No Linkage to Obesity in Candidate Regions of Chromosome 2 and 10 in a Selected Sample of Swedish Twins

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The aim of the current study was to investigate the importance of genetic and environmental effects in the variation of body mass index, and to investigate linkage for obesity to previously reported candidate regions on chromosome 2 and 10. A sample of 1422 twin pairs from the population based Swedish Twin Registry was used in order to estimate the genetic and environmental effects in the variation of body mass index by means of structural equation modeling. A selection of those, 51 concordant and 155 discordant for obesity, was used for the linkage analysis by implementing the "combined" Haseman-Elston approach. Heritability of body mass index ranged from 59–70%, implying that genetic effects were of importance for the variation of obesity, and there were significant sex and age differences. Linkage could not be verified in candidate regions of chromosomes 2 and 10, indicating that these genetic variants have a significant effect in extreme obese populations rather than in moderately obese Caucasians. However, the results were sensitive to issues related to power, minor effects of the genes, ethnic differences and the complex mechanism underlying obesity.

The complexity of the genetic architecture for human obesity, as well as the impact of several environmental factors, has made gene detection or polymorphism localization a very challenging task. Reviews of linkage and association studies (Comuzzie & Allison, 1998; Rankinen et al., 2002) showed that putative loci affecting obesity and obesity-related phenotypes have been revealed on all human chromosomes but Y. Many genetic findings have not been replicated and others have been associated with different phenotypes in different studies (Rankinen et al., 2002).

A review of twin, family and adoption studies showed estimates of heritability for body mass index (BMI), defined as weight in kilograms divided by the square of the height in meters, to be between 50% and 90% highlighting the importance of genetic factors to the variation in BMI (Maes et al., 1997). Furthermore, they reported a very low correlation between children and adoptive parents indicating the minor role of cultural transmission (adoptive children share the environment with their adoptive parents in contrast to their biological parents with whom they share genes).

Scanning the whole genome for genes affecting obesity related phenotypes, loci on chromosome 2 and 10 showed the strongest evidence for linkage in an affected sib-pair sample of extremely obese French individuals (Hager et al., 1998). The quantitative trait locus (QTL) on chromosome 2 was evidently linked to serum leptin levels and suggestively linked to body fat in family studies including extremely obese Mexican Americans and African-Americans (Comuzzie et al., 1997; Rotimi et al., 1999). Subsequent QTL studies on independent populations have also confirmed linkage to obesity on chromosome 10 (Hinney et al., 2000; Price et al., 2001).

The aim of the current study was to estimate the genetic and environmental effects in the variation of BMI in a sample of twin pairs from the population-based Swedish Twin Registry. Further, we wanted to investigate whether or not the reported obesity candidate loci on chromosome 2 and 10 are linked to BMI among obese twin pairs selected from the sample of twins. We used a selected sample, based on their phenotypic values, including extreme concordant and discordant dizygotic (DZ) twins, in order to maximize the power of detecting linkage (Gu et al., 1996; Gu et al., 1997; Risch & Zhang, 1995; Risch & Zhang, 1996; Zhang & Risch, 1996). DZ twins share on average half of their segregating genes making them an equally good sample for linkage analysis as ordinary siblings with the major advantage of twins being age matched.

Methods

Subjects

The study comprises twins from four sub-studies based on the Swedish Twin Registry (Lichtenstein et al., 2002): SATSA (Swedish Adoption Twin Study of Aging; Pedersen et al., 1984), OCTO-twin (Berg et al., 1992), GENDER (Malmberg et al., 1995) and the SALT (Screening Across Lifespan Twin)-pilot study (Lichtenstein et al., 2002). SATSA consist of twins that were above the age of 50 when contacted and were identified as having been reared apart,

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along with age and sex-matched pairs of twins who had been reared together (Pedersen et al., 1984). SATSA is a longitudinal study with a 3-year interval between measurement occasions, each wave containing both questionnaire and in-person-testing components. Phenotypic data used in this study were taken from the third in-person testing and the sample comprises 246 twin pairs. A more detailed description of collection procedures in the SATSA study has been given elsewhere (Pedersen et al., 1991). The OCTO-twin (Berg et al., 1992) comprises twin pairs that were above the age of 80 during the 3-year period of data collection. Phenotypic data were available from 351 twin pairs. The GENDER study comprises 249 opposite-sex twin pairs that were between the ages of 70 and 79 during data collection (Malmberg et al., 1995). A questionnaire and a health assessment were collected in order to explore sex differences in health and aging. Finally, 576 twin pairs were included in the SALT-pilot study (Lichtenstein et al., 2002), which aimed at screening all major complex diseases. Twins aged from 17 years and above were randomly chosen from the Swedish Twin Registry. They were contacted for a computer assisted telephone interview, concerning health status and were also asked to go to their local nurses' office for blood sampling.

In the total material blood was available from 246 pairs from SATSA, 289 from OCTO-twin, 246 from GENDER and 257 twin pairs from the SALT-pilot study. Fifteen individuals had participated in more than one sub-study of the above mentioned; therefore data from only one of the occasions was randomly selected for the analysis. Sixteen twin pairs had unknown zygosity and were therefore excluded. For 59 twin pairs phenotypic information was only available from one twin and for 25 pairs phenotypic information was missing from both individuals in the twin pair.

Informed consent was obtained from all subjects, in all four sub-studies. These studies were approved by the Ethics Committee of the Karolinska Institute, the Swedish National Data Inspection Authority and the IRB of the Pennsylvania State University.

BMI

BMI was measured by nurses in the SATSA, OCTO-twin and GENDER. In the SALT-pilot study it was either measured or self-reported from questionnaire where measured was missing (38.5%). However, the correlation between self-reported BMI and measured was $r = 0.95$ ($p < .0001$) for the sample of twins in the SALT-pilot study where both measures of BMI were available ($N = 702$). For the linkage study the BMI cut-off was chosen to be 29 kg/m^2 for both males and females, because it was approximately the 10th-percentile of the BMI distribution. Risch and Zhang (1995) proposed the 10th-percentile as cut-off point in selecting extreme concordant and discordant pairs. The absolute value of the difference in BMI within twin pairs had a lognormal distribution with an estimated mean of 3. Extreme concordant obese pairs were selected as those pairs where at least one twin had a BMI above the cut-off and the pair's difference in BMI was less than or equal to 3. Accordingly, discordant obese twin pairs were selected as those pairs with at least one twin above the cut-off and an intra-pair difference greater than 3. That is, the discordant sample included pairs where one twin had a BMI above the top 10th-percentile and the co-twin had a BMI below the bottom 30th-percentile of the BMI distribution. This amounted to a total of 99 concordant and 242 discordant DZ twin pairs. Genotypes were available from 51 concordant and 155 discordant pairs due to depleted blood samples for some pairs.

Age

The age distribution of the entire twin sample was skewed. Therefore subsequent analysis was done in three age groups 17–49, 50–69 and 70–94. The youngest age for the SATSA sample was 50, so that age was chosen as a cut-off point for the first age group. The GENDER study comprises opposite-sex twin pairs above the age of 70. With that in mind, 70 years of age was chosen as an arbitrary cut-off for the older age group.

Sub-studies

Differences in mean values and variances in BMI between sub-studies by age group were tested (PROC GLM in SAS). There were no systematic differences; therefore further statistical analysis proceeded as a joint analysis of the sub-studies.

Genotyping

Genomic DNA was isolated from peripheral leucocytes using standard methods and genotyped by PCR for informative simple sequence length polymorphism markers covering the previously reported chromosome 2 and chromosome 10 quantitative trait loci (QTL) (Comuzzie et al., 1997; Hager et al., 1998). For the reported chromosome 2 locus (*D2S367*), 5 markers were selected including *D2S352, D2S367, D2S177, D2S119* and *D2S2291*. Observed heterozygosity ranged from 0.76 to 0.86. The interval covers a region of approximately 16 cM, giving an average marker density of 3.9 cM and a maximum inter-marker distance of 5.9 cM.

For the reported chromosome 10 locus (*D10S197*), 8 markers were selected including *D10S1714, D10S211, D10S1662, D10S586, D10S572, D10S197, D10S588* and *D10S193*. Observed heterozygosity ranged from 0.73–0.88 except for marker *D10S586* that had an observed heterozygosity of 0.46. This region is located on the short arm of the chromosome 10 encompassing a genetic distance of approximately 14 cM, and it corresponds to an average marker density of 2 cM and a maximum inter-marker distance of 5.4 cM. Allele frequencies on each marker were estimated from our data. They did not deviate from those reported in the Genome Database (GDB) or deCODE (Kong et al., 2002). Genetic map distances were retrieved from the deCODE high-resolution genetic map of the human genome. All information concerning primer sequences and allele frequencies is publicly available at GDB. Primers were fluorescently labelled for electrophoresis on a laser fluorescent sequencing machine (ABI 377, PE Biosystems, CA, USA). Prior to electrophoresis, the PCR products for each individual genomic DNA sample were pooled according to the emission spectra of the fluorescent dyes and the fluorescence intensity.

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The electrophoresis data were analysed with the GeneScan® 3.1 computer software (PE Biosystems). Analyses and assignment of the marker alleles were done with Genotyper® 2.0 computer software using GeneScan 350 Tamra size standard (PE Biosystems).

Quantitative Genetic Analysis

Twin studies are ideal for estimating genetic and environmental effects of traits and diseases (Martin et al., 1997). Identical or monozygotic (MZ) twins share the same genes whereas fraternal or dizygotic twins (DZ) share on average half of their segregating genes. A broad measure of the similarity between twins is gained from calculations on the intraclass correlations (Neale, 1999). Comparisons between the intraclass correlations for MZ and DZ twins provide information about the effects that are present.

In general, the phenotypic variance is assumed to be due to three latent factors: additive genetic (A), shared environmental (C), and non-shared environmental factors (E, which also includes measurement error):

$$\mathrm{Var}(Y) = A + C + E$$

The correlation in MZ twin pairs is due to additive genetic and shared environmental factors (A + C). The correlation in DZ twins is assumed to be due to the sum of half the genetic, plus shared environmental factors ($^1/_2$ A + C). A genetic effect is indicated if twin similarity is greater among MZ than DZ pairs. Heritability is defined as the proportion of total phenotypic variation directly attributable to genetic effects (Falconer, 1989).

We fitted a series of models in order to test for sex differences (Neale & Martin, 1989). In the constrained model we assumed equal genetic and environmental variance components for males and females. That is, there were no sex differences in the genetic and environmental influences. The next step was to test whether there were sex differences in the relative importance of these effects by assuming one set of parameters for males in both same and opposite-sex twin pairs and similarly another set of parameters for females. This model is called the general sex-limitation model. If the latter model fitted better, a scalar sex-limitation model was tested that evaluated whether sex differences in the total phenotypic variance differed only by a scalar component. That is, the genetic and environmental variance in females is a scalar multiple of that in males, $a_f + c_f + e_f = k(a_m + c_m + e_m)$.

In all models the phenotypic means were adjusted for age. Outliers were identified by using estimated *z*-scores based on the measure of the Mahalanobis distance (Hopper & Mathews, 1982). Pairs with *z*-scores in excess of 2.9 for BMI were excluded from the variance-component analyses. For the models fitted, the degrees of freedom and twice the log-likelihood probability were computed by means of the structural equation-modeling package MX (Neale et al., 1999). Models were applied to raw data and a maximum likelihood approach was used to estimate the genetic and environmental components of variance. To compare two models a likelihood ratio test was used. The difference between twice the log-likelihood can be interpreted as a χ^2 statistic. A significant difference indicates that the model with fewer parameters to be estimated fits data worse.

Linkage Analysis

Linkage analysis of selective samples (extreme concordant and/or discordant pairs) violates important assumptions (i.e., normality) and can produce false positives or elevated type I errors when analysed. This can be overcome, either by incorporating the selection probabilities into the linkage calculations or by incorporating the phenotypic values from the individuals not selected for genotyping (Allison et al., 1999). It has also been shown that in the frame of the variance-components approach, conditioning on trait values leads to a likelihood ratio test that is valid and equal in power to alternative methods for analysing selected samples (Sham et al., 2000).

However, recently Sham and Purcell introduced a simpler alternative approach to variance-components models, the "combined" Haseman-Elston approach (Sham & Purcell, 2001; Sham et al., 2002). According to this, the dependent variable, which is a function of the weighted sum of squared sums and squared differences, is regressed onto the estimated proportion of identity-by-descent (IBD) sharing at a test locus. The weights are the variances of the squared sums and squared differences, respectively, and are expressed as functions of the sibling correlation. That is:

$$\frac{(X+Y)^2}{(1+r)^2} - \frac{(X-Y)^2}{(1-r)^2} + \frac{4r}{1-r^2} = \frac{4(1+r^2)}{(1-r^2)^2} Q\,(\hat{\pi} - .5) + \varepsilon$$

where X and Y are the mean-centred and standardised trait values, r is the sibling correlation of the trait in the population, $\hat{\pi}$ is the proportion of IBD sharing Pr(sharing 2 alleles IBD) + $^1/_2$ Pr(sharing 1 allele IBD), Q is the proportion of phenotypic variance explained by the additive effects of the QTL and ε is the error term. Estimated multipoint probabilities of allele sharing were derived from Merlin at each marker locus (Abecasis et al., 2002). The linkage analysis was done by utilizing the SAS statistical package (PROC REG). Linkage is usually presented in terms of LOD scores (logarithm of the likelihood ratio test for test of the significance of a QTL). A LOD score above 3 is considered as significant linkage (Lander & Kruglyak, 1995). An asymptotic estimate of the LOD score is given by the square of the *t*-value obtained from the "combined" Haseman-Elston regression divided by 2 times the natural logarithm of 10. The t^2 is expected to be distributed as a χ^2 with 1 *df*, as is the maximum likelihood test statistic usually employed in linkage analysis (Fulker & Cherny, 1996). Two times the natural logarithm of 10 is a constant for converting from common (base 10) to natural logarithms.

Genotyping errors and marker mutations often lead to unlikely double recombination in high-resolution maps. This consequently leads to a reduced estimated level of allele sharing IBD between sib pairs, which can profoundly affect linkage information. Therefore, likely genotyping errors and marker mutations were detected by SIBMED (Douglas et al., 2000). It is a multipoint approach based on hidden Markov models and it is designed for sib-pair data when parental genotypes are unavailable. It calculates the posterior probability of genotyping error or mutation for

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each sib-pair-marker combination, conditional on all marker data and an assumed genotype error rate (0.001). Hence, it removes the errors that have the largest impact on linkage results.

Results

Table 1 presents mean and standard deviation of BMI by age group, zygosity and sex in the whole sample of Swedish twins. Overall, men had a higher BMI compared to women. There were significant differences in variances between zygosities. This would consequently lead to a poorer fit in our variance-component models. Table 2 presents the intraclass correlations by age group, zygosity and sex. In general monozygotic twins had higher correlations compared to dizygotic twins indicating the importance of genetic effects. The correlation for opposite-sex twins is almost half of that for same sex dizygotic twins in the second age group indicating possible sex differences.

Table 3 presents estimates of the genetic and environmental effects in the variation of BMI by age group. Only the results from the best fitting model in each age group are shown. In the first age group a constrained model fitted data best. That is, the heritability estimates were the same for both males and females and were estimated to 70%. In the second age group a model with separate estimates for males and females fitted data best with heritabilities much higher in women (68%) compared to men (18%). There was also a significant shared environmental effect (47%) in men. Finally in the third age group, a scalar model fitted data best. That is, heritability is the same for both males and females (59%) but a variance difference between the sexes is allowed for.

The phenotypic distributions of the extreme concordant and discordant twin pairs from the selected sample of twins are shown in Figure 1.

The results of the linkage analysis with the "combined" Haseman-Elston approach, using genotypic data from extreme concordant and discordant twin pairs are shown in Figures 2 and 3. They are plots of asymptotic LOD scores at each marker. The sibling correlation was estimated from the whole sample of twins to 0.35. There were no significant LOD scores, indicating that linkage could not be verified on chromosomes 2 and 10.

Table 1

Mean ± Standard Deviation (*N* = Number of Individuals) of BMI (kg / m²) in a Swedish Sample of Twins by Age Group, Zygosity and Sex

	MZ		DZ		OS
	Men	Women	Men	Women	
17 ≤ Age < 50	24.3 ± 3.8 (97)	23.4 ± 3.5 (133)	25 ± 3.1 (111)	23.1 ± 3.2 (113)	23.7 ± 3.2 (235)
50 ≤ Age < 70	26.5 ± 2.7 (77)	24.8 ± 4.5 (69)	26.2 ± 3.3 (121)	26.3 ± 4.6 (163)	25.8 ± 3.8 (130)
Age ≥ 70	25.1 ± 3.5 (126)	24.4 ± 4.0 (230)	25.6 ± 3.4 (160)	25.1 ± 4.5 (352)	26.6 ± 3.9 (512)

Note: MZ: monozygotic twins
DZ: dizygotic twins
OS: opposite sex twins

Table 2

Intraclass Correlations (*N* = Number of Pairs) for BMI in a Swedish Sample of Twins by Age Group, Zygosity and Sex

	MZ		DZ		OS
	Men	Women	Men	Women	
17 ≤ Age < 50	0.79 (48)	0.79 (56)	0.30 (55)	0.14 (56)	0.32 (116)
50 ≤ Age < 70	0.54 (38)	0.80 (34)	0.40 (60)	0.37 (79)	0.21 (65)
Age ≥ 70	0.56 (57)	0.54 (102)	0.43 (70)	0.33 (156)	0.25 (250)

Table 3

Genetic (A), Shared (C) and Non-shared (E) Environmental Estimates of Variance for BMI (with 95% Confidence Intervals) Derived from the Best Fitting Model in Each Age Group

	Men			*Women*		
	A	C	E	A	C	E
17 ≤ Age < 50	0.70 .55–.77	0 0–.10	0.30 .23–.41	same as men		
50 ≤ Age < 70	0.18 0–.58	0.47 .12–.68	0.35 .22–.51	0.68 .42–.82	0.01 0–.13	0.31 .18–.54
Age ≥ 70	0.59 .46–.67	0 0–.08	0.41 .33–.51	same as men		

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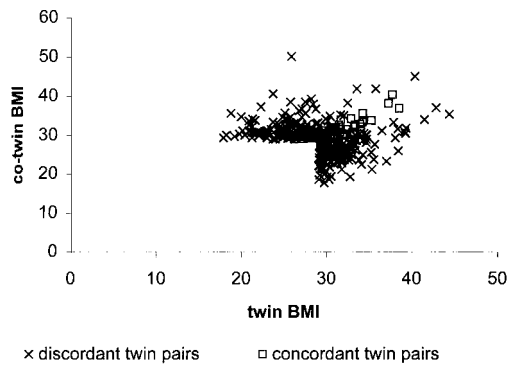

Figure 1

BMI distribution in concordant (N = 99) and discordant (N = 242) DZ twin pairs, respectively.

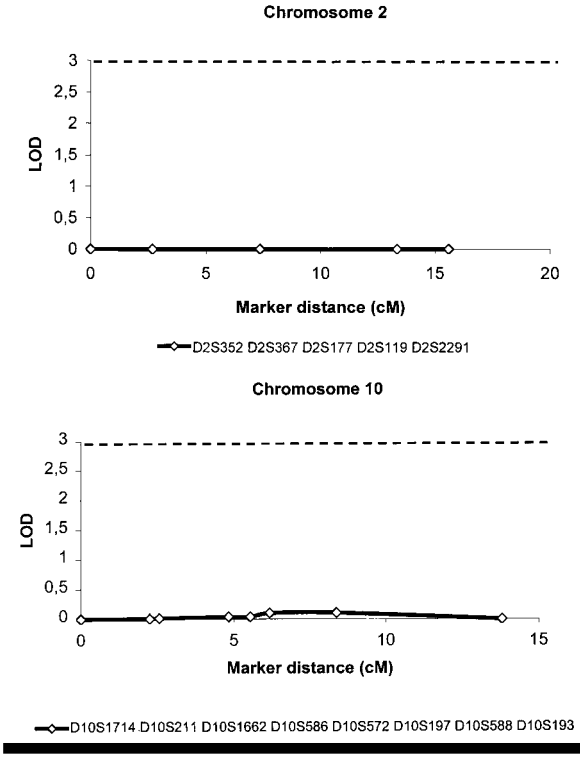

Figure 2

Plot of asymptotic LOD scores by each marker for chromosome 2 and 10, respectively.

Discussion

In previous studies two loci on chromosome 2 and 10 were linked to obesity among extremely obese individuals (Comuzzie et al., 1997; Hager et al., 1998; Hinney et al., 2000; Price et al., 2001; Rotimi et al., 1999). We have investigated whether the effects of these loci are also apparent in a moderately obese sample of Swedish twin pairs. However, we were unable to verify the findings on candidate regions of chromosome 2 and 10 in a selected sample of twins from the Swedish Twin Registry.

Heritability estimates ranged between 59–70% in the present study and were similar to other reported studies (Maes et al., 1997). The apparent effects of sex and age have also been reported in previous studies (Korkeila et al., 1991; Neale & Cardon, 1992). In the present study, the significant sex differences in the middle age group (50–70 years) could reflect true sex differences in the variation of BMI. Above the age of 50 major changes take place in the production of sex hormones in women. The shared environmental effect could reflect the accumulation of environmental experiences and changes in lifestyle habits such as smoking or diet that could be more important in men than in women. Subsequently one would expect to see a larger shared environmental effect in the older age group. However, in our opinion, a more likely explanation is that this represents a chance finding. Shared environmental effects are rarely found to be a major contributor to the variation of BMI (Maes et al., 1997). Therefore we believe that the shared environmental effect is probably much less than our observed (0.47). Sex differences are not persistent through all age groups but heritability was lower in the older age group compared to the younger. This may imply that individuals with a genetic predisposition to obesity have a lower survival probability.

Linkage to obesity related phenotypes on chromosome 2 was previously found in two separate populations (Comuzzie et al., 1997; Hager et al., 1998) and supported in animal studies (Rankinen et al., 2002). The proopiomelanocortin gene (*POMC*) is a candidate gene for this locus. Wardlaw (2001) stated that POMC neurons in the hypothalamus are important regulators of energy homeostasis and that the POMC-derived hormones and brain melanocortin receptors play a key role in this process.

The locus on chromosome 10 was initially reported to be linked to obesity in a study of French families (Hager et al., 1998). It has been subsequently verified in 2 independent studies (Hinney et al., 2000; Price et al., 2001). The results of these studies suggested that there is a major gene on chromosome 10p implicated in the development of human obesity. The region within its location covers a few known genes, none of which are an apparent candidate gene for obesity. Our study could not verify these findings.

There have been strategies and guidelines proposed on what is the optimal way of choosing sib-pairs for mapping quantitative trait loci (Allison, 1996; Dolan & Boomsma, 1998; Gu et al., 1997; Risch & Zhang, 1995; Risch & Zhang, 1996; Zhang & Risch, 1996). Risch & Zhang showed that only three types of sib pairs have substantial power to detect linkage for QTL, namely, those concordant for high values, those concordant for low values and those discordant for high or low values. However, extreme sib pairs are rare in the population limiting practicalities in sampling methods. In the current study the "sliding window rule" was used, since it allows a "window" of different scores at the top of the BMI distribution and hence allows a far greater proportion of eligible pairs to be used for the analysis (Allison, 1996). However, it does not yield equivalent power to Rich and Zhang's percentile based approach (Risch & Zhang, 1995). Power studies have shown that extremely large samples need to be screened in order to reach the adequate number of sib-pairs especially

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when parents are unavailable (Dolan & Boomsma, 1998; Risch & Zhang, 1996).

One limitation of our study was the limited power to detect a peak on the candidate regions of chromosome 2 and 10. Lately a better index of informativeness based on the non-centrality parameter was introduced as a more optimal way of selecting sib-pairs for linkage that could enhance power (Purcell et al., 2001). However, there could be more reasons preventing the verification of previous findings. Selection strategies as well as multiple testing corrections and corrections for ascertainment procedures are major statistical issues haunting most linkage and association studies. Power is also depended on the amount of QTLs responsible for the disease, which is usually unknown. In a series of simulation studies on linkage and replication of linkage results it was shown that "detectability" was improved as the number of QTLs decreased (Suarez et al., 1994). The results also suggested that even when a linkage claim in an oligogenic disorder is true it requires a much larger sample to replicate. In contrast a false linkage claim is unlikely to replicate.

We estimated power in our sample by altering certain parameters such as the increaser allele frequency and the proportion of variance explained by the QTL. We simulated, for each candidate region, 100 samples containing 2000 DZ pairs out of which we collected 51 concordant and 155 discordant sib-pairs according to the same selection criteria mentioned under the methods section. Parents were assumed to be unavailable. We assumed the same number of markers with the same distances as in our study. However, markers were set to have 10 equally frequent alleles. Simulations were done with the simulation package TWINSIM (http://www2.qimr.edu.au/davidD/twinsim.html). Assuming a QTL that explained 30% of the phenotypic variance (30% was explained by the remaining additive genetic effects, 40% was explained by unique environmental effects), and with the increaser allele frequency at 0.1 we had 14% and 10% power to detect suggestive linkage (LOD $\geq$ 2) at a QTL in candidate regions of chromosome 2 and 10, respectively. If the increaser allele frequency was fixed at 0.5 instead (sibling correlation 0.3), power was enhanced to 33% and 47%, respectively. Power for suggestive linkage exceeded 90% when both the increaser allele frequency and the variance explained by the QTL were assumed to be 50%. However, when the sibling correlation was 0.20 (30% of variance explained by QTL, 10% was explained by the remaining additive genetic effects and 60% was explained by unique environmental effects) power for suggestive linkage decreased. These power calculations highlight the importance of specifying known parameters correctly.

The effect of the loci could be different in different samples. The findings on chromosome 2 and 10 were found in extreme obese samples (mean BMI at 40) rather than moderately obese (Hager et al., 1998). This could indicate that genetic effects are more apparent in extreme obese compared to moderately obese humans. Previous linkage studies on chromosome 2 have shown linkage with leptin levels and not BMI, indicating that genetic mechanisms could act differently in separate obesity related phenotypes (Comuzzie et al., 1997). A genome-scan aimed at identifying QTLs that have a potential influence on BMI found only weak linkage signals on chromosome 2, indicating that the locus may have a smaller effect in some samples compared to others (Feitosa et al., 2002). Cultural and ethnic differences could also have limited our chances of verifying those findings. In Sweden, for example, the prevalence of obesity is less than half that of the United States (WHO, 1998). The apparent lack of concordance between studies could also reflect the fact that genetic determinants of inter-individual variation in obesity and related phenotypes are likely to be multiple and interacting, with most single variants producing only a moderate effect, which are hard to detect with linkage.

In conclusion, the current twin study shows an important heritability in the variation of BMI. Linkage to obesity in previously reported candidate regions on chromosomes 2 and 10 could not be verified, although our power to do so was weak. In spite the negative outcome of the linkage study it is of importance to publish both negative and positive findings. The results indicate that the candidate genes under study are of more importance to severe obese individuals or certain ethnic groups, rather than moderately obese Caucasians.

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Author Note

Electronic-Database Information

The URLs and accession numbers for data in this article are as follow:

The Genome Database

http://gdbwww.gdb.org/

For Chromosome 2: 247445, 248232, 63161, 62396, 658239.

For Chromosome 10: 655559, 62762, 644478, 247222, 244686, 62488, 247811, 62436.

Online Mendelian Inheritance in Man (OMIM)

http://www3.ncbi.nlm.nih.gov/Omim/

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For Chromosome 2: 176830

For Chromosome 10: 603188

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