Previously, we identified a locus on 11p influencing obesity in families with type 2 diabetes. Based on mouse studies, we selected TUB as a functional candidate gene and performed association studies to determine whether this controls obesity. We analyzed the genotypes of 13 single nucleotide polymorphisms (SNPs) around TUB in 492 unselected type 2 diabetic patients with known BMI values. One SNP (rs1528133) was found to have a significant effect on BMI (1.54 kg/m², P = 0.006). This association was confirmed in a population enriched for type 2 diabetes, using 750 individuals who were not selected for type 2 diabetes. Two SNPs in linkage disequilibrium with rs1528133 and mapping to the 3′ end of TUB, rs2272382, and rs2272383 also affected BMI by 1.3 kg/m² (P = 0.016 and P = 0.010, respectively). Combined analysis confirmed this association (P = 0.005 and P = 0.002, respectively). Moreover, comparing 349 obese subjects (BMI > 30 kg/m²) from the combined cohort with 289 normal subjects (BMI < 25 kg/m²) revealed that the protective alleles have a lower frequency in obese subjects (odds ratio 1.32 [95% CI 1.04–1.67], P = 0.022). Altogether, data from the tubby mouse as well as these data suggest that TUB could be an important factor in controlling the central regulation of body weight in humans. Diabetes 55:385–389, 2006

Until recently, type 2 diabetes was considered a disease of the elderly. Currently, worldwide, a large proportion of newly diagnosed patients are adolescents. The increase in the prevalence of type 2 diabetes, as well as the rapid spreading to a younger age at onset, is largely due to environmental factors, including modern eating habits and reduced physical activity. Obesity is a major risk factor for type 2 diabetes, and the obesity epidemic coincides with the type 2 diabetes epidemic. Genetic studies have suggested a common basis for both diseases. Recently, we identified a locus on 11p15 (95% CI 4,868,745–10,676,565 bps) influencing obesity in families with type 2 diabetes (1). The TUB gene was found to be the most relevant candidate gene within this locus, and we hypothesized that TUB was associated with obesity susceptibility.

TUB is the founding member of the tubby-like proteins and is conserved among vertebrate genomes (2). Interestingly, a loss of function mutation of tub results in the tubby mouse syndrome, which is characterized by late-onset obesity and neurosensory deficits. The tubby mouse begins to diverge in weight at ~12 weeks of age and ultimately reaches twice the weight of its wild-type littermates (3). Along with weight gain, tubby mouse shows insulin resistance, although it is not overtly diabetic. The high level of TUB expression in the hypothalamus, a brain region that is implicated in the control of systemic energy regulation (4), indicates that the obesity phenotype might result from defects in neuroendocrine control of satiety or metabolism. Recent experiments in the worm C. elegans show that tub-1 can increase adiposity when ablated (5). TUB is an attractive candidate gene for obesity as it is an important and fundamental factor in metabolism and obesity, although the role of TUB in obesity in humans has not been established.

To investigate the role of TUB in human obesity, 13 single nucleotide polymorphisms (SNPs) around TUB (Fig. 1A and online appendix Table 1 [available from http://diabetes.diabetesjournals.org]) were genotyped in 492 unrelated type 2 diabetic patients from the Breda cohort, for whom BMI values were available (Table 1). Linear regression analysis, adjusted for age and sex, revealed that the minor allele of SNP rs1528133 had a marked effect on BMI (+1.54 kg/m², P = 0.006) (Fig. 1B, Table 2). SNP rs1528133 is located 22 kb distal to TUB in the flanking gene RIC3, which has an unknown function. SNP rs1528133 is in strong linkage disequilibrium with SNPs in the 3′ end of TUB (Fig. 1C), indicating that the COOH-terminus of TUB may be associated with obesity. A replication study of rs1528133 and four SNPs located in the 3′ of TUB and in linkage disequilibrium with rs1528133 (rs2272382, rs2272383, rs750955, and rs1406095) was performed in a population enriched for type 2 diabetes Dutch cohort (RIVM) of 750 individuals (Table 1). We were able...
to validate our initial result and found a significant effect on BMI for the major alleles of both rs2272382 (−1.28 kg/m², \( P = 0.016 \)) and rs2272383 (−1.26 kg/m², \( P = 0.01 \)) (Fig. 1B, Table 2). These two SNPs are in a strong linkage disequilibrium with rs1528133 (\( D' = 0.8 \) and \( D'' = 0.9 \), respectively). Combining both cohorts (\( n = 1,242 \)) strengthened the effect, as rs2272382 and rs2272383 showed even stronger association with BMI (−1.12 kg/m², \( P = 0.005 \), −1.11 kg/m², \( P = 0.002 \)) (Fig. 1B, Table 2). These two SNPs remained significant in the combined cohort after stringent Bonferroni correction for five SNPs (\( P_{c} < 0.05/5 = 0.01 \)).

Comparing the 349 obese subjects from the combined cohort (BMI >30 kg/m²) with 289 normal subjects (BMI <25 kg/m²) revealed that the minor allele of SNP rs2272382 was significantly more common in the obese subjects (37.3%) than normal subjects (31.1%) (odds ratio 1.32 [95% CI 1.04–1.67], \( P = 0.022 \)), while the minor allele of rs2272383 showed borderline significance (1.23 [0.98–1.55], \( P = 0.078 \)) (Table 3). In addition, the genotype
TABLE 1
Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th>Trait</th>
<th>Breda cohort</th>
<th>RIVM cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>356 (173/183)</td>
<td>492 (224/267)*</td>
</tr>
<tr>
<td>Age at study (years)</td>
<td>49.39 ± 11.68*</td>
<td>73 ± 9.9</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td></td>
<td>63.0 ± 11.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>NA</td>
<td>27.8 ± 4.1</td>
</tr>
<tr>
<td>n (BMI &gt;30 kg/m²)</td>
<td>NA</td>
<td>129</td>
</tr>
<tr>
<td>n (BMI &lt;25 kg/m²)</td>
<td>NA</td>
<td>125</td>
</tr>
</tbody>
</table>

Data are means ± SD, unless otherwise indicated. *Not available for one subject. †Only available for 189 subjects. NA, not available.

To our knowledge, the potential contribution of TUB to human obesity was assessed only once (9). The authors have examined five polymorphic microsatellite markers around TUB in 716 Pima Indians comprising 217 nuclear families for sibpair linkage to BMI. No significant linkages were found in an analysis of all sibships or in an analysis restricted to discordant sibpairs. However, this linkage study was done in a different population and not with the same SNPs that were used by us.

Thus, the role of tubby as an important factor in metabolism and obesity has not been shown in humans. Our study is the first to provide an initial estimate of the association of the TUB gene with a quantitative measure of obesity. Recently, the effect of MC4R, a major gene for obesity, on weight regulation was estimated to be -0.52 kg/m² (10), and since the effect of TUB on weight regulation is more then twice as high (-1.3 kg/m²), this indicates the importance of TUB’s contribution to polygenetically regulated body weight.

Altogether, data from the tubby mouse as well as these data suggest that tubby has a protective role in the central regulation of body weight in humans. Mutations in TUB can lead to increased body weight and contribute to obesity. This discovery will reveal new molecular pathways in weight regulation that should lead to innovative therapies, preventive measures, and insights into the pharmacogenetics of such intervention strategies.
The effect of the different alleles of each of the SNPs on BMI (kg/m²)

<table>
<thead>
<tr>
<th>NCBI SNP reference</th>
<th>Allele</th>
<th>Breda cohort</th>
<th>RIVM cohort</th>
<th>Combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (492)</td>
<td>Effect on BMI* (95% CI)†</td>
<td>P value‡</td>
</tr>
<tr>
<td>rs2280729</td>
<td>Minor (T)</td>
<td>479§</td>
<td>0.01 (−0.85 to 0.86)</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Minor (C)</td>
<td>470§</td>
<td>−0.03 (−0.75 to 0.69)</td>
<td>0.88</td>
</tr>
<tr>
<td>rs2280726</td>
<td>Minor (G)</td>
<td>472§</td>
<td>0.16 (−0.64 to 0.95)</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Minor (A)</td>
<td>467§</td>
<td>−0.08 (−0.64 to 0.80)</td>
<td>−0.97</td>
</tr>
<tr>
<td>rs1406095</td>
<td>Minor (A)</td>
<td>470§</td>
<td>−0.15 (−2.12 to 1.23)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Minor (G)</td>
<td>471§</td>
<td>0.41 (−0.30 to 1.13)</td>
<td>−0.69</td>
</tr>
<tr>
<td>rs11041734</td>
<td>Minor (G)</td>
<td>470§</td>
<td>0.16 (−0.64 to 0.95)</td>
<td>0.61</td>
</tr>
<tr>
<td>rs4385931</td>
<td>Minor (C)</td>
<td>470§</td>
<td>−0.08 (−0.64 to 0.80)</td>
<td>−0.97</td>
</tr>
<tr>
<td>rs2272382</td>
<td>Minor (A)</td>
<td>470§</td>
<td>−0.15 (−2.12 to 1.23)</td>
<td>0.06</td>
</tr>
<tr>
<td>rs2242501</td>
<td>Minor (G)</td>
<td>470§</td>
<td>0.16 (−0.64 to 0.95)</td>
<td>0.61</td>
</tr>
<tr>
<td>rs2272383</td>
<td>Minor (G)</td>
<td>470§</td>
<td>0.16 (−0.64 to 0.95)</td>
<td>0.61</td>
</tr>
<tr>
<td>rs3750955</td>
<td>Minor (G)</td>
<td>470§</td>
<td>0.16 (−0.64 to 0.95)</td>
<td>0.61</td>
</tr>
<tr>
<td>rs1569128</td>
<td>Minor (C)</td>
<td>470§</td>
<td>−0.08 (−0.64 to 0.81)</td>
<td>0.05</td>
</tr>
<tr>
<td>rs1528133</td>
<td>Minor (C)</td>
<td>482§</td>
<td>1.54 (0.45–2.62)</td>
<td>−0.32</td>
</tr>
<tr>
<td>rs6578931</td>
<td>Minor (A)</td>
<td>479§</td>
<td>−0.08 (−0.64 to 0.81)</td>
<td>0.05</td>
</tr>
<tr>
<td>rs10769876</td>
<td>Minor (C)</td>
<td>470§</td>
<td>−0.08 (−0.64 to 0.81)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*All data were analyzed using a Gaussian linear regression, including age and sex as explanatory variables in the models. †The CIs were calculated using Woolf's method with Haldane's correction. ‡The P values were computed with a 95% two-sided \( \chi^2 \) test and are not corrected for multiple testing. Corrected P values are 0.004 (0.05/13) and 0.01 (0.05/5) for the Breda and RIVM/combined cohorts, respectively. $Number of subjects that were successfully genotyped. ¶P values remain significant after stringent Bonferroni correction.

allele(s) or genotypes were then added to the model. The inference criterion used for comparing the models is their ability to predict the observed data, i.e., models are compared directly through their minimized minus log likelihood. When the numbers of parameters in models differ, they are penalized by adding the number of estimated parameters, a form of the Akaike information criterion (11). For models where the allele(s) or genotypes were found to be significant, a P value was computed and is presented. Differences in allele and genotype distribution in case and control subjects were tested for significance using a 95% two-sided \( \chi^2 \) test. Odds ratios and the CIs were calculated using Woolf's method with Haldane's correction (12). For Hardy-Weinberg equilibrium, we compared the expected and observed genotypes in 2 × 3 tables (online appendix Tables 3, 4, and 5).
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REFERENCES


### TABLE 3
Association of TUB alleles with obesity in the combined cohort

<table>
<thead>
<tr>
<th>SNP (NCBI Reference)</th>
<th>Alleles</th>
<th>Group</th>
<th>Major allele [n (%)]</th>
<th>Minor allele [n (%)]</th>
<th>P value (allele)*</th>
<th>Odds ratio (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1406095</td>
<td>G,A</td>
<td>BMI &gt;30</td>
<td>357 (52.8)</td>
<td>319 (47.2)</td>
<td>0.981</td>
<td>1.00 (0.80–1.26)</td>
</tr>
<tr>
<td>rs2272382</td>
<td>G,A</td>
<td>BMI &lt;25</td>
<td>294 (52.9)</td>
<td>262 (47.1)</td>
<td>0.022</td>
<td>1.32 (1.04–1.67)</td>
</tr>
<tr>
<td>rs2272383</td>
<td>A,G</td>
<td>BMI &gt;30</td>
<td>415 (62.9)</td>
<td>247 (37.3)</td>
<td>0.078</td>
<td>1.23 (0.98–1.55)</td>
</tr>
<tr>
<td>rs3750955</td>
<td>C,T</td>
<td>BMI &gt;30</td>
<td>507 (74.6)</td>
<td>173 (25.4)</td>
<td>0.295</td>
<td>1.15 (0.89–1.49)</td>
</tr>
<tr>
<td>rs1528133</td>
<td>A,C</td>
<td>BMI &lt;25</td>
<td>524 (93.9)</td>
<td>34 (6.1)</td>
<td>0.297</td>
<td>1.26 (0.81–1.96)</td>
</tr>
</tbody>
</table>

*The P values were computed with a 95% two-sided χ² test. †Odds ratios and the 95% CIs were calculated using Woolf’s method with Haldane’s correction.