RESEARCH NOTE

Common variants in the gene for the serotonin receptor 6 (*HTR6*) do not contribute to obesity

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Introduction

We selected *HTR6* (serotonin receptor 6) as a candidate gene to test for associations with obesity since earlier studies have shown that mice with a disrupted serotonin receptor are less prone to become obese on a high-fat diet. We genotyped three tagSNPs (rs6658108, rs6699866 and rs9659997) and included one multimarker prediction test to cover the genetic information of the entire gene in our Belgian study population (1089 obese cases and 308 lean controls). Statistical analysis revealed no significant associations with obesity for all variants that were tested. Our data therefore indicate that common *HTR6* variants do not contribute to obesity in the tested population.

Obesity has become a worldwide health problem in the past decades. This disease is not only caused by a Westernized diet and a sedentary life style, but also by genetic factors as proven by twin, adoption and family studies. These studies have shown that genetic factors can explain up to 90% of variance in body mass index (BMI) (reviewed in Maes *et al*. 1997). Recent genome-wide association studies (GWAS) have identified a number of gene polymorphisms that modify BMI or the amount of body fat (Frayling *et al*. 2007; Chambers *et al*. 2008; Loos *et al*. 2008; Thorleifsson *et al*. 2009; Willer *et al*. 2009). Nevertheless, the combined effect of these variants accounts for only a small percentage of the variation in adiposity (Loos *et al*. 2008; Thorleifsson *et al*. 2009) suggesting that more obesity genes remain to be discovered. Alternatively, candidate gene studies have also been successful in discovering obesity genes (Yang *et al*. 2007). We consider the serotonin receptor 6 gene (*HTR6*) as a good candidate gene for obesity since earlier studies in mice have reported that disruption of the serotonin receptor 6 results in reduced sensitivity to diet-induced obesity (Frassetto *et al*. 2008). Furthermore, several anti-obesity drugs (fenfluramine, dexfenfluramine and sibutramine) target the serotonin system. Thus, we hypothesized that genetic variation in *HTR6* could have an effect on adiposity in response to a Westernized (high fat) diet and we initiated a case–control association study to test this assumption in humans.

Materials and methods

All subjects included in this study were of Belgian Caucasian origin, older than 20 years with Belgian Caucasian parents and at enrolment none were involved in an ongoing weight management programme. A total of 1089 obese patients were recruited in chronological order from the weight management clinic of the Antwerp University Hospital and had a body mass index (BMI) $\geq$ 30 kg/m^2. Patients were referred to clinic by their general practitioner or by another specialist or came on their own initiative and were not participating in a structured weight loss programme at the time of enrolment. Post-menopausal women as well as patients with diabetes or impaired glucose tolerance, on the basis of an oral glucose tolerance test (OGTT) and according to the WHO criteria (Alberti and Zimmet 1998; DeFronzo and Ferrannini 1991), were excluded from the study. A total of 308 control individuals were healthy volunteers with a BMI between 18.5 and 25 kg/m^2, recruited among employees from the Antwerp University Hospital and the Department of Medical Genetics. All subjects had given their written informed consent before participation and the study protocol was approved by the ethics committee of the Antwerp University Hospital.

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BMI was calculated as weight (in kg) over height (in m) squared. Waist circumference was measured at mid-level between the lower rib margin and the iliac crest, and hip circumference at the level of the trochanter major and the waist-to-hip ratio (WHR) was calculated. Visceral (VFA), subcutaneous (SFA) and total abdominal (TFA) fat areas were determined with a computerized tomography (CT) scan as previously described (Peeters *et al*. 2008). We have 80% statistical power to detect associations of single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) of 0.20 and a genotype relative risk of 1.3 (CaTS power calculator Skol *et al*. 2006). Maximum coverage of the genetic information while genotyping as few SNPs as possible was achieved by selecting tagSNPs from HapMap (http://www.hapmap.org/) (Frazer *et al*. 2007) with the Haploview software (Barrett *et al*. 2005). Aggressive Tagger analysis of 2-marker and 3-marker haplotypes with r^2 and LOD thresholds at 0.8 and 3.0, respectively (deBakker *et al*. 2005), indicated that genotyping only three SNPs (rs6658108, rs6699866 and rs9659997) and performing one multimarker prediction test would capture the information of all 10 known *HTR6* SNPs with a MAF > 5%. The CA haplotype of rs9659997 and rs6699866 captures the minor alleles of rs2872594 and rs4912138. LightSNiP assays (TIB-MolBiol, Berlin, Germany) were run in 5 μL reactions on a LightCycler 480 Real-Time PCR system (Roche, Penzberg, Germany) as described previously (Peeters *et al*. 2008). All LightCycler runs included blank samples as negative controls and samples with known genotype as positive controls. Analysis results of duplicate samples (6% of total) were 100% concordant.

Genotypes of rs2872594 and rs4912138 were imputed using PLINK software (Purcell *et al*. 2007). All results were checked for deviations from Hardy–Weinberg equilibrium (HWE) with a 0.01 cut-off *P* value. Genotype distribution differences between cases and controls were evaluated by chi-square analysis and odds ratios (OR) calculated by univariate logistic regression under an additive, dominant and recessive model with correction for age and gender. Associations between *HTR6* genotype and selected obesity quantitative traits in an additive model were evaluated with a Kruskal–Wallis test on data adjusted for age and BMI. Differences between mean values of these parameters, for a dominant or recessive model, were evaluated by Wilcoxon Rank-Sum tests on studentized residuals corrected for age and BMI. Quantitative traits were adjusted for age and BMI by linear regression. The probability of type I multiple testing errors was contained by controlling the false discovery rate (FDR). All statistical analyses were performed using SPSS version 15.0 (SPSS, Chicago, USA).

Results

Characteristics of the study population are shown in table 1. A total of 1089 obese patients and 308 lean control individuals were genotyped for *HTR6* variants rs6658108, rs6699866 and rs9659997 (table 2). The minor allele frequency (MAF) of these SNPs in our control population was 0.14, 0.20 and 0.37, respectively. Using the genotypes of rs9659997 and rs6699866, we could impute the genotypes of rs2872594 and rs4912138. As these two SNPs are in 100% linkage disequilibrium (LD), only the results for rs4912138 are shown. MAF for the imputed SNPs was 0.20 in our control population. All SNPs analysed were in HWE in both obese and control group (all $P > 0.01$; data not shown). Chi-square analysis indicated that the genotype distribution of the SNPs was the same for cases and controls (table 2). Also, none of the four variants contribute to the risk for obesity as the *P* values of the calculated odds ratios (ORs) were not significant for any of the models tested (table 2). We analysed the obesity parameters waist, WHR, TFA, VFA and SFA in the obese population for association with the tested SNPs to investigate whether a link with fat distribution is present and found no significant effect of *HTR6* genotype on any of these parameters after correcting for multiple testing (see table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). After a gender-specific subgroup analysis, we found a trend for association of rs6699866 and rs4912138 with waist in women. However, these associations do not stand upon correction for multiple comparisons (data not shown).

Table 1. Description of the study population.

	Normal weight (n = 308)	Obese (n = 1089)
Age (years)	36 (21–66)	42 (21–81)
Males/females	97/211	474/615
Weight (kg)	64.0 ± 7.7	110.7 ± 21.6
Height (cm)	168.3 ± 9.7	170.0 ± 9.6
BMI (kg/m^2)	22.1 ± 1.7	38.2 ± 6.2

Results are presented as mean ± standard deviation except for age, which is shown as median (range).

Discussion

We investigated whether polymorphisms in the *HTR6* gene contribute to obesity in a Belgian study population and found no significant differences between cases and controls in the genotype distributions of the analysed SNPs nor in the risk for obesity (OR not significant). *HTR6* genotype also had no significant impact on the mean values of five obesity quantitative traits in obese subjects. Although population stratification could explain the apparent lack of association, it is improbable since the occurrence of population structure in our study group has previously been ruled out (Peeters *et al*. 2008). A false negative result, then, is unlikely in view of the fact that we have sufficient statistical power to detect such associations. Nevertheless, gene variants with effect sizes below the detection limit of our study could be overlooked as they can only be picked up with a larger population sample size. Furthermore, it might still be possible that *HTR6* variants are associated with obesity in other populations due to

Table 2. Association analysis of *HTR6* variants with obesity risk.

Variant				MAF	χ^2 (P)*	Additive model	Dominant model	Recessive model
Rs66584108	G/G (%)	A/G (%)	A/A (%)			GG+AG+AA	GG vs. AG+AA	GG+AG vs. AA
Controls (n = 308)	226 (73.4)	76 (24.7)	6 (1.9)	0.14	0.93 (0.63)	P = 0.40	P = 0.37	P = 0.53
Cases (n = 1089)	779 (71.5)	279 (25.6)	31 (2.8)	0.16				
Rs6699866	C/C (%)	A/C (%)	A/A (%)			CC+AC+AA	CC vs. AC+AA	CC+AC vs. AA
Controls (n = 308)	193 (62.7)	104 (33.8)	11 (3.6)	0.20	0.071 (0.97)	P = 0.93	P = 0.90	P = 0.87
Cases (n = 1089)	688 (63.2)	360 (33.1)	41 (3.8)	0.20				
Rs9659997	T/T (%)	C/T (%)	C/C (%)			TT+CT+CC	TT vs. CT+CC	TT+CT vs. CC
Controls (n = 308)	123 (39.9)	145 (47.1)	40 (13.0)	0.37	0.80 (0.67)	P = 0.38	P = 0.42	P = 0.41
Cases (n = 1089)	407 (37.4)	526 (48.3)	156 (14.3)	0.38				
Rs4912138	G/G (%)	G/A (%)	A/A (%)			GG+GA+AA	GG vs. GA+AA	GG+GA vs. AA
Controls (n = 308)	194 (63.0)	103 (33.4)	11 (3.6)	0.20	0.176 (0.92)	P = 0.68	P = 0.68	P = 0.88
Cases (n = 1089)	700 (64.3)	352 (32.3)	37 (3.4)	0.20				

Number of cases and controls are given for each genotype group. MAF, minor allele frequency. χ^2 (P) for comparison of genotypes (2 degrees of freedom).

allelic heterogeneity. This should be investigated in replication studies in populations of a different ethnic origin. We thus conclude that genetic variation in *HTR6* does not play a major role in common obesity in a Belgian Caucasian population.

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