Genetic and environmental factors have been linked to obesity (MIM #601665) (1). The genetics of BMI have been well studied and have been reviewed recently (2). We have reported (3) that a locus for BMI maps to chromosome 6q23-25, with a peak logarithm of odds (LOD) score of 4.6.

Although BMI is an excellent overall indicator of obesity, it does not account for regional fat distribution, specifically abdominal adiposity. Waist circumference has been shown to be an independent risk factor for the development of diabetes, and some reports suggest that abdominal adiposity might be a more potent risk factor for the subsequent development of diabetes than more traditional measures of adiposity (4–7). The mechanism of this increased risk is hypothesized (8) to be related to the metabolically active adipose tissue found in the visceral region. Although abdominal adiposity is thought to be an important component of overall body fat, it has largely been overlooked in genetic analyses. Thus, we sought to perform linkage to waist circumference in the Framingham Heart Study original cohort and offspring studies.

We created a dataset using information from the original and offspring cohorts that were chosen at the examination visits where the mean age was similar: the original cohort in 1956–1960 and the offspring in 1987–1991. The parental and offspring generations were matched for mean age because obesity, specifically central adiposity, increases with age up to ~60 years; by matching cohorts by age, we hoped to create a more homogenous sample. We also looked at a priori–designated subsets of the sample: participants <65 and <60 years of age. Although obesity increases with age in young and middle-aged adults, declines are seen after age 60 years, which may confound the assessment of the genetic component.

Overall, 2,086 subjects were available for analysis (Table 1). The mean age was 48 years. The sample was 51% female. The mean waist circumference was 90 cm. Thirty-four percent of the sample was currently smoking, 14% were older than 60 years of age, and 6% were older than 65 years. The data are also presented by cohort or offspring status and by those aged <60 years. Forty percent of the overall sample was derived from the parental cohort and 60% from the offspring cohort. The heritability of waist circumference was 0.41 ($P < 0.001$).

In the overall sample, the highest LOD score was 3.3 on chromosome 6q23 at 138 cM (Fig. 1). When the sample was limited to those under the age of 60 years, the highest LOD score was 3.7 at the same location. The marker located at the region of peak linkage is D6S1009.

There were two other regions of linkage with LOD scores $>2.0$ on chromosome 2; LODs 2.06 at 4 cM and 2.00 at 127 cM. The nearest markers are D2S2976 and D2S410, respectively. Table 2 presents a list of all LOD scores $>1.20$.

We have found substantial evidence for linkage of waist circumference, a measure of central adiposity, to chromosome 6q23. We have previously reported (3) linkage to this region for BMI, suggesting that one or more genes underlying BMI and central adiposity reside in this region.

A major strength of our study is the ability to obtain
measurements of waist circumference of parent and offspring cohorts at roughly the same age. Aging is one of the most important determinants of body fatness, and obtaining age-specific measurements is important in order to correctly estimate the genetic component. Unfortunately, we did not have waist circumference data available when our subjects were younger. We have previously shown (3) for BMI that single-locus effects were most apparent at younger ages of adulthood. Thus, it is entirely possible that we have underestimated the true magnitude of the effect of genetics on waist circumference. Another limitation to this study is the temporal effect of obesity (9). A given waist circumference in the parental generation is not equivalent to the offspring generation. To circumvent this issue, we computed cohort-specific residuals, which effectively rank the relative waist circumference of each participant in the two generations.

Waist circumference is increasingly recognized as a critical component of the metabolic syndrome (10) and has been shown to further discriminate among individuals at increased risk for the metabolic syndrome and diabetes within defined BMI categories (11). Measures of adiposity have been shown to be independent risk factors for the development of diabetes (4–7), and some reports suggest that abdominal adiposity might be a more potent risk factor for the subsequent development of diabetes than overall adiposity (4–7). While hypothesized to be due to metabolically active adipose tissue found in visceral fat depots (8), specific mechanisms may include associated worsened insulin resistance (12) and closer coupling with levels of key metabolic risk factors (13). Taken together, these data suggest that central adiposity may be the most important component of body fat.

We have found linkage for waist circumference 6 cM away from our region of linkage to BMI. It is unlikely that waist circumference and BMI map to the same chromosomal region solely because of high correlations between the measures. Indeed, waist circumference and BMI are highly correlated (14). However, in our linkage analyses, we adjusted waist circumference for BMI, effectively removing the variation due to differences in BMI. Physiologically, this isolates the phenotype of central obesity from overall obesity, enabling the discovery of genes involved in this specific process. We also hypothesize that we may have isolated a locus for obesity that contains more than one gene involved in body fat regulation. Linkage to BMI on chromosome 6 demonstrated two peaks: one at 144 cM (LOD score of 4.64) among subjects with a mean age of 40.4 years (~6 cM from our peak region of linkage for waist circumference) and another at 166 cM (LOD score of 3.08) among subjects with a mean age of 60.0 years (3). Given that subjects in our dataset have a mean age of 48 years and our region of peak linkage is at 138 cM, these data hint at the possibility of an age-dependent gene regulating central adiposity, which might have been detected in the BMI linkage when subjects were predominantly younger. It is also possible that central obesity might precede the development of overall obesity, a process that might depend on the same sets of genes; however, further research is necessary to explore this hypothesis.

Variance components linkage analysis is sensitive to violations of the assumption of normally distributed data.
(15). This sensitivity can cause an error rate in excess of the specified error rate (usually 0.05). Allison et al. (15) show that this sensitivity can be caused by high rates of skewness, kurtosis, and small sample size. In this study, the skewness and kurtosis, reported in Table 1, are not excessive and our dataset is relatively large by genome-scan standards. Thus, there is little evidence that our results might be spurious due to violations of normality assumptions.

To the best of our knowledge, no published genome-wide linkage analyses exist that examine waist circumference, although data from segregation analyses have provided evidence for a major gene for waist circumference (16) and waist-to-hip ratio (17). There have been two analyses looking at abdominal visceral fat as measured by computed tomography, revealing linkage at 12q24.3 (LOD 2.88) (18) and 2p36 (LOD 2.49) (19), indicating that we have identified a novel locus for abdominal adiposity. Although there are no previously published genome-wide linkage analyses of waist circumference, several candidate gene studies have been performed. A thorough review of these studies is available (2).

Potential candidate genes lying near our region of peak linkage include the estrogen receptor (ESR1, located at 152 mB), the opioid receptor (OPRM1, located at 154 mB), and the neuromedin B receptor (NMBR, located at 142 mB), located roughly 15, 17, and 5 mB, respectively, from marker D6S1009. Polymorphisms in ESR1 have been shown (20) to be associated with waist circumference. Rats injected with an opioid antagonist demonstrated decreased food intake and decreased body weight (21), suggesting that OPRM1 is another potentially exciting candidate gene in this region. NMBR is a bombesin-like peptide receptor. Bombesin receptor subtype-3, part of this family of receptors, has been shown to regulate energy balance and adiposity.

In summary, we report linkage of waist circumference to the same locus where we have previously reported linkage to BMI. Further research is necessary to understand the genes involved in central adiposity.

RESEARCH DESIGN AND METHODS

The Framingham Heart Study began in 1948 with the enrollment of 5,209 men and women, 28–62 years of age at study entry, with subjects undergoing repeat exams every 2 years. In 1971, 5,124 men and women were enrolled into the offspring cohort of the Framingham Heart Study, which included the children or spouses of the children of the original cohort. Offspring subjects underwent examinations approximately every 4 years.

Each examination included an extensive cardiovascular disease assessment, 12-lead electrocardiogram, and blood testing. For this analysis, we only used information regarding age, sex, waist circumference, waist, height, and smoking status. Waist circumference was measured at the level of the umbilicus while the participant was standing. BMI was calculated by dividing weight (in kilograms) by height (in meters squared).

Leukocyte DNA was extracted from 5–10 ml of whole-blood or buffy-coat specimens. Aliquots of DNA from members of the largest Framingham Heart Study families were sent in four batches to the Mammalian Genotyping Service Laboratory at the Marshfield Clinic (Marshfield, WI), consisting of 330 pedigrees, ranging in size from 0 to 7. A 10-cM density genome-wide scan was performed (marker set 9, heterozygosity 0.77). Genotype data cleaning, including verification of family relationships and Mendelian inconsistencies, have been previously described (22).

Statistical analysis. Using SAS version 8.0 (Cary, NC), examination cycle and sex-specific residuals were computed. Exam-specific residuals were used to account for the increase in adiposity and obesity over time (9). Sex-specific residuals were used to account for sex differences in adiposity. Residuals included adjustment for age, age squared, BMI, and smoking status, using the following four equations: 1) cohort men: waist circumference = 6.984 + 0.136(age) – 0.001(age^2) + 0.915(BMI) – 0.008(smoking status); 2) cohort women: waist circumference = 14.808 + 0.035(age) + 0.0005(age^2) + 0.244(smoking status); 3) offspring men: waist circumference = 7.070 + 0.168(age) – 0.001(age^2) + 0.042(BMI) + 0.387(smoking status); and 4) offspring women: waist circumference = 6.966 + 0.054(age) – 0.0001(age^2) + 0.871(BMI) + 0.747(smoking status). Residuals were computed separately for the cohort and offspring and separately for men and women. Residuals were obtained from a regression-based linear model and were used to predict values of a given outcome (in this case, waist circumference) after correction for variation attributable to covariates. The residuals were computed by calculating the difference between the actual and predicted values obtained by linear regression. This way, the correction for covariates was fixed across the dataset. The use of residuals was necessary to standardize values between different examination cycles and sexes, as well as account for the effects of covariates. Residuals were standardized (mean = 0, SD = 1).

Variance components linkage analysis as implemented in Genehunter (23) was used to perform the genome-wide scan. This approach uses the genotype information at a locus to decompose the phenotypic variance into a component attributable to the locus (known as a quantitative trait locus or QTL), a polygenic component, and an environmental component. Genotype information at a locus is characterized by the probability that two related individuals share none, one, or two alleles identical by descent. Identical-by-descent computation grows exponentially with pedigree size in Genehunter. Nine of the largest families were split into smaller pedigrees. The resulting small loss of genetic information should be conservative with respect to linkage results.

All variance components were estimated by maximum likelihood. Linkage was tested by a likelihood ratio test in which the hypothesis that the QTL variance component is equal to zero was compared with it being greater than zero. The resulting χ² statistic was converted to a traditional LOD score by dividing by 2 × ln (10). It is possible to obtain estimates of the proportion of the total phenotypic variation due to the QTL. However, two recent articles (15,24) have shown that the estimate of this effect is strongly correlated with the LOD score estimate and poorly correlated with the true proportion. Therefore, we will not present effect estimates here. The Framingham Heart study is population based and not selected for any particular trait; therefore, no ascertainment correction is necessary. LOD scores >1.9 were considered suggestive, and LOD scores >3.3 were considered significant (25).

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REFERENCES

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