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Genomewide Linkage Analysis of Weight Change in the Framingham Heart Study

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Background: Weight gain adversely affects blood pressure, lipids, and glycemia. The genetic contribution to weight change is unknown.

Methods: Variance components linkage analysis using microsatellites was performed on 336 families from the Framingham Heart Study offspring cohort, using a 10-cM genome-wide linkage analysis. We evaluated linkage to two traits: short-term (8-yr) weight change and long-term (up to 24-yr) weight change. Models were adjusted for age, age squared, baseline weight, smoking status, and menopausal status.

Results: Mean short-term weight change ranged from 1.4–3.8 kg, and mean long-term weight change was 7.7 kg. The heritability of long-term weight change was 0.24; weight change was minimally heritable among younger individuals and over shorter follow-up intervals. We found significant evidence for linkage for long-term weight change, with a peak LOD score of 3.10 on chromosome 20 at 63.7 cM (nearest marker, D20S481). We also found suggestive evidence for linkage on chromosome 1 at 239.7 cM (LOD score, 2.28; nearest marker, D1S1644).

Conclusion: Long-term weight change is heritable, and evidence for linkage exists on chromosomes 1 and 20. Potential candidate genes include *MC3R*, *ASIP*, *AGT*, and *HSD11B1*. Additional research is necessary to uncover the genetic underpinnings of weight change that might contribute to associated adverse metabolic profiles. **(*J Clin Endocrinol Metab* 90: 3197–3201, 2005)**

OBESITY INCREASES THE risk of all-cause mortality (1), vascular disease (2), and nonvascular causes of death, including certain cancers (3). Weight gain adversely affects cardiovascular disease risk factors, including changes in lipids, fasting insulin, and blood pressure (4–6), and is one of the leading risk factors for type 2 diabetes (7, 8).

Risk factor profiles can be modified by minimal gains and losses in weight (8, 9), suggesting that overall measures of adiposity may not accurately reflect the dynamic interaction of weight change on metabolic profiles. Reductions in weight of 5–10% can result in significant improvements in lipoprotein profiles (10), insulin sensitivity (11), and blood pressure (12), whereas weight gain of more than 10 kg is associated with an increased risk for hypertension, elevated low density lipoprotein cholesterol, and higher triglycerides (13). Although we have previously reported quantitative trait loci (QTLs) for body mass index (BMI) and waist circumference to 6q23–24 and 11q14 in the Framingham Heart Study (14, 15), it is possible that different loci underlie the genetics involved with weight change over time.

In fact, changes in body mass and adiposity have been shown to be heritable, with estimates ranging from 0.23–0.86 for changes in BMI (16, 17), 0.70 for weight gain (18), and 0.45 for change in waist circumference (16). However, limited data exist looking at linkage to weight change. Thus, we determined heritability and linkage to weight change in the Framingham Heart Study offspring study.

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Abbreviations: BMI, Body mass index; IBD, identical by descent; QTL, quantitative trait locus.

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Subjects and Methods

The Framingham Heart Study began in 1948 with the enrollment of 5209 men and women, 28–62 yr of age at study entry, with subjects undergoing repeat exams every 2 yr (19). In 1971, 5124 men and women were enrolled in the offspring cohort of the Framingham Heart Study, which included the children or spouses of children of the original cohort. Offspring subjects underwent examinations approximately every 4 yr; the design and methodology of the offspring cohort has been previously described (20). The Boston Medical Center institutional review board approved the study, and all participants provided written informed consent.

Details regarding the methods of risk factor measurement and laboratory analysis have been described previously (21). Each examination included anthropometry, an extensive cardiovascular disease assessment, 12-lead electrocardiogram, and blood testing. Weight was measured to the nearest pound with the participant wearing only a gown without shoes or slippers and standing in the middle of the scale with weight equally distributed on both feet. For this analysis, we only used information regarding age, sex, weight, smoking status (defined as smoker at baseline or follow-up), and menopausal status (pre- or postmenopausal). BMI was calculated by dividing weight (in kilograms) by height (in meters squared). Adjustments were made for age and age squared to be consistent with our previously published work (14). We also adjusted for menopausal status, because the menopausal transition is associated with increases in body weight (22), increased visceral (23) and central fat depots (24), and higher BMI (24) compared with pre-

menopausal women. We adjusted for smoking status, because smoking cessation has been associated with body weight gain (25). We adjusted for baseline weight to investigate genetic influences on weight change independent of baseline weight, because the magnitude of absolute weight change depends on baseline weight.

Leukocyte DNA was extracted from 5–10 ml whole blood or buffy coat specimens using a standard protocol (26). Aliquots of DNA from members of the largest Framingham Heart Study families were sent in four batches to the Mammalian Genotyping Service Laboratory at Marshfield Clinic (Marshfield, WI), consisting of 336 pedigrees. A 10-cM density genome-wide scan was performed (average heterozygosity, 0.77). Genotype data cleaning, including verification of family relationships and Mendelian inconsistencies, have been previously described (27).

For the purpose of this analysis, we created three datasets using information from participant examinations that occurred at roughly 8-yr intervals (1971–1975 to 1979–1982, 1979–1982 to 1987–1992, and 1987–1992 to 1995–1998), enabling the creation of a weight change variable over 8 yr (short-term weight change), and a fourth dataset that examined long-term weight change (1971–1975 to 1995–1998) over approximately 24 yr. Participants were excluded from these analyses if they were under age 20 yr or had a BMI less than 18 kg/m^2 at any of the baseline examinations. Severe weight cyclers, defined as participants who repeatedly lost and gained 9 kg in at least three examination cycles, were also excluded from analyses. The rationale for this particular exclusion is derived from data from prior observational studies that suggest that individuals with weight fluctuation are at increased risk for all-cause mortality (28), indicating that it may be a different phenotype from more typical weight change over time. Because linkage signals are often the strongest when phenotypes are parsed into their most basic components, we removed the weight cyclers from our analysis. We also performed a secondary analysis in which we limited our study sample to participants under the age of 65 yr. Although obesity increases with age in young and middle-aged adults, declines are seen after age 60 yr (29), which may confound the assessment of the genetic component.

Statistical analysis

Using SAS (30), sex-specific residuals were computed using weight change, as defined by follow-up weight minus baseline weight. Sex-specific residuals were used to account for gender differences in adiposity. Residuals included adjustment for age, age squared, baseline weight, smoking status, and menopausal status (in women). Residuals were obtained from a regression-based linear model and were used to predict values of weight change 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. In 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 genders as well as to account for the effects of covariates. A residual value greater than 4 SD from the mean was reduced to the 4 SD value (*i.e.* Winsorized) to reduce the skewness and kurtosis of the data to an acceptable level (31). Specifically, the sex-specific residuals were derived for each of the four datasets separately (resulting in eight regression equations). Residuals were derived separately in men and women, then merged. The regression equations in women for weight change residual were: exams 1 to 6, $35.1 + (0.19 \times \text{age}) - (0.01 \times \text{age}^2) - (0.03 \times \text{wt}) + (1.94 \times \text{smok\_baseline}) - (7.15 \times \text{smok\_follow}) + (0.06 \times \text{menopausal status})$; exams 1 to 2, $0.23 + (\text{age} \times 0.53) - (0.01 \times \text{age}^2) - (0.01 \times \text{wt}) + (0.54 \times \text{smok\_baseline}) - (2.89 \times \text{smok\_follow}) + (0.33 \times \text{menopausal})$; exams 2 to 4, $-9.6 + (\text{age} \times 0.91) - (0.01 \times \text{age}^2) + (0.02 \times \text{wt}) + (4.84 \times \text{smok\_baseline}) - (5.48 \times \text{smok\_follow}) - (2.8 \times \text{menopausal})$; exams 4 to 6, $40.5 - (\text{age} \times 0.53) - (0.0003 \times \text{age}^2) - (0.05 \times \text{wt}) + (4.28 \times \text{smok\_baseline}) - (6.06 \times \text{smok\_follow}) + (2.01 \times \text{menopausal})$, where wt stands for weight, smok_baseline is smoking status at baseline, smok_follow is smoking status at follow-up, and menopausal is menopausal status. The regression equations among men for weight change residual were: exams 1 to 6, $45.6 - (0.52 \times \text{age}) - (0.004 \times \text{age}^2) - (0.06 \times \text{wt}) + (4.70 \times \text{smok\_baseline}) - (4.84 \times \text{smok\_follow})$; exams 1 to 2, $25.5 - (0.37 \times \text{age}) + (0.002 \times \text{age}^2) - (0.08 \times \text{wt}) + (1.50 \times \text{smok\_baseline}) - (1.34 \times \text{smok\_follow})$. exams 2 to 4, $23.9\text{-}(0.21 \times \text{age}) - (0.001 \times \text{age}^2) - (0.05 \times \text{wt}) + (5.03 \times \text{smok\_baseline}) - (4.22 \times \text{smok\_follow})$; and exams 4 to 6, $43.5 - (1.23 \times \text{age}) + (0.01 \times \text{age}^2) + (0.001 \times \text{wt}) + (8.22 \times \text{smok\_baseline}) - (9.44 \times \text{smok\_follow})$. R^2 values ranged from 0.02–0.13. For women, we included menopausal status as a covariate. Otherwise, models were identical for all datasets. Residuals were computed and then merged together for use in Genehunter to compute the heritability and linkage estimates.

Variance components linkage analysis (32, 33), as implemented in Genehunter (34), was used to perform the genomewide linkage analysis using residuals for weight change as the phenotype of interest. 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 (QTL)], a polygenic component and an environmental component. Genotype information at a locus is characterized by the probability that two related individuals share zero, one, or two alleles identical by descent (IBD). IBD computation grows exponentially with pedigree size in Genehunter. To make the IBD computation feasible, 19 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 χ^2 statistic was converted to a traditional LOD score by dividing by $2 \times \ln$ (10). It is possible to obtain estimates of the proportion of the total phenotypic variation due to the QTL. However, two recent studies (35, 36) have shown that the estimate of this effect is strongly correlated with the LOD score estimate and is poorly correlated with the true proportion. Therefore, we will not present effect estimates here. The Framingham Heart Study is population based and is not selected for any particular trait; therefore, no ascertainment correction is necessary.

Results

The characteristics of the four datasets are presented in Table 1. As expected, the sample aged with each progressive dataset; mean weight increased as well. The mean long-term weight change was 7.7 kg. The short-term (8-yr) weight change ranged from 1.4–3.8 kg. The most heritable trait was long-term weight change (0.24). Short-term weight change was minimally heritable, with estimates ranging from 0–0.12. Short-term weight change heritability was higher among younger individuals than among older individuals.

LOD scores greater than 1.0 are presented in Table 2 for all datasets. We found significant evidence for linkage for long-term weight change, with a peak LOD score of 3.10 on chromosome 20 at 63.7 cM (nearest marker, D20S481; Fig. 1). We also found suggestive evidence for linkage on chromosome 1 at 239.7 cM (LOD score, 2.28; nearest marker, D1S1644; Fig. 2). When the sample was limited to participants less than 65 yr of age, the peak LOD score on chromosome 20 was attenuated (LOD score, 2.32 at 63.7 cM).

Discussion

Long-term weight change is heritable, whereas short-term weight change is less heritable. We have found evidence for significant linkage to long-term weight change on chromosome 20 and evidence for suggestive linkage on chromosome 1.

Previous heritability studies have demonstrated that changes in measures of adiposity over time, including BMI, weight, and waist circumference, are heritable (16–18). These previous estimates were higher than ours, with estimates of heritability as high as 0.70 for weight gain.

Limited data exist on linkage to weight gain in the published literature. As part of the Genetic Analysis Workshop

TABLE 1. Baseline characteristics of the study sample

	Exams 1 to 2[a] (n = 1081)	Exams 2 to 4[a] (n = 1133)	Exams 4 to 6[a] (n = 1005)	Exams 1 to 6[a] (n = 1088)	Exams 1 to 6[a] age <65 yr[b] (n = 823)
Age at baseline (yr)	35 ± 9	41 ± 9	45 ± 8	34 ± 9	30 ± 6
% Female	52	50	50	52	51
Weight at baseline (kg)	71.9 ± 15.0	73.4 ± 15.1	77.1	71.6 ± 15.0	71.2 ± 15.2
Weight change (kg)	1.4 ± 6.1	3.7 ± 6.6	3.8 ± 7.2	7.7 ± 10.5	9.2 ± 10.6
BMI at baseline (kg/m^2)	25.2 ± 3.9	25.5 ± 4.2	26.7 ± 4.8	25.1 ± 4.0	24.8 ± 4.0
Smoking status					
Baseline smoker (%)	61	35	25	60	61
Follow-up smoker (%)	35	24	17	16	17
Postmenopausal status at baseline (%)	18	33	39	17	6
Skewness	0.20	0.43	0.35	0.57	0.65
Kurtosis	1.11	1.21	1.27	1.40	1.39
Heritability	0.12[c]	0.04	0	0.24[d]	0.19[c]

[a] Datasets 1 and 2 represent 1971–1975 to 1979–1982, 2 and 4 represent 1979–1982 to 1987–1992, 4 to 6 represent 1987–1992 to 1995–1998, and 1 to 6 represent weight change from 1971–1975 to 1995–1998.
[b] At baseline and follow-up.
[c] $P < 0.01$.
[d] $P < 0.001$.

proceedings, linkage analysis was performed on the slope of weight change in the Framingham Heart Study, which demonstrated a peak multipoint LOD score of 1.6 on chromosome 8 at 152 cM for body weight change and 1.9 on chromosome 8 at 102 cM for body weight gain and body weight change up to 50 yr of age (37). Different methodologies, including use of the regression slope instead of actual weight change, may contribute to differences from our findings.

The QTL that we identified on chromosome 20q13 has been replicated in several other linkage analyses that have examined BMI, percent body fat, and eating behavior, with LOD scores ranging from 2.2–3.2 (38). This region harbors several interesting candidate genes, including the melanocortin 3 receptor, *MC3R*, which has been shown to be associated with BMI (39) and severe obesity (40); the agouti-signaling protein, *ASIP*; *GHRH*, which has been associated with central adiposity (41); and the adenosine deaminase gene, *ADA*, which has been associated with BMI (42).

We also found suggestive linkage on chromosome 1 to weight change, with a LOD score of 2.28. Interestingly, this QTL is the same location where long-term fasting glucose values were mapped from the Framingham Heart Study (43), raising the possibility that weight change may be an important genetic component in the development of diabetes. Furthermore, the angiotensinogen (*AGT*) gene lies in this region, and polymorphisms in this gene have been associated with weight gain among treated hypertensive patients (44). This region also contains the 11β-hydroxysteroid dehydrogenase 1 gene (*HSD11B1*), which has been shown to be associated with obesity in expression studies (45).

Candidate gene analyses have been performed looking at weight change. Polymorphisms in the leptin receptor and the β_2-adrenergic receptor were shown to be associated with weight gain in a case-control study comparing participants who gained an average of 12.8 kg over 7 yr with those who remained weight stable (46). Polymorphisms in the serotonin 5-HT$_{2c}$ receptor gene have been associated with clozapine-induced weight gain among patients with schizophrenia (47). Candidate gene studies of overfeeding also shed light on genetic susceptibility to weight gain. In 12 pairs of identical male twins, polymorphisms in the *UCP* gene were involved in recovery from overfeeding (48), and polymorphisms in the β_2-adrenergic receptor (49) and the resistin gene (50) were shown to be associated with depot-specific fat accumulation after overfeeding. These data suggest that weight gain is an important phenotype that warrants additional study in the general population.

Strengths of our study include repeated measures of weight collected over a 27-yr period using standardized methods and well documented covariate data. Some limitations do exist. The predominantly Caucasian sample that comprises the majority of the Framingham offspring cohort may limit the generality of our findings. However, cardiovascular disease risk factor relationships from Framingham have been validated in six ethnically and geographically diverse cohorts and were found to be applicable in other

TABLE 2. LOD scores greater than 1.0 for all datasets

Weight change from exams 1 to 2[a]				Weight change from exams 1 to 6[a]			
Chromosome	Position (cM)	LOD score	Nearest marker	Chromosome	Position (cM)	LOD score	Nearest marker
2	195.4	1.10	D2S434	1	239.7	2.28	D1S1644
3	29.2	1.35	D3S3589	3	17.2	1.43	D3S1304
4	31.7	1.61	D4S2639	6	27.6	1.40	D6S1959
11	127.1	1.04	D11S4464	9	54.4	1.23	D9S304
13	77.4	1.09	D13S779	10	35.5	1.89	D10S1423
15	50.8	1.14	D15S1507	20	63.7	3.10	D20S481
21	9.2	1.28	D21S11	21	11.5	1.70	D21S1437

Datasets not featured (weight change from 2 to 4 and from 4 to 6) did not have LOD scores greater than 1.0.
[a] Datasets 1 and 2 represent 1971–1975 to 1979–1982, 2 to 4 represent 1979–1982 to 1987–1992, 4 to 6 represent 1987–1992 to 1995–1998, and 1 to 6 represent weight change from 1971–1975 to 1995–1998.

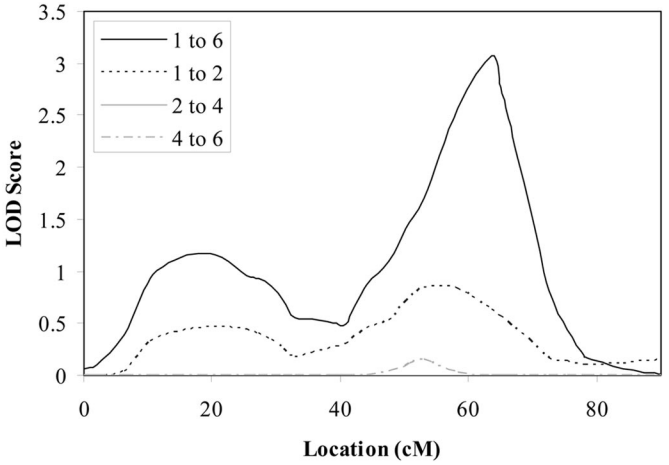

FIG. 1. Multipoint linkage analysis for weight change between exams 1 to 2, 2 to 4, 4 to 6, and 1 to 6: chromosome 20.

populations, reinforcing the generalizability of our data (51). Our genetic data were not collected until 1995; therefore, there may have been a survival bias, because subjects with a greater genetic predisposition to more severe weight change may have died prematurely. Our peak LOD score of 3.1 is significant for genome-wide significance, but there is a possibility that our results may represent a false positive finding. Our sample size and the marker spacing of approximately 10 cM in the genome-wide linkage analysis may limit our power to localize disease-influencing QTL in linkage to weight change. Although there may be additional QTLs not detected in our study, simulation studies using finer marker maps have demonstrated that the location error is low in maps using a marker spacing of 10 cM compared with 0.5 cM (52), suggesting that this should not have affected our results significantly. Lastly, we were unable to adjust for physical activity and diet in our multivariable regressions.

In summary, we have found evidence for linkage to long-term weight gain to two genomic regions. Both of these regions have been implicated in independent studies of obesity-related phenotypes. Additional studies are warranted to understand the genetic underpinnings to weight change, which affects blood pressure, lipids, and glycemia.

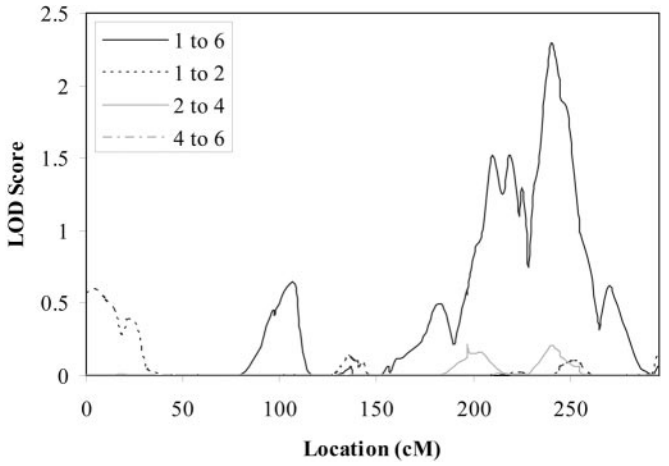

FIG. 2. Multipoint linkage analysis for weight change between exams 1 to 2, 2 to 4, 4 to 6, and 1 to 6: chromosome 1.

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