R125W coding variant in **TBC1D1** confers risk for familial obesity and contributes to linkage on chromosome 4p14 in the French population

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Stone et al. previously reported an association between the **TBC1D1** gene variant R125W (rs35859249) and severe obesity in women from US pedigrees. We attempted to replicate this result in 9714 French Caucasian individuals, combining family-based and general population studies. We confirmed an association with familial obesity (defined as body mass index (BMI) > 97th percentile) in women from 1109 obesity-selected pedigrees (Z-score = 2.70, P = 0.008). Analysis of 16 microsatellite markers on chromosome 4 restricted to the 42 pedigrees carrying the **TBC1D1** R125W variant allele also revealed a suggestive evidence of linkage with obesity (maximum likelihood binomial LOD of 2.73, P = 0.0002) on chromosome 4p14, where resides **TBC1D1**. In contrast, R125W variant was neither associated with BMI nor with obesity in a large population-based cohort. These results confirm a putative role of **TBC1D1** R125W variant in familial obesity predisposition.

INTRODUCTION

Stone et al. (1) previously identified a linkage to severe obesity in women at chromosome 4p15-14 in Caucasian US pedigrees. This interval was also linked to BMI in a Mexican-American cohort (2). More recently, Stone et al. (3) provided compelling data supporting the association of a non-synonymous polymorphism (R125W, rs35859249) in **TBC1D1** gene with severe familial obesity in women only. They also showed that this polymorphism accounted for the majority of the evidence of linkage on chromosome 4p15-14. In contrast, the 125W variant was not enriched in a set of 173 random obesity cases. Interestingly, by selecting pedigrees that segregated R125W with obesity, they were able to generate a new peak of linkage for obesity predisposition on chromosome 4q34–35, suggesting gene/gene interaction between **TBC1D1** and another obesity gene located at 4q34–35 (3). As recently outlined by the **INSIG2** studies (4–12), the replication of promising association data in additional samples is mandatory to exclude spurious conclusions (13). To our knowledge, no report confirmed the original report of association of **TBC1D1** with obesity. This prompted us to assess the contribution of R125W to obesity and BMI variation in 1109 French pedigrees with childhood or adult obesity and also in a large French general adult population (n = 4634).

RESULTS

The R125W minor allele frequency was 9.2% in pedigrees with childhood or adult obesity and was 10.1% in the general population, in agreement with frequency previously reported in the Utah population (9%) (3).
The genotypic distribution of R125W was in Hardy–Weinberg equilibrium ($P > 0.05$) in all the studied cohorts.

**Familial association between R125W and obesity**

We first genotyped the R125W polymorphism in 1109 French Caucasian pedigrees with obese children or adults ($n = 5080$). Phenotypic characteristics of the pedigrees are summarized in Table 1. We only tested the additive mode of inheritance, previously described as the best fitting mode of inheritance for the variant R125W (3). Using the 97th BMI percentile as threshold for obesity in children and adults, a borderline significant over-transmission of the 125W risk allele to obese subjects was found ($Z$-score = 1.99, $P = 0.05$). When restricting this analysis to women only, we observed a stronger familial association with obesity ($Z$-score = 2.70, $P = 0.008$). The same analysis restricted to men did not show any association with obesity ($Z$-score = 0, $P = 1$).

In order to tag the TBC1D1 locus and 10 kb upstream and 10 kb downstream of the gene, we have used the data of our recently genotyped Illumina 370 CNV duo arrays in 920 French women with extreme familial obesity and 920 French lean control women. Forty-five SNPs were genotyped in the Illumina array in this region and we performed case–control analysis under the additive model. Four SNPs (rs11096913, rs2925956, 1364951, rs13110318) harbored nominal evidence of association ($0.01 < P < 0.045$) but no SNP survived to a conservative Bonferroni correction (data not shown). It suggests that except for the R125W polymorphism, the gene variation in TBC1D1 does not play a major role in susceptibility to familial extreme obesity in French women.

**TBC1D1 R125W and linkage with obesity on chromosomes 4p14 and 4q34–35**

The pedigrees analyzed for linkage were a subset of the 1109 French Caucasian pedigrees with obese children or adults assessed for familial association between R125W and obesity. We selected 16 microsatellite markers covering the chromosome 4 with an average spacing between markers of 13.5 cm and previously genotyped in 395 French pedigrees with at least two sibs with a BMI ≥ 97th percentile (14–16). We then analyzed the linkage with obesity (BMI ≥ 97th percentile) on chromosome 4 in the whole pedigree sample, and in the sub-group of 42 pedigrees polymorphic for the TBC1D1 R125W variant (at least two obese sibs sharing at least one copy of the R125W variant allele), as previously conducted in US pedigrees (3). Non-parametric multipoint analyses in the whole pedigree sample did not reveal any suggestive linkage with obesity on chromosome 4 (Fig. 1). In contrast, we found a suggestive evidence of linkage with obesity on chromosome 4p14 (maximum likelihood binomial LOD: 2.73, $P = 0.0002$; closest marker D4S450; one-LOD unit: 4p15–4q13.3: D4S391 (52.40 cm)-D4S392 (79.98 cm)), when analyzing apart the 42 pedigrees polymorphic for the TBC1D1 R125W variant. The marker D4S405 was located close to the TBC1D1 gene (2.2 Mb on the physical map). We were unable to generate a new peak of linkage for obesity predisposition on chromosome 4q34–35 in this subgroup of 42 French pedigrees (Fig. 1). Gender-specific analyses did not show stronger evidence of linkage with obesity (data not shown).

**Association between R125W and obesity in a large French general population**

To assess the contribution of R125W polymorphism to BMI variation and obesity predisposition in a general population (not enriched in subjects with severe familial obesity), we genotyped the French prospective DESIR cohort ($n = 4,634$; Table 1). We first analyzed the R125W variant at baseline. We were not able to detect any association between the polymorphism and overweight (25 kg/m² ≤ BMI < 30 kg/m²) or moderate obesity (BMI ≥ 30 kg/m²; Table 2). We then analyzed the genotype distribution of R125W in subjects unaffected at baseline who developed overweight or obesity during the 9-year follow-up period. Again, people carrying the 125W polymorphism did not show higher incidence of overweight or obesity (Table 3, Fig. 2). We did not observe any association between R125W polymorphism and BMI or waist-to-hip ratio at baseline (Table 2) or using mixed model analyses on the whole data set (data not shown). Gender-specific analyses did not show any association with quantitative/binary traits, at baseline and during the follow-up (data not shown). Power calculation with alpha level = 0.05 indicates power higher than 80% to detect association between overweight or obesity at baseline or during the 9-year follow-up period and R125W considering an odd ratios of 1.45.

**DISCUSSION**

In this study, we were able to replicate the previous association between R125W and familial severe obesity in US women (3) in an independent French cohort. This result was based on the pedigree disequilibrium test analysis of a large number of obesity-selected French pedigrees ($n = 1109$), and was therefore robust to population stratification bias. Another confirmation of the work of Stone et al. (3) was the lack of association between TBC1D1 R125W variant and random mild obesity in a large general French population ($n = 4634$), even though we had a limited ($<80\%$) power to detect effect size lower than 1.45 in our design. This observation suggests that cases with familial forms of severe obesity are a high-value resource to identify susceptibility genes, but may be not fully representative of most common forms of the disease. This may also explain why promising associations with familial forms of obesity, obtained from positional cloning approaches (17,18) have not been reproducibly replicated in other cohorts (19–21). These observations combined with other recent reports (22,23) suggest that stratifying according to the family history of the disease may be of interest in replication studies, to avoid misleading conclusions.

Stone et al. (3) previously showed that the TBC1D1 R125W polymorphism accounted for the majority of the evidence of linkage on chromosome 4p15-14 in Caucasian US pedigrees. In this previous report, the HLOD score for obesity dropped from 9.0 to 2.8 after discarding the women with at least one copy of the R125W variant allele (3). Our results further
support a potential contribution of TBC1D1 R125W polymorphism to the linkage with obesity on chromosome 4p15-14 in French pedigrees. Non-parametric multipoint analyses in the whole pedigree sample did not reveal any linkage with obesity on chromosome 4. However, we found a suggestive evidence of linkage with obesity on chromosome 4p14 (LOD maximum likelihood binomial: 2.73, \( P = 0.0002 \); closest marker D4S405 in the vicinity of TBC1D1 gene) in the subgroup of 42 pedigrees carrying the TBC1D1 R125W polymorphism (at least two obese sibs sharing at least one copy of the R125W variant allele). A Genethon map was used (cm). Physical position (in Mb, NCBi Build 35 assembly) of the markers was indicated in brackets.

In summary, the replication of a possible role of TBC1D1 coding gene variation in genetic predisposition of severe familial obesity in French combined to a potential involvement of TBC1D1 protein in adipogenesis process (26) and in insulin pathways through GLUT4 translocation (27) further support the candidacy of TBC1D1 as a ‘thrifty’ gene as recently proposed by Koumanov et al. (28).

**MATERIAL AND METHODS**

**Subjects**

A total of 9714 subjects were genotyped for TBC1D1 R125W. We tested familial association with obesity using 674 pedigrees with at least one obese child including 3181 individuals: 971 obese children, 288 non-obese children, 1282 parents and 640 grandparents. From these 1109 families, we selected a subgroup of 395 pedigrees with at least two sibs with a BMI > 97th percentile for further linkage analysis. Among these 395 pedigrees, 42 pedigrees displayed cosegregation between obesity and R125W (at least two obese sibs sharing at least one copy of the R125W variant allele). We were unable to generate an interacting linkage peak on chromosome 4q34–35 in the 42 French pedigrees carrying the TBC1D1 R125W variant (Fig. 1), as previously observed in US pedigrees (3). Further replication is required to definitively conclude about such an interaction between chromosomes 4p14 and 4q34–35, as the number of pedigrees (\( n = 42 \)) involved in the present analysis and in the US initial report (\( n = 26 \)) is not sufficient to reach definite conclusions. In addition, it remains possible that Stone et al., (3) by selecting pedigrees that segregated R125W with obesity, have enriched their subset for a disease-causing allele at 4q34–35 by chance. The recent identification of a novel obesity locus on chromosome 4q35 in American Indian populations is concordant with this hypothesis (25).

**Table 1.** Characteristics of the different cohorts. We detailed the sample size, sex ratio, mean and SD for age, BMI and BMI Z-score traits in the different studied cohort.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Sample size (% men)</th>
<th>Mean age (SD)</th>
<th>Mean BMI (SD)</th>
<th>Mean BMI Z-score (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese children (childhood obesity pedigrees)</td>
<td>PDT*</td>
<td>971 (49)</td>
<td>11.14 (3.22)</td>
<td>28.21 (6.57)</td>
</tr>
<tr>
<td>Non-obese relative children (childhood obesity pedigrees)</td>
<td>PDT*</td>
<td>288 (50)</td>
<td>21.83 (10.45)</td>
<td>25.93 (8.03)</td>
</tr>
<tr>
<td>Parents (childhood obesity pedigrees)</td>
<td>PDT*</td>
<td>1282 (45)</td>
<td>41.11 (6.73)</td>
<td>30.33 (7.26)</td>
</tr>
<tr>
<td>Grand parents (childhood obesity pedigrees)</td>
<td>PDT*</td>
<td>640 (38)</td>
<td>66.64 (7.23)</td>
<td>29.11 (5.38)</td>
</tr>
<tr>
<td>Obese offsprings (adult obesity pedigrees)</td>
<td>PDT*</td>
<td>653 (30)</td>
<td>40.85 (13.73)</td>
<td>40.19 (8.02)</td>
</tr>
<tr>
<td>Non-obese offsprings (adult obesity pedigrees)</td>
<td>PDT*</td>
<td>377 (42)</td>
<td>34.15 (15.04)</td>
<td>24.98 (3.28)</td>
</tr>
<tr>
<td>Parents (adult obesity pedigrees)</td>
<td>PDT*</td>
<td>733 (36)</td>
<td>53.60 (12.72)</td>
<td>34.08 (9.02)</td>
</tr>
<tr>
<td>Grand parents (adult obesity pedigrees)</td>
<td>PDT*</td>
<td>101 (37)</td>
<td>68.14 (9.71)</td>
<td>31.99 (7.64)</td>
</tr>
<tr>
<td>DESIR total population (at baseline)</td>
<td>Case–control, QT analyses*</td>
<td>4634 (50)</td>
<td>47.29 (10.05)</td>
<td>24.72 (3.82)</td>
</tr>
</tbody>
</table>

*PDT, pedigree disequilibrium test.
*QT, quantitative trait.

**Figure 1.** Results of the maximum likelihood binomial (MLB) multipoint analyses on chromosome 4. The obesity affection trait (defined by BMI > 97th percentile) was studied. The y-axis indicates the LOD score. Solid curves correspond to the analysis of 395 French families with at least two sibs with a BMI > 97th percentile. Dashed curves correspond to the analysis of a subgroup of 42 pedigrees co-segregating for TBC1D1 R125W polymorphism (at least two obese sibs sharing at least one copy of the R125W variant allele). A Genethon map was used (cm). Physical position (in Mb, NCBi Build 35 assembly) of the markers was indicated in brackets.
We also genotyped 4634 subjects (men and women aged between 30 and 65 years) who were participants of the DESIR prospective study in a general population (data from the Epidemiological Study on the Insulin Resistance syndrome), a 9-year follow-up study that aims to clarify the development of the insulin resistance syndrome (29). They came from 10 health examination centers in the western central part of France, and all participants signed informed consent. The study protocol was approved by all local ethic committees and an informed consent was obtained from each subject before participating in the study.

Genotyping

The rs35859249 SNP was genotyped with the TaqMan technology (Applied Biosystems). Conditions for the TaqMan reaction were 95°C for 10 min and followed by 50 cycles of 92°C for 15 s and 60°C for 1 min. There was a genotyping success rate of 98.3%, and a concordance rate of 100% from 781 duplicated DNA.

Study of body mass index (BMI) and waist to hip ratio (WHR) according to the R125W genotypes in the whole DESIR population at baseline has been detailed (mean ± S.D. (n)).

Table 2. TBC1D1 R125W genotype frequencies, according to overweight and obesity status at baseline, and odds ratios for disease status in comparison with lean controls at all four three-yearly examinations: the DESIR study

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Total (n)</th>
<th>OR additive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean (BMI &lt; 25 kg/m²)</td>
<td>2114 (79.98)</td>
<td>498 (18.84)</td>
<td>31 (1.17)</td>
<td>2643</td>
<td>–</td>
</tr>
<tr>
<td>Overweight (25 ≥ BMI &lt; 30)</td>
<td>1230 (81.89)</td>
<td>257 (17.11)</td>
<td>15 (1)</td>
<td>1502</td>
<td>0.89 [0.76–1.03] P = 0.13</td>
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<tr>
<td>Obesity (BMI ≥ 30 kg/m²)</td>
<td>337 (80.05)</td>
<td>83 (19.71)</td>
<td>1 (0.24)</td>
<td>421</td>
<td>0.92 [0.74–1.20] P = 0.66</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>24.74 ± 3.80 (3681)</th>
<th>24.71 ± 3.93 (838)</th>
<th>23.52 ± 3.6 (47)</th>
<th>4566</th>
<th>P-value additive</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.86 ± 0.09 (3676)</td>
<td>0.85 ± 0.09 (833)</td>
<td>0.86 ± 0.09 (47)</td>
<td>4566</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 3. TBC1D1 R125W genotype frequencies, according to incident overweight and obesity status after 9 years of follow-up, and odds-ratios in comparison with lean controls at all four three-yearly examinations: the DESIR study

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Total (n)</th>
<th>OR additive</th>
</tr>
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<tr>
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<td>498 (18.84)</td>
<td>31 (1.17)</td>
<td>2643</td>
<td>–</td>
</tr>
<tr>
<td>Overweight (25 ≤ BMI &lt; 30)</td>
<td>296 (77.3)</td>
<td>77 (20.1)</td>
<td>10 (2.61)</td>
<td>383</td>
<td>1.27 [0.97–1.54] P = 0.08</td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30 kg/m²)</td>
<td>288 (82.3)</td>
<td>62 (17.7)</td>
<td>0 (0.0)</td>
<td>350</td>
<td>0.85 [0.62–1.08] P = 0.15</td>
</tr>
</tbody>
</table>

Figure 2. Incidences of overweight (A) and obesity (B) in the French general population according to the TBC1D1 R125W genotypes. The prospective value for overweight and obesity was measured using Cox-survival models adjusted for age and gender. For the TBC1D1 125W variant allele, the hazard ratios (HR) for overweight and obesity were 1.05 [0.89–1.24] (P = 0.6) and 0.88 [0.67–1.14] (P = 0.3) respectively.

Statistical analysis

Tests for deviation from Hardy–Weinberg equilibrium and for association in case–control used the DeFinetti program (http://linkage,rockefeller.edu/soft/). Logistic regression was used for overweight and obesity status, adjusting for confounding variables baseline age and sex. For familial association test, we used pedigree disequilibrium test implemented in UNPHASED software. To assess the effect of genotype on the incidence of new disease in participants who were healthy at baseline, we used Cox proportional hazard
regression models. We used linear regression models for quantitative trait analyses. The linkage analysis was performed with the maximum likelihood binomial method implemented in the MLBGH software (30). SPSS 10.1 software was used for general statistical analyses. For statistical power calculation we used the program QUANTO (31).

ACKNOWLEDGEMENTS

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Conflict of Interest statement. None declared.

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