were analyzed as quantitative traits in univariate genome-wide scans. All twin pairs were from independent families. Significant and suggestive linkage was interpreted as corresponding to LOD scores of at least 3.3 and 1.9, respectively.<sup>28</sup> Simulated *P*-values were calculated on a genome-wide basis, with the phenotype data randomly reassigned to the genotypes for each pair in a minimum of 500 random reassignments, with *P* < 0.05 considered as significant linkage. The approximate support interval for linkage was defined using the –1 LOD score interval. Genome-wide linkage scan data with a LOD ≥ 1 and corresponding (within 5 cM) to a linkage for the same phenotype reported from other studies were considered supportive of previously established linkage.

A  $\chi^2$  test was used to confirm that SNP data were in Hardy–Weinberg equilibrium. Single-point Monks–Kaplan association analysis,<sup>29</sup> in the QPDTPHASE program,<sup>30</sup> was used to test the relationship between SNPs and central fat mass. Haplotype analysis used the QPDTPHASE program,<sup>30</sup> which calculates the expected covariance over a

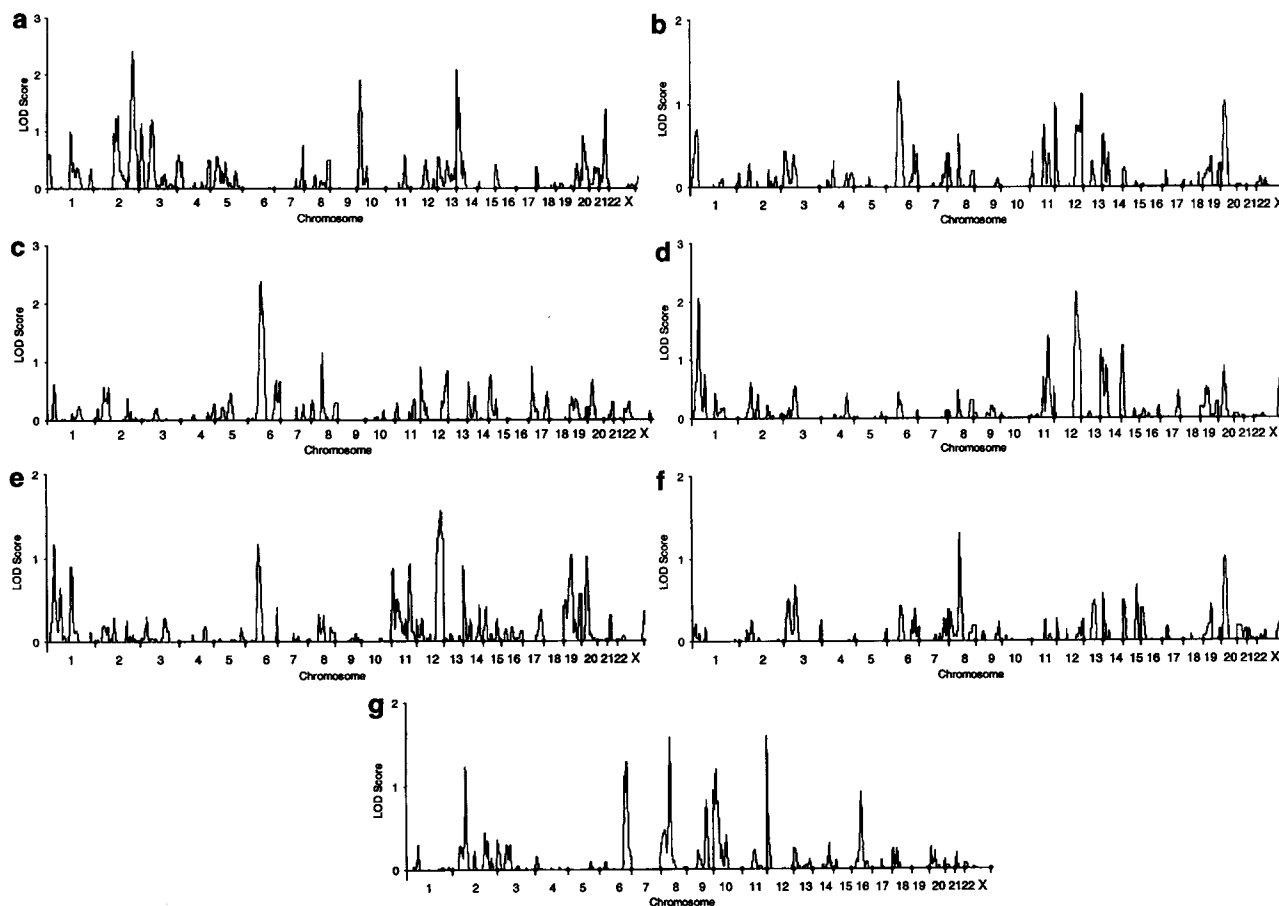
maximum-likelihood distribution of haplotype frequencies. Analysis of anthropometric data was performed using Statistica (Statsoft Inc. 1998, Ver 6.0, Tulsa, OK, USA).

### Results

The mean age of the twin cohort was 47.0 ± 11.5 years (range 18.4–79.1). These women had a mean BMI on the upper boundary of normal BMI (25.1 ± 4.6 kg/m<sup>2</sup>, range 13.9–52.4; Table 1), consistent with a general trend towards overweight in the UK population. Overall data for obesity-related phenotypes were consistent with age-matched singleton population-based samples.<sup>31</sup>

NPL analyses of obesity-related traits provided support for previously established linkage of central fat mass to 12q24 (141 cM; Figure 1d) with LOD 2.2, and for BMI to 8q11 (67 cM; Figure 1f) with LOD 1.3. There were novel suggestive linkages for total fat percentage, maximal at 6q12–13 (LOD 2.4; 81 cM; Figure 1c), and total lean mass at 2q37 (LOD 2.4; 246 cM; Figure 1a). An additional novel linkage for central fat mass at 1p36 (LOD 2.1; 48 cM; Figure 1d) was also observed. Empirical *P*-values generated using a permutation approach for these linkages did not achieve conventional genome-wide significance. The approximate support interval for the central fat mass–chromosome 12q linkage extended between 130 and 165 cM (Figure 2; flanked by markers D12S79 and D12S1723). The approximate support interval for the chromosome 6q linkage for percentage total fat was 35 cM, and extended between 70 and 105 cM (flanked by D6S426 and D6S262).

As the twins in this cohort represent a distribution of individuals from lean to obese (Table 1), we also performed an analysis of data for lean body mass to localize genes that control leanness. Total lean mass showed suggestive linkage to 2q37 (LOD 2.4; Figure 1a), with an approximate support interval of 22 cM, which extended between 235 and 257 cM (flanked by D2S2308 and D2S338). Additional linkage for total lean mass with LOD > 2 was to 14q11 (Figure 1a). It should be noted that total lean mass, unadjusted for total mass, is not necessarily indicative of true leanness (i.e. obese subjects can have a high lean mass). Furthermore, since total lean mass and total fat mass change substantially and in a nonparallel way over the age



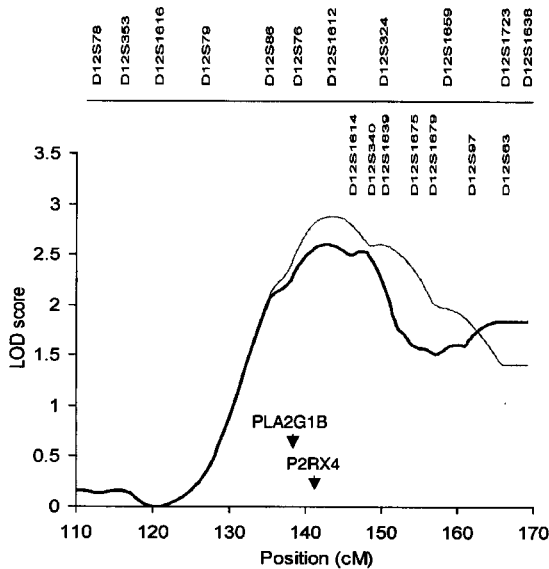
**Figure 1** Genome-wide scans for QTL that regulate (a) total lean mass, (b) total fat mass, (c) total fat percentage, (d) central fat mass, (e) central fat mass percentage, (f) body mass index and (g) WHR in 1094 twin pairs. Equivalent LOD scores (LOD) were calculated from the NPL Z-scores output by MAPMAKER/SIBS, using the relationship  $Z^2/4.6052 = \text{LOD}$ .

range of the subjects studied, these data must be interpreted cautiously. Additional weaker evidence of linkage ( $\text{LOD} \geq 1$ ) in the cohort was seen to nine other genomic regions with obesity-related traits (Table 2).

The traits we analyzed could be expected to have some level of correlation. Genome-wide linkage scans for the most strongly correlated variables: total fat and total fat percentage ( $r=0.93$ ,  $n=2030$ ) and central fat mass and central fat mass percentage ( $r=0.94$ ,  $n=2044$ ), do show substantial similarity. Nevertheless, for total fat and total fat percentage, the magnitude of LOD scores for comparable linkage peaks on chromosome 6 at 81–83 cM varied by almost two-fold (Figure 1b and c). Similarly, with central fat mass and central fat percentage, the magnitude of the LOD scores on chromosome 1 at 48 cM differed greatly (Figure 1d and e). While there was a strong phenotypic correlation between total fat mass and central fat mass ( $r=0.89$ ,  $n=2033$ ), the genome scans for these variables showed less concordance and may still be potentially relevant to general overweight and obesity, or regional deposition of fat on the trunk, respectively. Data for other

obesity-related traits such as BMI and WHR showed lesser correlation with central fat mass (ie  $r=0.81$ ,  $n=2008$  and  $r=0.494$ ,  $n=1986$ , respectively) and result in correspondingly unique linkage data (Figure 1f and g).

As the 12q linkage for central fat mass (Table 2) was among the largest we defined and supported a previous report,<sup>32</sup> we focused additional efforts on that region. NPL analysis of data on the base set of markers for the expanded cohort of 1243 twin pairs reinforced and enhanced the level of linkage on chromosome 12q24 for central fat mass (LOD 2.9) and central fat mass percentage (LOD 2.1). Due to resource constraints, fine mapping for the 12q24 linkage was undertaken with only seven additional microsatellite markers (Figure 2). As the support interval was relatively large and asymmetric, we placed fine mapping markers towards the q-telomere to increase the IC in this region. NPL analysis of the fine-mapping data for central fat in the expanded cohort yielded a LOD of 2.6 (143 cM). This analysis increased confidence in the linkage results by removing reliance on only a few microsatellite markers and narrowed the support interval to 22 cM (Figure 2). The



**Figure 2** Maximum NPL for the central fat mass phenotype on chromosome 12q in 1243 twin pairs. Linkage analysis results are presented for the expanded twin cohort with the core markers used in the initial scan (—) and after genotyping of additional microsatellite markers (---). The top row of marker names are the core markers, fine-mapping markers are defined on the second row.

mean IC in the interval rose from 0.61 to 0.72 as a result of the fine mapping.

In all, 26 genes, implicated as potential candidates in the 12q24 region, were identified from public domain resources. Analysis of data for 45 SNPs in these genes (Table 3) provided evidence of association with central fat in women, for two genes (Figure 3). The lowest *P*-value with central fat mass was with SNP rs5637 in exon 3 of secretory phospholipase A<sub>2</sub> precursor gene (*PLA2G1B*; *P* = 0.0067, *n* = 1066). Allele frequencies for rs5637 were 0.853 for G-allele and 0.147 for A-allele, with the A-allele carriers having lower mean central fat mass (Table 4, Figure 3a). The SNP rs1179387 in intron 3 of *PLA2G1B* also showed association with central fat mass (*P* = 0.0069, *n* = 1059, allele frequencies: A = 0.852, C = 0.148). Lewontin's *D'* for these two SNPs was 0.99, illustrating that the two SNPs are in strong linkage disequilibrium. Haplotype analysis provided maximum evidence of association with a four-SNP haplotype encompassing rs2009391, rs5634, rs5637 and rs1179387 (Table 5, *P* = 0.0026, T–T–A–G, frequency: 0.14).

One SNP within the ligand-gated cation channel gene *P2RX4* (Figure 3b) also showed nominal association with central fat mass in the study cohort: rs1182945 in intron 2 (*P* = 0.017, *n* = 1055, allele frequencies: A = 0.668,

**Table 2** Genomic location of maximum NPL for obesity-related phenotypes in unselected female twins with LOD scores above 1

Chromosome	Position <sup>a</sup> (cM)	Phenotype	No. of individuals <sup>b</sup>	Marker <sup>c</sup>	Maximum NPL Z	Maximum LOD
1	48	Central fat mass	2048	D1S199	3.1	2.1
1	48	Central fat percentage	2044	D1S199	2.3	1.2
2	82	Waist/hip ratio	2116	D2S337	2.4	1.2
2	83	Waist circumference	2116	D2S337	2.8	1.7
2	246	Total lean mass	2150	D2S2344	3.3	2.4
3	86	Total lean mass	2150	D3S1285	2.4	1.2
6	81	Total fat percentage	2030	D6S257	3.3	2.4
6	82	Central fat percentage	2044	D6S257	2.3	1.2
6	83	Total fat mass	2150	D6S257	2.4	1.3
6	175	Waist/hip ratio	2116	D6S1719	2.5	1.3
8	60	Waist/hip ratio	2116	D8S505	2.7	1.6
8	67	Body mass index	2146	D8S601	2.5	1.3
8	70	Waist circumference	2116	D8S285	2.7	1.6
8	71	Total fat percentage	2030	D8S285	2.3	1.2
10	17	Waist/hip ratio	2116	D10S189	2.4	1.2
10	24	Total lean mass	2150	D10S1779	3.0	1.9
11	124	Central fat mass	2048	D11S925	2.6	1.4
12	0	Waist/hip ratio	2116	D12S352	2.7	1.6
12	141	Central fat mass	2048	D12S1612	3.2	2.2
12	155	Central fat percentage	2044	D12S1659	2.7	1.6
12	167	Total fat mass	2150	D12S1723	2.3	1.1
14	1	Total lean mass	2150	D14S261	3.1	2.1
14	3	Central fat mass	2048	D14S261	2.3	1.2
20	27	Total fat mass	2150	D20S189	2.2	1.0
20	27	Body mass index	2146	D20S189	2.2	1.0
22	42	Total lean mass	2150	D22S1151	2.5	1.4

<sup>a</sup>Distance of the linkage peak from the p telomere.

<sup>b</sup>Number of individuals differs depending on the availability of phenotype data.

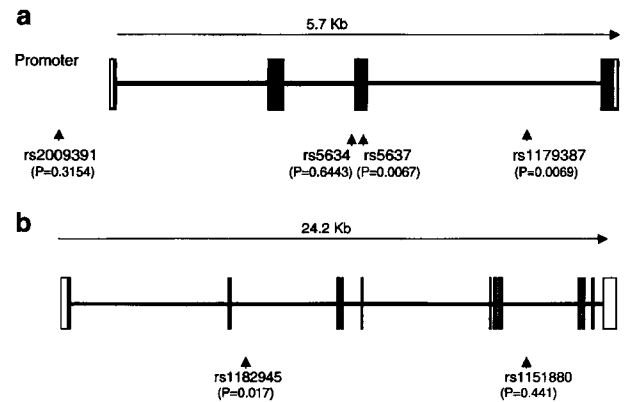
<sup>c</sup>Closest microsatellite marker genotyped to the apex of the linkage peak.

**Table 3** Positional candidate genes studied for association with central fat mass and *P*-values for Monks–Kaplan association test with central fat mass in the association cohort

<i>Gene</i>	<i>dbSNP reference number (rs)</i>	<i>Position Chr12</i>	<i>P-value</i>
ACACB	34269	108 060 225	0.4
	34280	108 066 955	0.9
	1016331 <sup>H</sup>	108 087 016	0.6
TBX5	1895584	113 269 255	0.7
TBX3	539709	113 582 034	0.6
NOS1	816358	116 160 028	0.8
	733334	116 195 409	0.14
	567581	116 220 232	0.5
PBP	904662	117 044 553	0.9
HSPB8	1966544	118 088 842	0.2
PRKAB1	278145	118 574 819	0.4
	278152 <sup>H</sup>	118 579 999	0.4
PLA2G1B	1179387 <sup>H</sup>	119 223 631	0.0069
	5637	119 225 485	0.0067
	5634 <sup>H</sup>	119 225 557	0.6
	2009391 <sup>H</sup>	119 228 763	0.4
COX6A1	968474 <sup>H</sup>	119 341 250	1
ACADS	575437	119 636 151	0.5
	9204 <sup>H</sup>	119 640 498	0.4
TCF1	1169288 <sup>H</sup>	119 879 370	0.9
	1169302 <sup>H</sup>	119 895 022	0.19
P2RX7	208307	120 066 576	0.5
P2RX4	1182945	120 118 690	0.017
	1151880 <sup>H</sup>	120 130 371	0.4
PSMD9	483416	120 800 833	0.7
DIABLO	12870 <sup>H</sup>	121 217 700	0.7
FLJ13491	883562	121 985 599	0.3
PITPNM2	1984658	122 008 306	0.5
CDK2AP1	6633	122 270 689	0.5
GTF2H3	1051793	122 669 275	1
ATP6VOA2	1040156 <sup>H</sup>	122 748 244	0.3
FLJ22471	1882491 <sup>H</sup>	122 979 115	0.2
NCOR2	8546 <sup>H</sup>	123 333 907	0.16
	1271309	123 345 585	0.3
	1244085	123 354 135	0.09
	906302 <sup>H</sup>	123 375 337	0.5
	1244053	123 415 092	0.9
	872224	123 504 713	0.7
SCARB1	1814575	123 804 271	0.8
	5888	123 809 628	0.17
AACS	914610	124 080 119	0.5
FLJ31978	527824	127 880 953	0.6
	470576 <sup>H</sup>	127 942 190	0.07
SFRS8	897646	130 790 890	1
	755437	130 926 532	0.7

<sup>H</sup>SNP data present in HapMap.

*T* = 0.332). Carriage of the A-allele in this SNP was associated with higher mean central fat mass (Table 4), although it should be noted that, due to the nature of the Monks–Kaplan association test, the *P*-value is based on within-family association and not a direct test of the genotypic means. Pairwise *D'* values were calculated for the SNPs genotyped in *PLA2G1B* and *P2RX4*, and, despite the relatively close physical mapping of the two genes, no evidence of linkage disequilibrium was found between the genes.



**Figure 3** Position of SNPs (designated by reference SNP number) studied in (a) *PLA2G1B* and (b) *P2RX4*. *P*-values for Monks Kaplan association test with central fat mass are shown below each SNP. □ = untranslated region, ■ = exon.

**Table 4** Genotype frequencies and central fat mass data for individuals grouped by genotype for two SNPs in positional candidate genes on chromosome 12q24

<i>Gene (SNP)</i>	<i>Genotype</i>	<i>Mean ± SD (g)</i>	<i>n</i>
<i>PLA2G1B</i> (rs5637)	A/A	1256 ± 802 <sup>a</sup>	28
	A/G	1267 ± 784	272
	G/G	1387 ± 869	766
<i>P2RX4</i> (rs1182945)	T/T	1341 ± 847	116
	A/T	1342 ± 846	456
	A/A	1363 ± 841 <sup>b</sup>	483

Significant difference by Monks–Kaplan single-point association test. <sup>a</sup>*P* = 0.0067. <sup>b</sup>*P* = 0.017.

## Discussion

This study of female twins provides evidence of support for previously established linkage of central fat mass to 12q<sup>32</sup> and for BMI to 8q.<sup>33,34</sup> The use of a positional candidate approach to identify QTLs in the region resulted in the detection of statistically significant associations between SNPs in the *PLA2G1B* and *P2RX4* genes and central fat mass in women.

The deposition of fat on the trunk and waist is a predominant risk factor for disorders such as metabolic syndrome, non-insulin-dependant diabetes and cardiovascular disease.<sup>8,35</sup> The metabolic properties of visceral and subcutaneous adipocytes are also known to differ, with visceral cells being more sensitive to lipolytic stimuli and less sensitive to the inhibitory effect on lipolysis by insulin.<sup>35–37</sup> Furthermore, the prevalence of abdominal obesity and its relationship to generalized obesity differs with ethnic group, gender<sup>38,39</sup> and menopause.<sup>8</sup> We found support for the previously established linkage observed in the Québec Family Study,<sup>32</sup> which used computed tomography (CT) to estimate fat mass in 521 subjects. Our study

**Table 5** Effect of the PLA2G1B haplotypes on central fat mass in female dizygous twins

Haplotypes <sup>a</sup>	G-T-G-T	T-T-G-T	T-T-A-G	G-C-G-T
Haplotype frequency (%) <sup>b</sup>	51	30	14	4
Central fat mass (g) <sup>c</sup>	1362 ± 30	1341 ± 39	1274 ± 57 <sup>d</sup>	1464 ± 107

<sup>a</sup>Results are mean ± standard error for haplotypes encompassing SNPs rs2009391, rs5634, rs5637 and rs1179387.

<sup>b</sup>Rare haplotypes (frequency less than 3%) not included.

<sup>c</sup> $P = 0.03$  overall association with central fat mass.

<sup>d</sup> $P = 0.0026$  for individual haplotype compared to all others.

included nearly twice as many subjects, but CT was not used. CT is widely regarded as the method of choice for precise measurement of abdominal subcutaneous and visceral fat, but involves considerable radiation. Absorptiometry does not provide the same detail, but has been shown to be a reproducible technique for assessment of obesity-related traits, including central fat mass.<sup>8,25</sup> In another study, linkage for obesity was also found to 12q24 in patients on antipsychotic medication.<sup>40</sup> Recently, Li *et al*<sup>41</sup> have shown significant linkage for obesity-related traits: percentage fat (LOD 3.79;  $P = 0.00001$ ), BMI (LOD 2.98;  $P = 0.0001$ ) and waist circumference (LOD 2.86;  $P = 0.00014$ ) to chromosome 12q23–24. Their fine-mapping data localized linkages for those traits to 116–125 cM on chromosome 12. There does not appear to be an overlap between the –1 LOD approximate support interval reported in that paper and our data; however, the proximity of the linkages is intriguing. Studies of non-insulin-dependant diabetes in humans<sup>42–44</sup> have also highlighted this genomic region as important, raising the possibility that QTLs in the region may be implicated in several disease states. We also found linkage of central fat mass to chromosome 1p36 (LOD 2.1). Another study<sup>33</sup> found strong evidence of linkage on chromosome 1 (45–48 cM) for BMI; however, they did not consider measures of central obesity. In contrast, we did not find linkage of BMI to this region of chromosome 1 and so, despite the recognition of potential concordance of the linkage peaks, we remain cautious about the possible overinterpretation of this data.

Our data showed evidence of support for linkage of BMI to chromosome 8p12–q22.1. This linkage had previously been reported in 10 large families of Mexican Americans from a fine-mapping study of 8p12–q22.1 (LOD 3.21 at 63 cM),<sup>34</sup> which is within 4 cM of the linkage for BMI that we report. Participants in that study had an increased BMI (29.7 kg/m<sup>2</sup>) compared to our subjects, but were not morbidly obese. Linkage of BMI to 8q12 has also been observed in a large cohort of white children and young adults from the Rochester Family Heart study.<sup>33</sup> That study also provided evidence of linkage for BMI to 20p12–13, a finding supported by our data. Other presently unsupported linkage areas from this genome scan, particularly those with LOD > 2, may aid in prioritizing regions for further study among numerous linked regions reported to date.<sup>13</sup> In a recent review, Perusse *et al*<sup>13</sup> reported that there

are at least 204 QTLs for obesity-related phenotypes from 50 genome-wide linkage scans, with 38 genomic regions reported as being supported by data from at least two studies. Care must be taken when interpreting this data and further studies may be required on the genomic regions and positional candidates identified in this study to clarify their relevance.

Our work on fine mapping in the expanded cohort helped to confirm suggestive linkage of central fat mass to 12q24, narrow the support interval (LOD 2.6; 143 cM) and facilitate a study of positional candidates in the region. It is well known that the principle candidate gene within a linkage region, identified on the basis of known function, may not be the source of the observed linkage, so we studied SNP in a substantial number of the potential candidate genes in the region. There are over 120 genes within the support interval, but due to resource limitations we chose only 26 genes for study and attempted to expeditiously sample possible QTLs. The number of pairs we used in the association study was defined by power calculations which suggested that a QTL accounting for 10% of the phenotypic variance, with a  $D'$  of 0.5 between the marker and the QTL, no dominance and a minor allele frequency of 0.2, would require 552 sib pairs to achieve 80% power with an  $\alpha = 0.001$ . In comparison, for an effect size of 5%, the number of pairs required was estimated as 1112. Our data is useful in emphasizing both the size of cohorts needed for such studies and the scale of investigation that may generally be required to uncover QTLs in positional candidate studies.

We found evidence of association between SNPs in the PLA2G1B gene and central fat mass. Phospholipases (PLA2) are small lipolytic enzymes that release fatty acids from the second carbon group of glycerol. The best known of these are the digestive enzymes secreted as zymogens by the pancreas. PLA2G1B is the pancreatic form and is thought to hydrolyse phospholipids to increase free fatty acids and lysolipids. Huggins *et al*<sup>45</sup> demonstrated previously that PLA2<sup>-/-</sup> mice fed a high-fat diet were resistant to diet-induced obesity. In the light of that result, there may be a plausible role for genetic variation in the PLA2G1B gene in protection against diet-induced obesity. The SNP rs5637, which showed the strongest association with central fat mass, results in synonymous coding for serine. In comparison, rs1179387 is in intron 3 of the PLA2G1B gene, suggesting

that both SNPs may be in linkage disequilibrium with a functional variant(s), yet to be determined.

The product of the *P2RX4* gene belongs to the family of purinoceptors for ATP. This receptor functions as a cell surface ligand-gated ion channel with high calcium permeability. Expression of the gene in the rat has shown *P2RX4* levels to be high in the brain, suggesting a possible role in appetite control. In humans, the gene is expressed at high levels in the pancreas, stomach and small intestine, and at lower levels in the brain. Interestingly, ethanol has been found to inhibit the opening of channels regulated by *P2RX4* in *Xenopus* oocytes.<sup>46</sup> As ethanol is known to effect the regulation of leptin,<sup>47</sup> reduced levels of which stimulate appetite, there is a possibility that *P2RX4* could also exert effects on fat metabolism via a link with ethanol or leptin. SNP rs1182945, which showed potential association with central fat mass, is in intron 2 of the *P2RX4* gene and so it seems likely that a functional change, in linkage disequilibrium with this SNP, is responsible for the observed effect on central fat mass.

Other studies of candidate genes in the 12q24 region<sup>48,49</sup> have found association of BMI with genetic variation in the *SCARB1* gene, but we did not find any association between the two SNPs we studied and either central fat mass or BMI. Mutations in the *TCF1* gene are thought to be responsible for Mature Onset Diabetes of the Young in some patients (*MODY3*).<sup>50–53</sup> However, we found no association between the SNP in this gene and central fat mass in female twins.

We performed a substantial number of statistical tests in the association study and present these data without correcting for multiple testing. Application of the conservative Bonferroni correction – assuming the SNPs are uncorrelated – would be one method to account for this issue, but this is considered too conservative by most researchers.<sup>54</sup> Presentation of the data without correction may be important for later meta-analysis. We cautiously advocate only that SNP in *PLA2G1B* and *P2RX4* show possible association, which will need to be verified by replication. We also performed only a superficial sampling of SNPs in potentially relevant genes. We did this in an attempt to gain insight and to aid in prioritization of genes and SNPs in the region for further study. This approach has yielded two potentially relevant associations, but much work remains to be performed in the region.

In summary, despite early disappointment with the positional cloning approach for the discovery of complex disease genes, recent successes, the current support for established linkages and the use of selected intermediate phenotypes provide considerable room for optimism. If these results for *PLA2G1B* and *P2RX4* can be verified through replication or functional analysis, the recognition that these polymorphisms are specifically associated with accumulation of abdominal fat could be a major contribution to combating the development of disorders such as the metabolic syndrome and other related cardiovascular conditions.

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