

TUB is a candidate gene for late-onset obesity in women

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Abstract

Aims/hypotheses We recently reported significant associations between BMI and three *TUB* single nucleotide polymorphisms (SNPs) in two Dutch cohorts enriched for type 2 diabetes. Here, we attempted a replication of these associations in a large population-based cohort of female twins comprehensively phenotyped for measures of general and central obesity.

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Methods Two *TUB* SNPs (rs2272382, rs2272383) and a third (rs1528133), 22 kb distal to *RIC3*, were genotyped in 2694 Europid women from the St Thomas' UK Adult Twin Registry (Twins UK) (mean age±SD: 47.6±12.7 years; 42.8% postmenopausal). We explored the hypothesis that *TUB* is a candidate gene for late-onset obesity in humans through testing the interaction of the SNPs by menopausal status.

Results In the whole cohort, none of the three SNPs showed a significant main effect on measures of general or central obesity. However, for central obesity the rs2272382 SNP showed a significant interaction with menopausal status ($p=0.036$). Postmenopausal women homozygous for the minor allele of rs2272382 showed significantly more general obesity ($p=0.022$) and central obesity ($p=0.009$) than carriers of the major allele. Differences (beta [95% CI]) between the two genotype groups were 0.92 kg/m² (0.03–1.81) for BMI ($p=0.036$), 2.73 cm (0.62–4.84) for waist circumference ($p=0.013$) and 2.43% (0.27–4.60) for per cent central fat ($p=0.027$). These associations were confirmed by a sibling transmission disequilibrium test for central obesity, waist circumference and per cent central fat.

Conclusions/interpretation We have replicated associations of *TUB* SNP rs2272382 with measures of general and central obesity in normal postmenopausal women. These findings confirm *TUB* as a candidate gene for late-onset obesity in humans.

Keywords Association study · Central obesity · General obesity · Replication · *TUB* gene

Abbreviations

DZ dizygotic
GEE generalised estimating equation
GPCR G-protein-coupled receptor

LD	linkage disequilibrium
MZ	monozygotic
Sib-TDT	sibling transmission disequilibrium test
SNP	single nucleotide polymorphism
TUB	tubby homologue (mouse)

Introduction

The tubby-like proteins comprise a highly conserved group, which may function as transcription factors [1] and/or as adaptor molecules for downstream signalling of insulin [2]. A loss-of-function mutation of the mouse *Tub* gene results in the tubby mouse syndrome, which is characterised by late-onset obesity with insulin resistance, as well as neurosensory defects (retinal and cochlear degeneration) [3]. As the *Tub* gene is predominantly expressed in the hypothalamus, obesity in tubby mice might reflect defects in neurons of the hypothalamic satiety centre, where the location of insulin and leptin receptors matches that of *Tub* [3]. Phosphorylation of *Tub* by the insulin receptor kinase increases its binding capacity for proteins with SH2 domains [2] and it may mediate insulin signalling in the brain, where insulin plays an important role in energy homeostasis [4].

The role of the *TUB* gene in human obesity has not been established. Recently, Shiri-Sverdlov et al. [5] reported a significant association between single nucleotide polymorphism (SNP) rs1528133 and BMI in 492 individuals with type 2 diabetes, which was replicated in a population of 750 individuals enriched for the disease. SNP rs1528133 is located 22 kb distal to *TUB* in the flanking gene *RIC3* and is in strong linkage disequilibrium (LD) with SNPs in the 3' end of *TUB*. The resistance to inhibitors of cholinesterase 3 homologue (*RIC3*) protein is thought to be involved in assembly of neurotransmitter-gated receptors in the endoplasmic reticulum and trafficking to the cell surface, thereby influencing the efficacy of synaptic transmission [6].

In the sample of 750 individuals, it was found that a significantly higher BMI associated with the minor alleles of two further *TUB* SNPs, rs2272382 (intron 3–exon 4 boundary) and rs2272383 (3' untranslated region), an effect that was confirmed in the combined cohorts. Moreover, the minor alleles were more common in obese individuals (BMI >30 kg/m²) than lean control individuals (BMI <25 kg/m²) drawn from these cohorts. These data suggest that *TUB* could be influential in controlling the central regulation of body weight in humans.

The aim of our study was to replicate the previously observed associations with the three *TUB* SNPs in diabetic patients and individuals enriched for type 2 diabetes [5] in a large sample of European female twins from the general population. We report confirmation of association of rs2272382

with measures of general and central obesity, but only in postmenopausal women, which accords with the association of the mouse homologue *Tub* with late-onset obesity.

Methods

Participants The St Thomas' UK Adult Twin Registry (Twins UK) comprises unselected, mostly female, volunteers ascertained from the general population through national media campaigns in the UK [7]. Means and ranges of quantitative phenotypes in Twins UK were similar to an age-matched sample from the general population [8]. Informed consent was obtained from all participants before they entered the studies, which were approved by the local research ethics committee. For this study, we included 2,694 participants with phenotype data on at least one of the obesity-related measures and genotype data on at least one SNP. This sample consisted of 408 monozygotic (MZ) pairs, 892 dizygotic (DZ) pairs and 94 single participants from DZ pairs. Information on menopausal status was available in 2,198 individuals (42.8% postmenopausal). Characteristics of the participants are shown in Table 1.

Zygosity, body composition and biochemical analyses Zygosity in the Twins UK sample 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 calculated as weight divided by height squared (kg/m²). Waist circumference (cm) was measured at the level midway between the lower rib margin and the iliac crest. Body composition (total and central fat mass) 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 an RIA (Linco Research, St Louis, MO, USA).

SNP genotyping The SNPs rs2272382, rs2272383 and rs1528133 were genotyped by KBiosciences (Hoddesdon, UK), using the KASPar system. This is a fluorescence-based allele-specific PCR with improved robustness and discriminating power over conventional Amplification Refractory Mutation System approaches (<http://www.kbioscience.co.uk/chemistry/chemistry-intro.htm>). Genotyping accuracy for all SNPs was 98% as assessed by inclusion of duplicates (pairs of MZ twins) in the arrays, and negative controls (water blanks) were included on each plate. Genotyping success rate for the three SNPs varied between 95.2 and 98.1%.

Association analyses Preliminary analyses were performed using STATA 8 (StataCorp, College Station, TX, USA).

Table 1 General characteristics of participants

Variable	Whole cohort		Premenopausal ^c		Postmenopausal ^c	
	<i>n</i> ^a	Mean±SD	<i>n</i> ^a	Mean±SD	<i>n</i> ^a	Mean±SD
Age (years)	2,694 ^b	47.0±12.2	1,258	38.0±8.7	940	56.0±7.0
Leptin (ng/ml)	2,348	16.3±11.8	1,240	15.0±11.3	881	18.1±12.4
BMI (kg/m ²)	2,615	24.7±4.4	1,219	24.2±4.5	905	25.5±4.3
Weight (kg)	2,616	65.3±11.8	1,219	64.6±12.3	906	66.5±11.7
Waist (cm)	2,561	78.3±10.2	1,200	76.2±9.9	881	81.1±10.4
Total fat (kg)	2,610	23.4±8.7	1,221	21.2±8.6	934	25.5±8.6
Total fat (%)	2,539	35.6±8.0	1,183	32.8±7.7	913	38.3±7.4
Central fat (kg)	2,581	1.3±0.7	1,206	1.1±0.7	923	1.5±0.7
Central fat (%)	2,581	31.2±11.5	1,206	27.0±10.7	923	34.9±10.8

^a Number of participants with genotype data on at least one SNP

^b Number of participants with phenotype data on at least one of the obesity-related measures and genotype data on at least one SNP

^c A total of 496 participants have missing menopausal information

With the exception of per cent total fat and per cent central fat all 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 *df* in one twin of each pair chosen at random to prevent inflated significance. Association analyses were performed using generalised estimating equations (GEEs), which allow for the relatedness between twins and yield unbiased standard errors and *p* values [9]. More specifically, a recessive model (homozygotes of a minor allele vs carriers of the common allele) was tested for SNPs rs2272382 and rs2272383, and a dominant model (carriers of a minor allele vs homozygotes of the common allele) was tested for SNP rs1528133, following the procedure and significant findings of Shiri-Sverdlov et al. [5]. Age and menopausal status were included as covariates in the models. In consideration of the tubby mouse syndrome characterised by late-onset obesity and both cohorts studied by Shiri-Sverdlov et al. [5] involving only older individuals (mean ages of 70.3 years for their Breda cohort and 56.1 years for their RIVM cohort), we explored the hypothesis that *TUB* is a candidate gene for late-onset obesity in humans through testing the interaction of the SNPs by menopausal status.

To control for population stratification bias, DZ twin pairs discordant for genotype were also used in a sibling

transmission disequilibrium test (Sib-TDT) association analysis as described elsewhere [10, 11].

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 per cent total fat) and one for central obesity (waist circumference, central fat mass and per cent central fat). To reduce the likelihood of generating false-positive associations through multiple testing, results of individual variables characterising obesity (i.e. serum leptin, BMI, per cent total fat, waist circumference and per cent central fat) could only be declared significant if initial tests with the general and central obesity scores yielded a positive association for at least one of these combined variables.

Assuming a phenotypic sibling correlation of 0.3, a sample of 840 DZ pairs is adequate to detect a locus effect of 0.5% with 80% power (and alpha=0.05). Thus, the current study of 892 DZ pairs and an additional 418 MZ pairs and 94 DZ singletons provided even greater power.

Results

Table 2 shows genotype and allele frequencies of the three SNPs in our cohort, which are compatible with the study by

Table 2 Genotype and allele distributions

SNP	Genotype			Total ^a	MAF (95% CI)	H-W test ^a	
	11	12	22			χ^2	<i>p</i> value
rs2272382	645	570	145	1,360	0.316 (0.299, 0.334)	1.29	0.256
rs2272383	545	650	173	1,368	0.364 (0.346, 0.382)	0.94	0.332
rs1528133	1,209	165	9	1,383	0.066 (0.057, 0.076)	1.64	0.200

^a Tested in one of each MZ pair, one of each DZ pair and all singleton DZ twins
H-W test, Hardy–Weinberg equilibrium test; MAF, minor allele frequency

Shiri-Sverdlov et al. [5]. Pairwise LD, quantified by r^2 , was modest for all pairwise combinations of the three SNPs: rs2272382 vs rs2272383: 0.17; rs2272382 vs rs1528133: 0.02; rs2272383 vs rs1528133: 0.09. None of the loci showed deviation from Hardy–Weinberg equilibrium.

In the whole cohort, none of the three SNPs showed a significant main effect on measures of general or central obesity. Analyses of individual obesity variables confirmed the lack of effect (see Table 3). Only homozygotes of the minor allele of rs2272382 had a larger waist (79.3 ± 0.65 vs 78.0 ± 0.21 cm, $p=0.035$) than carriers of the major allele. However, for central obesity rs2272382 (but not the other two SNPs) showed a significant interaction with menopausal status ($p=0.036$). Therefore, we performed the analyses for all obesity variables in pre- and postmenopausal women separately for this SNP (Table 4). In the premenopausal group, we did not find any significant associations with any of the variables. However, within the postmenopausal women several effects were found for rs2272382. Homozygotes for the minor allele of rs2272382 showed significantly more general obesity ($p=0.022$) and central obesity ($p=0.009$) than carriers of the major allele (11 and 12; Table 4). Differences (beta [95% CI]) between the two genotype groups were 0.92 kg/m^2 (0.03–1.81) for BMI ($p=0.036$), 2.73 cm (0.62–4.84) for waist circumference ($p=0.013$) and 2.43% (0.27–4.60) for per cent central fat ($p=0.027$).

In DZ twin pairs discordant for their genotype at the rs2272382 locus these associations were confirmed by Sib-TDT ($p<0.05$) for central obesity, waist circumference and per cent central fat and borderline significant for BMI ($p=0.072$) (Table 4). In these DZ pairs, who are naturally matched for age and a range of possible environmental confounders, a relatively large effect size was observed. For example, individuals homozygous for the minor rs2272382 allele on average had a 4.6 cm larger waist circumference, a 1.6 kg/m^2 higher BMI, and a 3.4% larger per cent central fat than their co-twin individuals heterozygous or homozygous for the major allele. However, in all postmenopausal women, this SNP only explained between 0.32 and 0.57% of the variance of these measures of general and central obesity.

Discussion

The aim of our study was to replicate associations of SNPs in the *TUB* gene and measures of obesity in a large population-based cohort of female twins. Although results were largely negative in the whole cohort, we confirmed associations of *TUB* SNP rs2272382 with multiple measures of general and central obesity in postmenopausal women only.

SNP rs2272382 locates in the intron 3–exon 4 boundary of the *TUB* gene but does not change the splicing site, so generation of alternative inert transcripts are unlikely. The

SNP is probably in LD with an as yet unidentified functional variant in or close to the *TUB* gene. We explored the data from the HapMap project (<http://www.hapmap.org>) in a 150 kb region including both the *TUB* and *RIC3* gene and found that rs2272382 was not located within any haplotype block. We further tested the pairwise LD pattern between this SNP with the other 98 SNPs having minor allele frequencies $\geq 5\%$ in the HapMap CEPH data and only observed two SNPs (rs4578424 and rs4575312) with a pairwise $r^2 > 0.5$ with rs2272382. However, both SNPs are located in intronic regions. Furthermore, sequencing all *TUB* exons in 12 unrelated individuals did not yield any non-synonymous SNPs (R. Shiri-Sverdlov, unpublished data).

The exact mechanism linking the loss-of-function mutation in tubby mice to adult-onset obesity has not been elucidated. The *TUB* gene is expressed throughout the brain [3, 12]. The high level of expression in the hypothalamic regions implicated in the systemic control of energy regulation [3, 13] suggests that the obesity phenotype might result from defects in neuroendocrine control of satiety or metabolism. Alterations in the concentration of some of the molecular mediators important in hypothalamic control initiated by leptin have been reported in *Tub* mutant mice. Orexigenic neuropeptide Y was upregulated in the dorso-medial and ventro-medial nuclei of mature obese tubby mice and downregulated in the arcuate nucleus, where a 20% reduction in proopiomelanocortin concentration was also found [14].

The tubby homologue (mouse) (*TUB*) protein locates initially at the plasma membrane then translocates to the nucleus following activation of $G\alpha_q$, the α subunit of heterotrimeric G-proteins, which transduces signals from 7-transmembrane receptors. Several G-protein-coupled receptors (GPCRs) are thought to have a role in energy homeostasis, satiety and obesity [15–19]. Of these, serotonin, bombesin, melanin concentrating hormone, melanocortin 4 receptor and dopamine D1 receptors are all GPCRs linked to $G\alpha_q$ signalling, for which *TUB* might function as a downstream effector. Like the tubby mouse, the serotonin receptor (5HT_{2c}) knockout mouse shows late-onset obesity and hyperinsulinaemia without substantial dysregulation of glucose levels [20]. Both 5HT_{2c} [21] and *TUB* [22] are most highly expressed in the para-ventricular nucleus. *TUB* could be a $G\alpha_q$ -responsive transcription factor that mediates extracellular signalling to the nucleus [1], influencing gene transcription. In the absence of functional *TUB*, dysregulation of GPCR genes could elicit the tubby mouse phenotypes. Variants in the human gene could have a comparable effect on bodyweight. However, there is no firm indication yet that *TUB* can act as a transcription factor. In transfected cells *TUB* is tyrosine phosphorylated by the insulin receptor in response to insulin treatment [2]. *TUB* might have a role in insulin signalling and could possibly integrate insulin and GPCR-mediated signals [2]. We found no association of any

Table 3 The effect of the three *TUB* polymorphisms on obesity-related variables in all women

	GEE						Sib-TDT			
	Number	Mean±SEM		<i>p</i> value ^a	Beta (95% CI)	Pairs	Mean±SEM		<i>p</i> value	Difference (95% CI)
		11 and 12 ^b	22 ^c				11 and 12 ^b	22 ^c		
rs2272382										
General obesity ^d	947/864/238	-0.02±0.02	0.06±0.07	0.304	0.07 (-0.06, 0.20)	98	0.07±0.09	0.10±0.11	0.824	0.03 (-0.20, 0.25)
Central obesity ^e	1,095/980/269	-0.02±0.02	0.04±0.06	0.398	0.05 (-0.07, 0.17)	104	0.05±0.09	0.07±0.10	0.796	0.03 (-0.17, 0.22)
Leptin (ng/ml)	1,049/931/258	16.2±0.26	16.5±0.73	0.887	0.30 (-1.25, 1.85)	104	16.6±1.24	16.9±1.24	0.882	0.24 (-2.92, 3.39)
BMI (kg/m ²)	1,172/1,039/282	24.7±0.09	24.9±0.27	0.376	0.27 (-0.27, 0.81)	108	24.9±0.39	25.3±0.44	0.429	0.38 (-0.57, 1.33)
Total fat (%)	1,143/1,007/270	35.5±0.17	35.6±0.49	0.836	0.10 (-0.84, 1.04)	106	35.6±0.75	35.6±0.80	0.967	0.03 (-1.61, 1.68)
Waist (cm)	1,149/1,012/280	78.0±0.21	79.3±0.65	0.035	1.42 (0.18, 2.66)	107	78.3±0.91	79.9±1.13	0.149	1.60 (-0.66, 3.87)
Central fat (%)	1,161/1,023/275	31.1±0.25	31.2±0.69	0.862	-0.12 (-1.44, 1.21)	107	31.8±1.04	31.4±1.13	0.379	-0.42 (-2.64, 1.80)
rs2272383										
General obesity ^d	839/985/257	-0.01±0.02	-0.06±0.06	0.530	-0.04 (-0.17, 0.09)	109	0.01±0.10	0.01±0.09	0.941	-0.01 (-0.23, 0.21)
Central obesity ^e	951/1,124/300	-0.00±0.02	-0.10±0.06	0.290	-0.06 (-0.17, 0.05)	110	-0.04±0.10	0.01±0.09	0.666	0.05 (-0.16, 0.25)
Leptin (ng/ml)	915/1,069/290	16.3±0.27	15.7±0.60	0.789	-0.58 (-2.04, 0.89)	116	16.3±1.07	15.9±0.94	0.733	-0.38 (-2.57, 1.82)
BMI (kg/m ²)	1,013/1,189/322	24.7±0.09	24.6±0.24	0.822	-0.09 (-0.60, 0.42)	115	24.6±0.44	24.6±0.37	0.789	0.00 (-0.89, 0.89)
Total fat (%)	987/1,157/309	35.5±0.17	35.3±0.47	0.791	-0.12 (-1.00, 0.77)	118	35.4±0.70	35.6±0.77	0.803	0.21 (-1.47, 1.90)
Waist (cm)	986/1,169/316	78.2±0.22	78.0±0.56	0.829	0.02 (-1.17, 1.21)	111	77.6±1.08	78.4±0.87	0.284	0.86 (-1.34, 3.05)
Central fat (%)	1,005/1,172/317	31.3±0.25	30.2±0.63	0.179	-0.86 (-2.11, 0.39)	119	31.2±1.04	31.0±0.99	0.876	-0.18 (-2.50, 2.14)
rs1528133										
General obesity ^d	1,870/224/11	0.00±0.02	-0.04±0.07	0.468	-0.05 (-0.18, 0.08)	81	0.09±0.12	0.04±0.11	0.686	-0.05 (-0.29, 0.19)
Central obesity ^e	2,117/277/15	0.00±0.02	-0.05±0.06	0.217	-0.07 (-0.19, 0.04)	85	0.13±0.11	0.03±0.11	0.401	-0.10 (-0.33, 0.13)
Leptin (ng/ml)	2,035/250/16	16.4±0.26	16.0±0.69	0.835	-0.19 (-1.75, 1.38)	88	17.3±1.37	17.9±1.50	0.804	0.64 (-2.58, 3.85)
BMI (kg/m ²)	2,248/297/18	24.7±0.09	24.6±0.24	0.525	-0.19 (-0.72, 0.34)	91	25.5±0.56	25.2±0.52	0.713	-0.25 (-1.34, 0.83)
Total fat (%)	2,194/280/13	35.7±0.17	34.8±0.51	0.061	-0.89 (-1.81, 0.04)	90	36.5±0.85	36.0±0.91	0.547	-0.56 (-2.40, 1.28)
Waist (cm)	2,196/295/18	78.3±0.22	77.9±0.56	0.450	-0.53 (-1.75, 0.69)	89	79.8±1.27	79.0±1.05	0.658	-0.76 (-3.28, 1.75)
Central fat (%)	2,227/286/15	31.3±0.24	30.4±0.69	0.165	-0.93 (-2.24, 0.38)	90	32.6±1.24	32.0±1.34	0.672	-0.58 (-3.31, 2.14)

^a Age-adjusted *p* values^b 11 for rs1528133; ^c 12 and 22 for rs1528133^d Factor score based on factor analysis of serum leptin, BMI, weight, total fat mass and per cent total fat^e Factor score based on factor analysis of waist circumference, central fat mass and per cent central fat

Table 4 The effect of rs2272382 polymorphism on obesity-related variables in pre- and postmenopausal women

	GEE		Sib-TDT					
	Number	Mean±SEM		Pairs	Mean±SEM		p value	Difference (95% CI)
		11 and 12	22		11 and 12	22		
Premenopausal								
General obesity	519/458/121	-0.20±0.03	-0.19±0.10	50	-0.03±0.14	-0.14±0.16	0.520	-0.10 (-0.43, 0.22)
Central obesity	522/463/124	-0.34±0.03	-0.35±0.09	49	-0.15±0.14	-0.30±0.16	0.277	-0.16 (-0.44, 0.13)
Leptin (ng/ml)	571/489/133	14.9±0.3	15.0±0.9	53	15.4±1.9	15.9±1.8	0.853	0.44 (-4.34, 5.23)
BMI (kg/m ²)	555/485/132	24.2±0.1	24.2±0.4	52	24.7±0.7	24.5±0.6	0.766	-0.19 (-1.49, 1.11)
Total fat (%)	544/470/125	32.8±0.2	32.3±0.7	50	33.8±1.1	32.2±1.2	0.218	-1.52 (-3.98, 0.93)
Waist (cm)	543/480/131	76.1±0.3	77.0±1.0	51	76.8±1.4	77.5±1.6	0.659	0.71 (-2.49, 3.90)
Central fat (%)	555/479/127	27.0±0.3	26.3±1.0	50	29.3±1.5	26.6±1.6	0.077	-2.72 (-5.73, 0.30)
Postmenopausal								
General obesity	343/334/96	0.22±0.04	0.43±0.09	36	0.26±0.13	0.53±0.15	0.121	0.27 (-0.08, 0.62)
Central obesity	358/353/103	0.28±0.04	0.50±0.09	36	0.30±0.13	0.67±0.15	0.013	0.38 (0.08, 0.67)
Leptin (ng/ml)	369/355/103	17.8±0.4	19.1±1.3	38	18.5±2.0	19.4±2.1	0.707	0.96 (-4.20, 6.13)
BMI (kg/m ²)	374/368/107	25.4±0.2	26.0±0.4	37	25.4±0.5	27.0±0.8	0.072	1.64 (-0.15, 3.43)
Total fat (%)	387/368/103	38.1±0.3	39.3±0.7	36	38.3±1.1	39.8±1.0	0.241	1.49 (-1.05, 4.04)
Waist (cm)	363/357/106	80.6±0.4	82.9±1.1	37	80.0±1.4	84.6±2.0	0.027	4.68 (0.57, 8.78)
Central fat (%)	388/372/106	34.6±0.4	36.8±0.9	37	35.0±1.6	38.4±0.6	0.042	3.43 (0.14, 6.72)

^a Age-adjusted *p* values under recessive model (genotype 22 homozygotes compared with allele 1 carriers)

SNPs with serum leptin, which suggests that TUB is not involved in this signalling pathway.

This is the first reported association of a variant in the *TUB* gene with late-onset obesity in humans. The current study had a number of strengths, including the large sample size, which was approximately twice the size of the combined cohorts studied by Shiri-Sverdlov et al. [5], the more comprehensive list of phenotypes representing general and central obesity and the availability of accurate measures of body fat by dual-emission X-ray absorptiometry scans. Furthermore, we confirmed associations by Sib-TDT in the DZ twins discordant for their genotypes, excluding population stratification bias as a possible source of spurious associations. We limited the number of tests performed by creating factor scores for general and central obesity and we only tested those specific genetic models that showed significant results in the previous study [5]. Furthermore, we explored the hypothesis that *TUB* is a candidate gene for late-onset obesity in humans through testing the interaction of the SNPs by menopausal status, because the two cohorts studied by Shiri-Sverdlov et al. [5] involved only older individuals (86.5% were >50 years of age) and the onset of obesity in the tubby mouse is characterised by a late onset. In their combined sample, Shiri-Sverdlov et al. [5] observed a BMI difference (95% CI) of 1.12 kg/m² (0.34–1.91) between genotype groups for the recessive model of rs2272382. In our postmenopausal women this was 0.92 kg/m² (0.03–1.81) overall and 1.64 kg/m² (–0.15, 3.43) for the naturally matched 37 DZ pairs discordant for their genotypes. Finally, it should be noted that our findings in the twin individuals can be considered as representative of the UK female population as a whole [8]. We have previously found few differences between twins and singletons in the population generally, the only indication being that MZ twins had a slightly lower weight and a smaller variance for weight than DZ twins and singletons [8].

In summary, we have replicated association of *TUB* with measures of general and central obesity in normal postmenopausal women confirming *TUB* as a candidate gene for late-onset obesity in humans. In tubby mice alteration of the expression of different neuropeptides involved in appetite control and feeding behaviour has been observed in recent studies [14, 23, 24]. Investigation of effects of *TUB* variants on modulation of food intake in humans may therefore be a promising avenue for future research.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

- Boggon TJ, Shan WS, Santagata S, Myers SC, Shapiro L (1999) Implication of tubby proteins as transcription factors by structure-based functional analysis. *Science* 286:2119–2125
- Kapeller R, Moriarty A, Strauss A et al (1999) Tyrosine phosphorylation of tub and its association with Src homology 2 domain-containing proteins implicate tub in intracellular signaling by insulin. *J Biol Chem* 274:24980–24986
- Kleyn PW, Fan W, Kovats SG et al (1996) Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. *Cell* 85:281–290
- Bruning JC, Gautam D, Burks DJ et al (2000) Role of brain insulin receptor in control of body weight and reproduction. *Science* 289: 2122–2125
- Shiri-Sverdlov R, Custers A, van Vliet-Ostapchouk JV et al (2006) Identification of TUB as a novel candidate gene influencing body weight in humans. *Diabetes* 55:385–389
- Castillo M, Mulet J, Gutierrez LM et al (2006) Role of the RIC-3 protein in trafficking of serotonin and nicotinic acetylcholine receptors. *J Mol Neurosci* 30:153–156
- Spector TD, Williams FM (2006) The UK adult twin registry (Twins UK). *Twin Res Hum Genet* 9:899–906
- Andrew T, Hart D, Snieder H, de Lange M, Spector TD, MacGregor AJ (2001) Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res* 4: 464–477
- Tréguët D-A, Ducimetière P, Tiret L (1997) Testing association between candidate-gene markers and phenotype in related individuals, by use of estimating equations. *Am J Hum Genet* 61:189–199
- Dong Y, Zhu H, Wang X et al (2004) Obesity reveals an association between blood pressure and the G-protein beta3-subunit gene: a study of female dizygotic twins. *Pharmacogenetics* 14:419–427
- Spencer-Jones NJ, Wang X, Snieder H, Spector TD, Carter ND, O'Dell SD (2005) Protein tyrosine phosphatase-1B gene PTPN1: selection of tagging single nucleotide polymorphisms and association with body fat, insulin sensitivity, and the metabolic syndrome in a normal female population. *Diabetes* 54:3296–3304
- North MA, Naggert JK, Yan Y, Noben-Trauth K, Nishina PM (1997) Molecular characterization of TUB, TULP1, and TULP2, members of the novel tubby gene family and their possible relation to ocular diseases. *Proc Natl Acad Sci USA* 94:3128–3133
- Santagata S, Boggon TJ, Baird CL et al (2001) G-protein signaling through tubby proteins. *Science* 292:2041–2050
- Guan XM, Yu H, Van der Ploeg LH (1998) Evidence of altered hypothalamic pro-opiomelanocortin/neuropeptide Y mRNA expression in tubby mice. *Brain Res Mol Brain Res* 59:273–279
- Ohki-Hamazaki H, Watase K, Yamamoto K et al (1997) Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* 390:165–169
- Huszar D, Lynch CA, Fairchild-Huntress V et al (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88:131–141
- Chen AS, Marsh DJ, Trumbauer ME et al (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet* 26:97–102
- Tecott LH, Sun LM, Akana SF et al (1995) Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. *Nature* 374: 542–546
- Szczyepka MS, Rainey MA, Kim DS et al (1999) Feeding behavior in dopamine-deficient mice. *Proc Natl Acad Sci USA* 96:12138–12143
- Coleman DL, Eicher EM (1990) Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. *J Heredity* 81:424–427

21. Julius D, MacDermott AB, Axel R, Jessell TM (1988) Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science* 241:558–564
22. He W, Ikeda S, Bronson RT et al (2000) GFP-tagged expression and immunohistochemical studies to determine the subcellular localization of the tubby gene family members. *Brain Res Mol Brain Res* 81:109–117
23. Backberg M, Madjid N, Ogren SO, Meister B (2004) Down-regulated expression of agouti-related protein (AGRP) mRNA in the hypothalamic arcuate nucleus of hyperphagic and obese tub/tub mice. *Brain Res Mol Brain Res* 125:129–139
24. Wang Y, Seburn K, Bechtel L et al (2006) Defective carbohydrate metabolism in mice homozygous for the tubby mutation. *Physiol Genomics* 27:131–140