Further Evidence for a QTL Influencing Body Mass Index on Chromosome 7p from a Genome-wide Scan in Dutch Families

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Obesity is a rapidly growing threat to public health, driven by the increased occurrence of high caloric diets and sedentary lifestyles. Within this environment, genetic influences may largely determine inter-individual differences in obesity-related traits. To map genes involved in weight regulation, we performed a genome-wide linkage scan for body mass index (BMI), a reliable measure of total body fat, in 192 Dutch families including 315 twins and 210 siblings with data on BMI. Using variance components linkage analysis, regions with LOD-scores greater than 2 were observed on 6p25.1 (LOD-score, 2.13) and 7p21.1 (LOD-score, 2.40). LOD-scores higher than 1 were found on chromosomes 3, 13, 15 and 21. Of note, evidence for the putative quantitative trait locus for BMI on 7p was obtained previously from such diverse populations as Mexican-Americans, Asians and Nigerians, suggesting that the underlying genes may effect weight regulation in diverse environments. An obvious positional candidate in the 7p linkage region is the gene encoding neuropeptide Y (*NPY*) that controls satiety and food intake.

In addition to having a direct adverse effect on physical and mental well-being, obesity is a major risk factor for diverse clinical outcomes including diabetes type II, hypertension, coronary heart disease, osteoarthritis and various forms of cancer (Pi-Sunyer, 2002; Bianchini et al., 2002). During the past decade, the prevalence of obesity has been growing rapidly worldwide (World Health Organization, 2003). This increased prevalence is commonly attributed to the availability of high caloric diets and sedentary lifestyles, particularly in western societies. Not everyone is similarly affected by these recently arisen environmental circumstances, which may be explained by genetic influences. Body mass index (BMI) is a simple measure closely correlated with total body fat (Borecki et al., 1991). Inter-individual differences in BMI can be attributed to genetic influences for 35–55% according to family studies (Rice et al., 1999; Fuentes et al., 2002) and 65–85% according to twin studies (Schousboe et al., 2003). The latter study design may be more sensitive in the presence of allelic and gene–gene interactions and does not suffer from residual confounding effects of age.

Although the relevance of genetic influences to variation in BMI is undisputed and genes underlying rare Mendelian forms of obesity have been identified (Chagnon et al., 2003), knowledge about genes regulating body weight in the population at large is still limited. Knowledge of these genes may reveal biological pathways that are critical to the development of obesity through interaction with present-day environmental factors. A common strategy to map and subsequently positionally clone such genes is a genome-wide linkage scan. We adopted this strategy in order to map quantitative trait loci (QTLs) affecting BMI in a study among 192 families comprising 803 individuals from the Netherlands Twin Registry.

Materials and Methods

Participants and Phenotyping

In 1991, the Netherlands Twin Register (NTR) started a longitudinal survey study of health and lifestyle (Boomsma et al., 2002). To this aim, questionnaires were sent out in 1991, 1993, 1995, 1997, and 2000 to adolescent and adult twins and their family members. Twin pairs were asked to participate in all waves; parents were asked to participate in 1991, 1993 and 1995; siblings and spouses were included since 1995 and 2000, respectively.

In the present study, we used BMI as a measure of adiposity since it is highly correlated with other measures of fat mass (Borecki et al., 1991). BMI was

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calculated as weight in kilograms divided by the square of height in meters. Both weight and height data were available for 7509 twins and siblings. They were born between 1909–1982 (median year of birth, 1972). Participants were asked to indicate their weight in all questionnaires and, in addition, weight was measured during a visit to the study center for a subset of participants. Large discrepancies over time or extreme weights were checked and discarded from the analysis if unresolved. For the purpose of linkage analyses, available data on the most recently reported weight of both twins at the same age was used to minimise confounding effects of age. For non-twin offspring, the most recent weight was always used. Only weights reported at ages of 20 years and older were considered in the analysis. As the correlation between measured and self-reported weight is high within the Netherlands Twin Registry (0.92), we did not distinguish between self-reported (95.2% of data) and measured weight (4.8%).

To establish height, all survey data collected between 1991 and 2000 at ages of 20 years and older were used. Most individuals completed questionnaires in more than one survey and the mean reported height was used for calculating BMI. Differences in height across questionnaires were checked and height data were discarded when there was no consistency across questionnaires and when differences were larger than 5 cm (1.4% of total sample). In a subset, height was also measured at the study center. When measured height was available at the age of 20 years or older (12% of data), this was used in the analyses instead of self-reported height. The correlation between self-reported and measured height for this sample is .93.

A genome-wide scan was performed on a subset of the Netherlands Twin Registry. The original aim of this scan was to map quantitative trait loci for anxious depression. To this end families with sibling pairs who scored either extremely discordant or concordant for anxious depression-related traits were selected (Boomsma et al., 2000). The selected individuals (n = 2724) were asked to provide a buccal swab for DNA extraction. Of the 1962 (72%) who returned a buccal swab, 917 individuals were selected for genotyping on the basis of belonging to families with larger sib ships and available amount of genomic DNA. After excluding 1 individual with an excessive genotyping error rate, 2 individuals with uncertain identities and 2 families with systematic Mendelian errors that could not be resolved, 192 families remained for which data on BMI were available for two or more offspring. These families comprised 525 offspring with data on BMI of which 315 were twins and 210 additional siblings, 293 twins were dizygotic and 22 twins from separate families were monozygotic and formed a sibling pair with a non-twin sib. In addition, 254 parents and 24 additional offspring were genotyped for whom no BMI data were available. The latter were included in the analysis to improve the estimate of the proportion of alleles shared identical-by-descent (IBD).

DNA Collection, Genotyping and Error Checking

Details on the collection of DNA have been described elsewhere (Boomsma et al., 2002; Meulenbelt et al., 1995). Genotyping was conducted by the Mammalian Genotyping Service of the Center of Medical Genetics, Marshfield, WI, USA, using the 10 cM spaced short tandem repeat polymorphism screening set 10 (Yuan et al., 1997). On the autosomes, 379 markers were measured and the genotyping success rate was 92.0% (SD = 0.17%). Pedigrees were checked for Mendelian errors with the software Unknown (Schaffer, 1996) and familial relationships in the entire sample with the GRR software (Abecasis et al., 2001). Mendelian errors were removed by assigning missing values to the genotypes if the errors appeared incidental. Unlikely double recombinants were identified using Merlin (Abecasis et al., 2002). Excessive recombination was observed for five markers suggesting a systematic error. These markers were not included in the final analyses: two markers on chromosome 1 (D1S468 and D1S1627), two markers in a group of five very closely mapped markers on chromosome 11 (D11S1985 and D11S2006), and one marker on chromosome 20 (D20S159). All other unlikely genotypes were removed from the data set using the pedwipe procedure from Merlin.

Statistics

Multipoint IBD-estimation and variance components linkage analysis that included covariates was carried out using Merlin 0.9.12 (Abecasis et al., 2002). The distribution of BMI was slightly positively skewed and transformed with the natural logarithm to obtain a normal distribution. Sex, age, squared age and cubic age were significantly associated with BMI and included as covariates in the linkage analysis. Allele frequencies were estimated using all individuals for estimation of the proportion of alleles shared IBD (Broman, 2001). Marker positions were taken from the Decode map if available (Kong et al., 2002). For markers not mapped by Decode, the position was taken from the Marshfield map (Broman, 2001) and the position on the Decode map was estimated using linear interpolation from adjacent markers with known Decode map positions. Since Merlin uses the Haldane mapping function, Kosambi cMs were transformed to Haldane cMs prior to IBD-estimation. IBD was estimated every 4 Haldane cM. For presentation purposes, Haldane cMs were transformed back to Kosambi cMs after the linkage analysis. The 1-LOD-drop support interval was used as an estimate for the 90% confidence interval of the location of the genetic variation underlying mapped QTLs (Dupuis & Siegmund, 1999).

Results

A total of 192 two-generation families comprising 803 individuals were studied. For 525 offspring (315

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Table 1

Characteristics of Twins and Siblings from the Netherlands Twin Registry with Data on BMI who Were and Were not Part of the Genome-wide Scan

Characteristic	Genotyped		Not genotyped	
N	525		7509	
Male (%)	217	(41.3)	2959	(39.4)
Mean age (*SD*)	32.6	(11.9)	29.7	(10.6)
Mean BMI (*SD*)	23.6	(3.8)	22.9	(3.4)

twins and 210 sibs), data on BMI were available (mean size sib ship = 2.7). The mean BMI of individuals in the genome-wide scan (23.6 kg/m^2) was 0.72 kg/m^2 greater than of those who were not (N = 7509; 22.9 kg/m^2; Table 1). After adjustment for age and sex, only a minor difference in mean BMI remained (0.35 kg/m^2; p = .014).

The heritability of BMI was estimated at .79 after adjustment for age and sex, which is consistent with a previous report on this population (Schousboe et al., 2003). The result of the autosomal genome scan for QTLs affecting BMI is presented in Figure 1. Regions with LOD-scores greater than 2 were found on chromosome 6p25.1 at marker SE30 (maximum LOD score [MLS] = 2.13) and on chromosome 7p21.1 at marker D7S1802 (MLS = 2.40). Figure 2 depicts the chromosome 6 and 7 results in more detail. On chromosome 6, the 1-LOD-support interval was flanked by markers D6S942 and D6S1006 covering the region from 0 to 30.2 cM (approximately 12.9 mb). On chromosome 7, markers D7S3051 and D7S1808 encompassed the 1-LOD-drop support interval extending from 32.3 to 43.8 cM (approximately 9.8 mb).

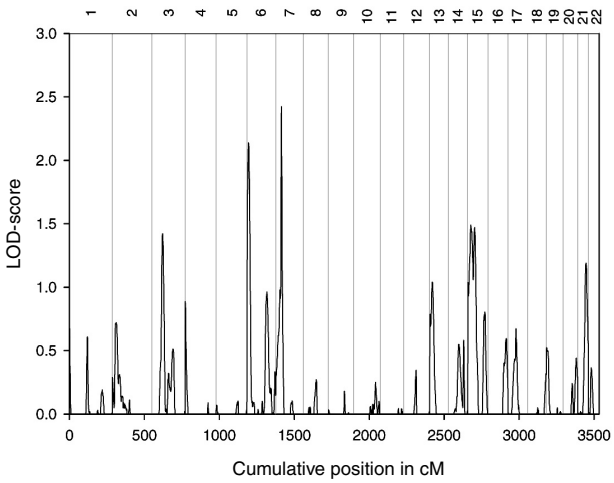

Figure 1

Result of genome-wide linkage scan for BMI in 192 Dutch families.

LOD-scores between 1.0 and 1.5 were observed on chromosomes 3 (MLS = 1.42 near D3S2409), 13 (MLS = 1.04 between D13S787 and ATA5A09), 15 (MLS = 1.49 near D15S165), and 21 (MLS = 1.19 between D21S2055 and D21S1411).

Discussion

In a genome-wide scan for QTLs influencing BMI, we observed LOD-scores greater than 2 on chromosomes 6p25.1 and 7p21.1. Importantly, linkage with BMI on chromosome 7p has now been replicated in families from various countries: Dutch (LOD-score = 2.40; this study), Mexican-American (p = .042 in a linkage analysis of candidate regions; Bray et al., 1999), Chinese and

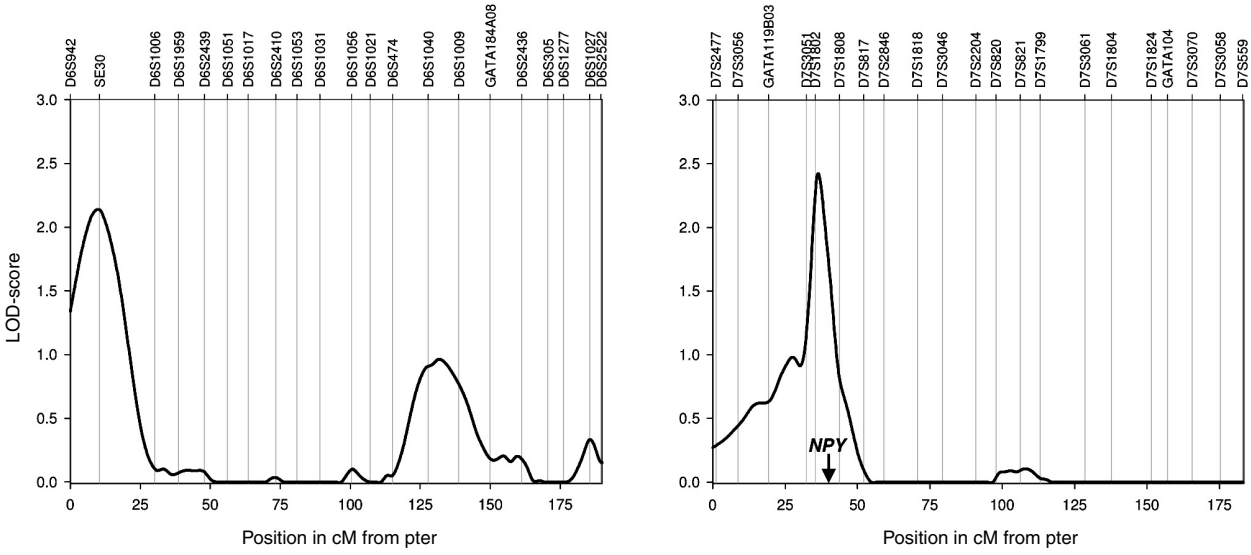

Figure 2

Linkage with BMI observed on chromosomes 6 (left) and 7 (right).

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Japanese (LOD-score = 2.66; Wu et al., 2002) and Nigerians (LOD-score = 3.07; Adeyemo et al., 2003). In addition, linkage with fat-free mass was observed in the same chromosomal region among French Canadians (LOD-score = 2.72; Chagnon et al., 2000). Hence, the putative BMI QTL on 7p may be common to diverse ethnic populations and might exert its effect on obesity in diverse environments.

The most obvious positional candidate in the linkage region is the gene encoding neuropeptide Y (NPY) and maps within the 1-LOD-drop support interval of our study (Figure 2). NPY is released from the hypothalamus in response to fasting and hypoglycaemia and undergoes feedback inhibition after food intake (Jequier & Tappy, 1999). NPY thus regulates food intake, as is consistent with the observation that infusion of NPY in the central nervous system of animal models induces binge eating (Stanley et al., 1986). Indicative of NPY's relevance to human obesity is the association of the –880I/D (Bray et al., 2000) and Leu7Pro (Mattevi et al., 2002) gene variants with BMI. Application of combined linkage/association analysis (Fulker et al., 1999; Beekman et al., 2004) will allow testing whether genetic variation at the NPY locus indeed contributed to the linkage observed on 7p or that other genes are involved.

Given the multifactorial nature of human obesity, other chromosomal regions can be assumed to harbour additional genes influencing BMI. The limited statistical power in genome-wide scans, including ours, means that QTLs will be missed and absence of replication across studies can be expected even for true effects. Replicated linkage with BMI, which we did not detect in our study, was reported for chromosome 7q (LOD-scores of 2.36 [Feitosa et al., 2002]), 4.7 and 3.2 ([Wu et al., 2002]) and 2 ([Arya et al., 2004)]). The leptin gene (LEP), a satiety factor (Jequier et al., 1999), is an obvious candidate in this chromosomal region. Recently, genetic variation in the gene encoding glutamic acid decarboxylase (GAD2) was found to contribute to the reported linkage with morbid obesity on 10p11-12 and was associated with obesity and higher scores for hunger and disinhibition (Boutin et al., 2003). Interestingly, the enzyme encoded by GAD2 catalyses the formation of γ-aminobutyric acid (GABA), which is suggested to interact with neuropeptide Y to stimulate food intake (Ovesjo et al., 2001). If the role of genetic variation in NPY, LEP and GAD2 in human obesity is confirmed in more extensive studies, it is appealing to speculate that dysregulation of physiological signals to control food intake is a key to understanding the increasing prevalence of obesity in societies where high caloric foods are abundant and readily available.

In addition to chromosome 7p, a LOD-score greater than 2 was observed on 6p in our study. No obvious candidate genes have yet been located in this linkage region (Chagnon et al., 2003). Linkage with BMI on 6p has not been found by other genome-wide scans. It may, therefore, be that we identified a new BMI QTL or, alternatively, that our finding is a false positive signal. Given the relatively large gap between the marker showing the maximum LOD-score and the adjacent marker qter, genotyping additional markers may assist in discriminating between these alternative explanations.

In conclusion, BMI is a highly heritable trait and we obtained further evidence that chromosome 7p harbours a gene or multiple genes affecting this trait in man. In order to obtain a more complete map of QTLs influencing BMI, it will be necessary to combine the data from multiple genome-wide scans.

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