Brief Genetics Report

Polymorphisms in the Amino Acid Transporter Solute Carrier Family 6 (Neurotransmitter Transporter) Member 14 Gene Contribute to Polygenic Obesity in French Caucasians

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Positional candidate gene analysis of the obesity-linked chromosome Xq24 locus identified two obesity-associated single nucleotide polymorphisms (SNPs) in the membrane amino acid transporter encoding the SLC6A14 (solute carrier family 6 [neurotransmitter transporter], member 14) gene in the Finnish population. Since we previously reported a modest evidence of linkage for this region in French obese families, we analyzed these SNPs in 1,267 obese adult case and 649 lean control subjects. SNPs 20649 C>T (odds ratio 1.23, 95% CI 1.04–1.45; P = 0.013) and 22510 C>G (1.36, 1.16–1.59; P = 0.0001) were shown to be associated with obesity in the French population. In addition, pedigree disequilibrium test results showed a modest excess of both at-risk SNP alleles in affected offspring (P = 0.05 and P = 0.08 for SNPs 20649 C>T and 22510 C>G, respectively). The SNP 22510 C>G at-risk G allele was associated, both in adult women with moderate obesity and in 234 obese girls, with higher body fat and modified perception of hunger and satiety (0.003 < P < 0.06). In conclusion, these data confirm an association of the SLC6A14 gene locus with obesity. Diabetes 53:2483–2486, 2004

The technique of utilizing a genome-wide scan of multiplex obese families followed by a positional candidate gene analysis has been proposed for identifying new and unexpected genes underlying common polygenic obesity. Indeed, the complete sequencing and annotation of the human genome combined with extensive databases of single nucleotide polymorphisms (SNPs) make the identification of complex gene haplotypes in linkage disequilibrium with frequent disease traits possible. The interest of this approach was recently supported by identification of the GAD2 (chromosome 10p12) and SLC6A14 (chromosome Xq24 [solute carrier family 6 [neurotransmitter transporter], member 14]) genes as candidate genes for obesity in the French and Finnish populations, respectively (1,2). Both genes are found to be highly expressed in the central nervous system, especially in those regions of the hypothalamus where feeding behavior is regulated and act by modulating the orexigenic effect of γ-aminobutyric acid (3) or by affecting tryptophan availability for the synthesis of serotonin (4,5). However, replication studies are required to confirm the role of susceptibility genes in complex traits and to evaluate their overall contribution to disease in different population types.

Located in the 3’ untranslated region of the SLC6A14 gene is the SNP 22510 C>G (rs2071877). This SNP was identified (2) to be strongly associated with obesity in the Finnish population. Furthermore, a haplotype including this SNP, together with SNP 20649 C>T (rs2011162, located in intron 12), showed a significantly different allele frequency in obese versus control subjects (2). However, the fact that the at-risk allele of SNP 22510 C>G in the initial Finnish study (G allele) was found to be inverted in the Swedish-Finnish replication sample (C allele) reflects a potential genetic heterogeneity and necessitates the analysis of other populations (6). The Finnish population has been relatively isolated for a long period, which may lead to differences in mechanisms of disease or to different at-risk SNP haplotypes compared with more admixed Caucasian populations. In addition, no correlation was observed between genotype combination and obesity-related phenotypes that would have provided additional evidence for the potential physiological effect of SLC6A14 in the regulation of energy balance. These issues, which are not trivial in complex trait analysis, prompted us to assess the contribution of the two obesity-associated SNPs of the SLC6A14 gene in the French obese population.

We genotyped SNPs 20649 C>T and 22510 C>G in 1,267 obese and in 649 unrelated nonobese normoglycemic...
TABLE 1
Comparison of genotypic and allelic distribution of SNPs 20649 C>T and 22510 C>G of the SLC6A14 gene among 1,267 obese subjects, 320 French-Caucasian obese children (BMI ≥97th percentile), and 649 control subjects

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes in women</th>
<th>Genotypes in men</th>
<th>Allele frequencies (men and women)</th>
<th>Allelic effect (OR [95% CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>P</td>
</tr>
<tr>
<td>20649C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>182</td>
<td>148</td>
<td>44</td>
<td>—</td>
</tr>
<tr>
<td>Obese†</td>
<td>408</td>
<td>328</td>
<td>54</td>
<td>0.021</td>
</tr>
<tr>
<td>Obese children</td>
<td>79</td>
<td>69</td>
<td>11</td>
<td>0.229</td>
</tr>
<tr>
<td>22510C/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>GG</td>
<td>GC</td>
<td>CC</td>
<td>—</td>
</tr>
<tr>
<td>Obese†</td>
<td>322</td>
<td>369</td>
<td>100</td>
<td>0.004</td>
</tr>
<tr>
<td>Obese children</td>
<td>57</td>
<td>77</td>
<td>26</td>
<td>0.532</td>
</tr>
</tbody>
</table>

Data represent the number of individuals per genotype and their status. Genotype frequencies were compared in females, and allelic frequencies were compared in males and males and females (two-sided Fisher's exact test). *Two-sided Fisher's exact test; † moderate and morbid obesity combined.

subjects, all of French-Caucasian origin. Both SNP 20649 C>T and 22510 C>G were associated with obesity (odds ratio [OR] 1.23, 95% CI 1.04–1.45; P = 0.013, and 1.36, 1.16–1.59; P = 0.0001, respectively) (Table 1). The risk allele of SNP 20649 C>T was the C allele, with a frequency of 0.72 in obese subjects versus 0.68 in control subjects. With regard to SNP 22510 C>G, the G allele was found to be the risk allele, with a frequency of 0.64 in obese subjects versus 0.56 in control subjects. Thus, in a large sample set of obese subjects, we confirm the observation of Suvioilahti et al. (2), namely, that in Finnish men (117 obese and 182 control subjects) the most frequent alleles for both SNPs are at risk for obesity. When we analyzed men and women separately, only women appeared to contribute to the obesity association of SNP 20649 C>T (Table 1). Both sexes contribute to the observed association with SNP 22510 C>G. In addition, the frequencies of the SLC6A14 SNPs in 326 obese French-Caucasian children (BMI >97th percentile) were compared with the 649 control adults. No significant association with childhood obesity was observed for both SNPs (Table 1). However, the at-risk G allele of SNP 22510 C>G showed an intermediate frequency in obese children (0.59) compared with obese adults (0.64) and with control adults (0.56).

Thereafter, haplotype analyses of SNP 22510 C>G and 20649 C>T were performed in the 1,267 obese and 649 control subjects using the UNPHASED suite of software for case-control data (7). The haplotype including SNPs 20649 C>T and 22510 C>G did not provide stronger association than SNP 22510 C>G alone (data not shown). Nominal evidence for linkage between the chromosome Xq24 locus and obesity, showing a maximal logarithm of odds score near marker DXS1001 of 1.42, was previously identified in obese French-Caucasian families having at least one subject with a BMI >40 kg/m² and one sibling with a BMI >27 kg/m² (8). Although this less-than-suggestive linkage may be spurious, we investigated the familial association of SLC6A14 SNPs in our original 188 nuclear families (comprising 612 individuals). Using both the UNPHASED suite to assess association and/or association and linkage and the pedigree disequilibrium test, no strong familial association was detected for both SLC6A14 SNPs (Table 2). However, a weak excess of the C at-risk allele of SNP 20649 C>T was observed in affected offspring (P = 0.05). Further, a nonsignificant excess of the at-risk haplotype (C-G) for SNPs 20649 C>T and 22510 C>G in affected offspring was observed (Table 2).

We further explored the potential contribution of SLC6A14 gene variants in food intake behavior as this gene encodes for an amino acid transporter that may affect tryptophan availability for serotonin synthesis. Serotonin is a neurotransmitter involved in a wide spectrum of behaviors, including feeding, via serotonin receptor signaling (4,5). To investigate this further we examined the relationship between the three scores of the Three-Factor Eating Questionnaire (TFEQ) (9) and the SLC6A14 gene variants in the obese cohort. We analyzed men and women separately because these scores are very sex dependent. SLC6A14 gene SNPs were associated with two scores of the TFEQ in obese women who had BMIs between 30 and 40 kg/m², but not in morbidly obese women with BMI >40 kg/m². Moderately obese women who were homozygous for the at-risk allele of SNP 22510 C>G showed higher Z scores of BMI (corrected by age and sex [zBMI]) and hunger and disinhibition scores (P = 0.003, P = 0.05, P = 0.035, respectively) (Fig. 1A and B). A similar result was observed between TFEQ scores and SNP 20649 C>T (P = 0.009, P = 0.038, P = 0.014, for zBMI and hunger and disinhibition scores, respectively; data not shown). No association was observed in either the total obese adult group or in the obese adult men subgroup. In children, the...
TFEQ is not a validated questionnaire. However, we observed a possible association between SNP 22510 C>G and subjective sensation of satiety during meals in obese girls, accessed by an in-house questionnaire administrated by a trained physician. Indeed, 20.5% of GG carriers (n = 78), 9.6% of GC carriers (n = 115), and 4.9% of CC carriers (n = 41) answered that they lack the feeling of satiety that occurs during a usual meal (data not shown; Fisher’s exact test, P = 0.021 and P = 0.008, under a dominant model). Furthermore, 94.9% of GG carriers versus 85.4% of CC carriers showed a BMI >90th percentile (P = 0.06 and P = 0.03 under dominant model; data not shown), suggesting that this SNP could be associated with a more severe phenotype in obese girls.

The present case-control study confirms the Finnish association of the SLC6A14 gene with obesity. In line with the weak evidence of linkage in the SLC6A14 gene region, no strong familial association was observed. However, the excess of the at-risk allele C of SNP 20649 C>T that was seen in the affected offspring in the pedigree disequilibrium test suggests that the observed association does not result from a stratification bias. It may also indicate that other unknown SNPs located in the same locus may also participate in the genetic risk for obesity. Although we believe that the contribution of the SLC6A14 gene locus to the genetic background of common obesity is confirmed by the present study performed in the more admixed French population, further studies will be required to assess the functional effect, if any, of SNPs 20649 C>T and 22510 C>G. In this regard, it will be useful to compare the gene SNP haplotype structure in the two populations to firmly identify the truly functional SNP (or a combination of several SNPs associated with obesity).

The mechanism by which SLC6A14 may participate in obesity and possibly in the control of food intake behavior is still uncertain. Genes involved in the serotonin signaling pathway have been previously associated (10,11) with several obesity-related phenotypes. Here, the presence of an association between 22510 C>G and the disinhibition and hunger scores of the validated TFEQ questionnaire, although modest and only observed in moderately obese women, might suggest a role of the SLC6A14 gene on food intake behavior. Disinhibition defines the loss of food intake control under a variety of situations and is well correlated to binge eating and overeating in nonobese and obese subjects (12,13). The questions relative to the perception of satiety we used in children have yet to be validated and thus might be taken with caution, but the fact that a subjective lack of satiety perception is observed more in GG obese girl carriers might suggest a role of SLC6A14 on several other dimensions of food intake behavior. A more detailed analysis of food intake behavior in variant allele carriers might suggest a role of SLC6A14 on several other dimensions of food intake behavior. A more detailed analysis of food intake behavior in variant allele carriers is thus warranted. In our study, why no phenotypic effect on food intake could be identified with variants of this chromosome X gene in males remains to be elucidated, and further analyses of additional populations are necessary to fully address this issue.

In conclusion, Finnish data, together with the present study, suggest that the SLC6A14 gene locus may be part of the genetic susceptibility for common obesity.
RESEARCH DESIGN AND METHODS

Association studies of the SLC6A14 gene were performed using a set of 1,267 unrelated obese patients (mean BMI 41.3 ± 8.55 kg/m² and age 48 ± 13 years, 835 women and 432 men). In addition, SLC6A14 SNPs were genotyped in 326 French-Caucasian obese children (BMI ≥ 97th percentile, mean zBMI 4.06 ± 1.23, age 11.1 ± 3.13 years, 166 girls and 160 boys). We used 649 unrelated nonobese and normoglycemic subjects as our control group (mean BMI 22.6 ± 2.7 kg/m², age 58 ± 11 years, 380 women and 269 men), including 376 unrelated nonobese and normoglycemic husbands and wives from type 2 diabetic families and a second set of 273 unrelated nonobese and normoglycemic subjects selected from 294 families from a general population recruited on a geographical basis in two towns in northern France (the so-called Fleurbaix-Laventie study). The familial association test was performed using a set of 188 nuclear families who were previously described (1). In obese adults, parameters of food-eating behavior were assessed by the TFEQ (9), which evaluates the cognitive restraint of eating, disinhibition, and hunger. The range of scores for hunger was 1–14, for disinhibition 1–18, and for restraint 1–21. Because the TFEQ is not a validated questionnaire in obese children, we asked several short questions related to food intake behavior during the clinical investigation of young obese children (234 obese girls and 231 obese boys). Eight questions were asked by a trained physician or a dietician. They were related to food intake behavior during a meal (presence or absence of hyperphagia and rapidity of food ingestion) and between meals (presence or absence of nibbling). Among the eight questions on this in-house questionnaire, one was related to the sensation of satiety after a meal.

Genotyping. The two SNPs were genotyped with the LightCycler and the LightTyper assays (Roche Diagnostics, Basel, Switzerland), based on hybridization probes and fluorescence resonance energy transfer between fluorescein and LC Red 640 (Roche Diagnostics) (14). The conditions are available from the authors.

Statistical analysis. Fisher’s exact test was applied to compare allelic frequencies between case and control subjects (http://www.nugenob.com) and a Medical Research Council grant (G 0000477).

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REFERENCES