0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(8):3171–3176

doi: 10.1210/jc.2007-0418

Linkage of Genes to Total Lean Body Mass in Normal Women

Gregory Livshits, Bernet S. Kato, Scott G. Wilson, and Tim D. Spector

Sackler Faculty of Medicine (G.L.), Tel Aviv University, Tel Aviv 69978, Israel; Twin Research and Genetic Epidemiology (G.L., B.S.K., T.D.S.), Unit St. Thomas' Hospital, Kings College London, London SE1 7EH, United Kingdom; and Department of Endocrinology and Diabetes (S.G.W.), Sir Charles Gairdner Hospital, Nedlands, Western Australia 6009

Background: Total lean body mass (LEAN-tot) is one of the three major components of body weight. Its deterioration is a risk factor for frailty. Despite this, there are few studies examining the contribution of genetic factors.

Objective: Our objective was to examine the contribution of genetic factors for LEAN-tot variation, including a genome-wide search for the genes.

Research Methods: Dual-energy x-ray absorptiometry measurements of LEAN-tot were obtained from each of the 3180 United Kingdom females (509 monozygotic and 1081 dizygotic twin pairs). Contribution of genetic factors was assessed using variance component analysis. A genome-wide linkage analysis was performed on the dizygotic twins using a modified version of the Haseman-Elston method.

Results: Age, body height, total fat, and bone mass were correlated with LEAN-tot, and commonly explained 52% of the LEAN-tot variation. The crude heritability estimate was 74.0 ± 4.0%, after adjustment for the aforementioned factors; 65.2 ± 4.6% was attributable to independent genetic effects. Significant ($P < 0.001$) genetic correlations were found between LEAN-tot and bone mass, and LEAN-tot and total fat. Adjusted only for age, LEAN-tot showed no significant linkage. After adjustment for all covariates, significant linkage (LOD = 4.49 and 3.62) was observed at chromosome 12q24.3 and 14q22.3, respectively. Additional peaks of interest were on 7p15.3-15.1 (LOD = 2.86) and 8p22 (LOD = 2.83).

Conclusions: LEAN-tot measured by dual-energy x-ray absorptiometry is highly heritable, independent of other body measures. This first genomic search for genes associated with the lean component of body mass suggests significant linkage to quantitative trait loci on chromosomes 12 and 14. **(*J Clin Endocrinol Metab* 92: 3171–3176, 2007)**

THERE IS A GROWING body of evidence indicating that all three main components in body composition (bone mass, lean body mass, and fat mass) are important for good health, and all three experience age-dependent changes and degeneration (1–3). The data suggest that although a relative amount of fat, in general, increases with aging, the other two components clearly decrease with age, eventually leading to diminished fitness and growing frailty of the individuals (3, 4). However, although there are extensive published data concerning the fat and bone mass, data on lean mass are scarce, despite their significant contribution to various components of individual fitness. It has been shown, for example, that regardless of sex and race, lean body mass is the major predictor of the left ventricular mass, whereas fat mass contribution is rather minor (1). Several studies proposed that lean mass affects bone mass more strongly than fat mass (5, 6), and this can be due to common genetic factors (7, 8). Moreover, some studies even suggested that familial lean mass resemblance is a genetic mechanism by which femoral neck bone mineral density is inherited (9, 10).

First Published Online June 5, 2007

Abbreviations: ACACB, Acetyl-coenzyme A carboxylase-β; BMD-tot, total bone mineral density; DXA, dual-energy x-ray absorptiometry; DZ, dizygotic; FAT-tot, total fat; FFM, fat-free mass; IBD, identical-by-descent; LEAN-tot, total lean body mass; MZ, monozygotic; QTL, quantitative trait loci; r_E, environmental correlation; r_G, genetic correlation.

Heritability, candidate genes and genome-wide linkage analyses for bone mass and fat mass have been widely reported in numerous publications (11, 12). Surprisingly, however, there are only very few studies that examined familial resemblance of lean mass (13–15). Moreover, we are not aware of any genome-wide linkage or association study of lean mass. This may have added importance because substantial common genetic effects are shared with bone mass and structure (8, 15).

Dual-energy x-ray absorptiometry (DXA) provides a definitive method of accurately determining these three body composition components, and allows investigation of their independent effects on various aspects of human physiology in normalcy and in pathology (16). The present study used DXA body composition data in a very large population-based sample of female twins and provides the first quantitative trait loci (QTL) from a genome-wide linkage scan of total lean body mass (LEAN-tot).

Subjects and Methods

Sample

The data examined in the present study were from the TwinsUK Adult Twin Registry, described in detail elsewhere (17). All participants gave written informed consent before entering the study, and the St. Thomas' Hospital research ethics committee approved the project. The present study comprised 1018 monozygotic (MZ) and 2162 dizygotic (DZ) twins having available whole body densitometry data for body components. All participants were female volunteers ascertained from the general population through national media campaigns in the United Kingdom, and unselected for any disease or trait.

Phenotype

Each individual in the sample was assessed for body weight and height in addition to body composition measurements. Body composition was measured using the whole body DXA method, following manufacturer's recommendations (QDR 4500W system; Hologic, Inc., Bedford, MA). All scan printouts were reviewed by an expert reader to ensure proper positioning and analysis. The subject lay supine on a bed and was scanned from head to toe for determination of total bone mineral density (BMD-tot), total lean tissue (LEAN-tot) mass, and total fat (FAT-tot) mass, as described by us elsewhere (13). Note that although LEAN-tot includes mostly muscle mass, it also includes tendons and soft tissue, such as viscera.

Genotyping

DNA was extracted using the BACC2 DNA extraction kit (Nucleon Biosciences, Coatbridge, Lanarkshire, UK) from 10 ml venous blood collected into 1.6 mg/ml EDTA. Genome-wide linkage analysis included the genotyping of 737 highly polymorphic markers, using standard fluorescence-based genotyping methodologies (18) from the ABI Prism linkage mapping set (Applied Biosystems, Foster City, CA), and ordered according to the combined linkage-physical map of the human genome (19). Consistencies among genotypes, family relationships, and twin zygosity were routinely investigated, and this included analysis of patterns of Mendelian inheritance and identity-by-state relationships. Random duplicate genotyping was routinely undertaken throughout the study and indicated a mean genotyping error rate of less than 1% for the microsatellite genotyping. Discrepant genotypes and MZ twins identified by this routine were excluded from further analyses.

Statistical analysis

First, all MZ and DZ twin study participants were included in analysis of distribution and adjustment, using the multiple linear regression technique (StataCorp, version 9; StataCorp LP, College Station, TX). Three types of adjustment were undertaken: 1) age and age-derivatives were used for adjustment of variation of LEAN-tot to obtain the crude heritability and linkage signal estimates of the trait; 2) to examine the correlation between the three body composition components, free of body size effect, each of them was adjusted for age and body height; and 3) LEAN-tot was adjusted for age, height, FAT-tot, and BMD-tot to test the residuals created at this stage for lean-specific heritability and linkage.

To estimate heritability, we used quantitative-model fitting to twin data. Briefly, this approach is based on comparisons of the correlations between MZ and DZ twin pairs, and allows for the partitioning of observed phenotypical variance into additive genetic component, and common and/or unique environmental components (20). In this study the variance component estimates were obtained by the maximum-likelihood based method implemented in the MAN-7 software package (21).

Because our data showed significant correlation between the three body composition components, we also conducted a bivariate genetic analysis between each pair of these variables (21). This analysis represents an extension from univariate to multivariate models, and allows exploration of the question as to whether the origin of the covariance between the different variables is genetically and/or environmentally determined. The general model estimates contribution of the common genetic factors, genetic correlation (r_G) and shared environment, environmental correlation (r_E) between two traits. The statistical significance of r_G and r_E was studied using nested models, and examining the change in χ^2 values between the models. A more detailed description is given elsewhere (20). We used this analysis to ascertain the extent to which encountered phenotypical correlations are attributable to common (pleiotropic) genetic and shared environmental effects. To achieve this, we estimated r_G and r_E between the BMD-tot and FAT-tot, both adjusted for age and body height, as well as between the BMD-tot and LEAN-tot adjusted for the same covariates.

Linkage analysis

Only DZ twins were examined at this stage. A total of 2162 individuals with LEAN-tot were genotyped and available for the analysis. Twin pairs formed independent families. The estimation of the proportion of alleles shared identical-by-descent (IBD) by a twin pair was obtained using GENEHUNTER 2 software (Whitehead Institute, MIT, Cambridge, MA). Multipoint genome-wide linkage analysis was conducted using generalized linear modeling in Stata (StataCorp LP). This regression technique is based on optimal Haseman-Elston methods (22), in which the square of the sibling difference in LEAN-tot phenotype (adjusted for age and other covariates) is regressed on the estimated proportion of alleles IBD. This method is algebraically equivalent to other likelihood techniques (23, 24). The prioritized regions were selected if the LOD score obtained was suggestive, *i.e.* more than 1.9 (23). The confirmation of the positive results was made by computing the genome-wide significance level (empirical *P* value) using a permutation approach (25). To obtain empirical *P* value estimates of the established LOD scores of interest, 1000 permutations of the data sets were performed for each LOD score, keeping both IBD structure and family structure intact. To keep twin pairs reserved, we computed the squared difference for each twin pair, which implies for 1081 DZ twin pairs we obtain 1081 "squared differences," *i.e.* we used the same phenotype as our phenotype for the genome scan. Phenotype values were permuted among individuals between the twin pairs. To obtain additional confirmatory evidence for the presence of the potential QTL in the chromosomal region(s) showing significant LOD scores, we used a bootstrap approach (26). Basically, the bootstrap is a procedure that involves choosing random samples with replacement from a data set and analyzing each sample the same way. Similarly to permutations, the bootstrap samples were sampled from the dependent variable consisting of 1081 squared differences. In this study the random samples of the same size as the original data set were drawn 2000 times with replacement. In each bootstrap replication b ($b = 1, \ldots, 2000$), we conducted a genome scan and each time estimated corresponding LOD scores.

It should be mentioned that concerning the X-chromosome, female twins share at least one paternal allele; the oversharing on X-chromosome may lead to a different distribution of the test statistic compared with autosomes. Because "Stata" package does not correct for the aforementioned bias and because of the poor coverage on X-chromosome, we will not consider X-chromosome associated linkage peaks in this study.

Results

Descriptive statistics and heritability estimates

Table 1 provides the basic descriptive statistics of the studied traits for MZ and DZ twins separately. All measurements

TABLE 1. Basic descriptive statistics for MZ and DZ twins

	MZ twins				DZ twins			
	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
Age (yr)	48.00	18.32	75.66	13.44	46.35	18.41	79.02	12.12
Weight (kg)	64.29	36.80	120.20	11.16	66.36	35.10	140.00	12.57
Height (cm)	162.19	144.00	180.00	6.16	162.74	141.00	191.00	6.20
BMI (kg/m^2)	24.45	15.12	46.15	4.10	25.06	13.22	56.88	4.64
LEAN-tot (g)	38,243	23,571	63,309	5,132	39,033	20,884	67,825	5,298
FAT-tot (g)	22,669	5,236	53,307	8,085	23,537	6,281	87,220	9,098
BMD-tot (g/cm^2)	1.125	0.790	1.460	0.112	1.144	0.768	1.966	0.112

BMI, Body mass index.

in both samples were very similar. LEAN-tot significantly correlated ($P < 0.001$) with age, anthropometrics, and FAT-tot and BMD. Table 2 summarizes the results of multiple regression analyses of LEAN-tot on all the potential covariates in the total sample. As seen, body height, FAT-tot, BMD-tot, and age were retained in the regression equation, collectively explaining some 51.5% of the LEAN-tot total variation. The distribution of lean data adjusted for age, age and height, and for all significant covariates closely followed the normality assumptions by the Kolmogorov-Smirnov test ($d = 0.018$; $P > 0.10$).

Adjusted for age only (adjustment No. 1) as well as for age and all other significant covariates (adjustment No. 3), correlations in LEAN-tot between both MZ and DZ twins were very sizeable and highly significant ($P < 0.001$ in all instances; Fig. 1). Model fitting analysis of LEAN-tot adjusted for age only showed that these correlations include large additive genetic ($h^2 = 0.740 \pm 0.040$) and minor but significant common environment components ($c^2 = 0.104 \pm 0.038$). Estimates of the familial effects of the LEAN-tot phenotypes adjusted for age and all other significant covariates showed that the most parsimonious model included a slightly lower contribution of the genetic factors ($h^2 = 0.652 \pm 0.046$) and modest common environment effects ($c^2 = 0.165 \pm 0.043$). Constraining any of these two effects to zero, in any of the aforementioned two analyses was rejected by the likelihood ratio test ($P < 0.001$).

Bivariate variance component analysis. After adjustment of each of the three body composition components for age and body height (adjustment No. 2), substantial phenotypical correlations were observed between LEAN-tot and BMD-tot ($r = 0.422$; $P < 0.001$), and LEAN-tot and FAT-tot ($r = 0.402$; $P < 0.001$). However, the correlation between BMD-tot and FAT-tot was low ($r = 0.078$), although formally statistically significant ($P < 0.05$). The aforementioned correlation pattern was observed consistently in both MZ and DZ twins.

Our bivariate genetic analysis included simultaneous estimates of variance components and adjustment for significant covariates (adjustment No. 2). Both genetic and environmental correlations between LEAN-tot and BMD-tot were statistically highly significant by likelihood ratio tests ($r_G = 0.286 \pm 0.019$ and $r_E = 0.336 \pm 0.031$) and all statistically different from zero ($P < 0.001$). Similarly, high and significant estimates were obtained in bivariate analysis of LEAN-tot and FAT-tot ($r_G = 0.479 \pm 0.017$ and $r_E = 0.501 \pm 0.022$). Our data showed no detectable common genetic factors between BMD-tot and FAT-tot ($r_G = 0.000$), however, shared environmental effects for these two variables were significant ($r_E = 0.209 \pm 0.032$).

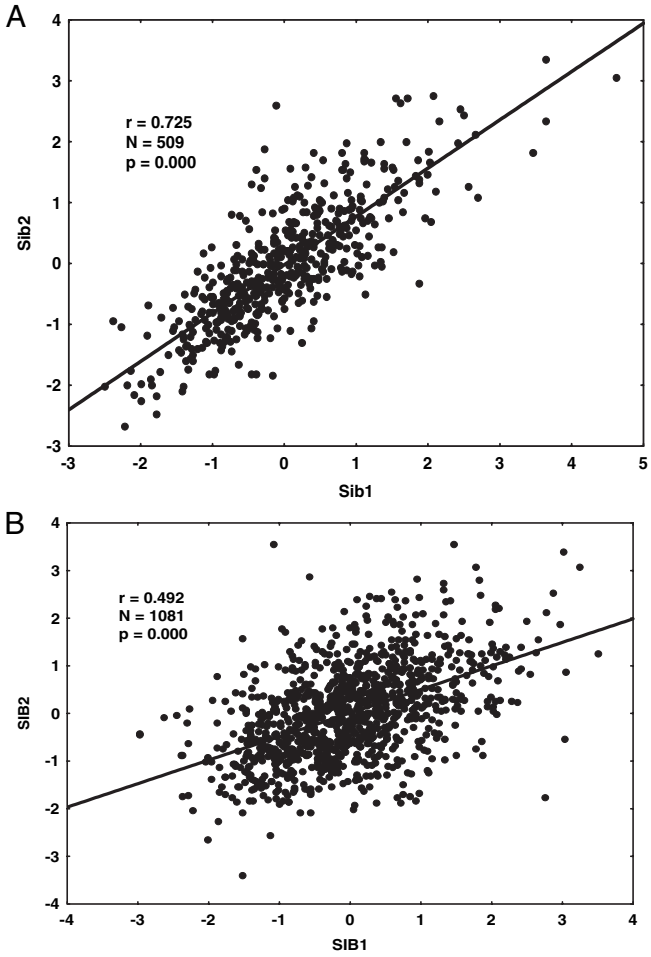

FIG. 1. Sibling correlations in LEAN-tot between MZ (A) and DZ (B) twins. The data were adjusted for all significant covariates (Table 2).

Linkage analysis

First, LEAN-tot adjusted for only age (adjustment No. 1) was subjected to the multipoint linkage analyses with all available markers. The analysis revealed no significant linkage signals, and there was only one suggestive peak on X-chromosome at 160cM: LOD = 1.94. However, when LEAN-tot was adjusted for all significant covariates (adjustment No. 3), significant and suggestive linkage peaks were observed on chromosomal regions 1p36.13, 2q31.1, 7p15.3–15.1, 8p22, 12q24.3, and 14q22.3 (Table 3). Table 3 shows only empirical LOD scores higher than 1.9. The maximum LOD score of 4.49 at the marker D12S1675 was observed on chromosome 12q24.3. Significant LOD = 3.62 at marker D14S276 was also observed on chromosome 14q22.3. Using permutation technique, we computed empirical *P* values for all six

TABLE 2. Regression summary for dependent variable: LEAN-tot in the total sample of United Kingdom twins

Covariate	β	SE of β	Multiple R^2	R^2 change	*P* value
Intercept					<0.001
Height	0.4093	0.0121	0.2759	0.2759	<0.001
BMD-tot	0.3371	0.0126	0.3978	0.1219	<0.001
FAT-tot	0.3561	0.0120	0.5030	0.1053	<0.001
Age	−0.1022	0.0129	0.5149	0.0119	<0.001

aforementioned LOD scores (Table 3). Of these, four linkage peaks appeared reliable: chromosomes 7, 8, 12, and 14, with $P = 0.0004$ for chromosome 12 being the most impressive result. Finally, as an additional test of the reliability of the LOD scores observed for these four chromosomes, we conducted the bootstrap analysis of the data. It should be mentioned that 95% confidence intervals in all instances included nonsignificant and not indicative LOD scores (<1.9). However, as seen in Table 4 for chromosome 12q and 14q, the mean and 50% LOD scores were significant (>3.0), and marginally significant (>2.8) for the remaining two chromosomes. In case of the 12q, the proportion of the bootstrapped LOD scores greater than three was observed in 72%.

Discussion

In this study, using a very large community based sample of more than 3000 MZ and DZ female twins, we showed that age, height, and total body fat and BMD explain some 51.5% of lean-body mass variation. Body height was a major predictor, with approximately 28% of the variation attributable to its effect, with FAT-tot mass explaining 11.9% and BMD 10.2%. Interestingly, we found almost negligible correlation of fat mass and BMD ($r = 0.078$), suggesting that the correlation between BMD and body mass index observed in this and other studies is attributable primarily to lean rather than fat mass of the body. These results are in good agreement with published data showing the importance of lean mass as a predictor for osteoporosis (5, 14) and concluding that it is a better predictor of BMD than fat mass (6, 27). Moreover, our data clearly suggest that this correlation is caused by genetic and environmental effects shared by LEAN-tot and BMD-tot, whereas the small correlation between FAT-tot and BMD-tot can be ascribed to common environmental factors only (*e.g.* shared diet or childhood exercise patterns). However, of interest is the fact that we observed substantial correlation between total lean and fat mass ($r = 0.402$), which was attributable to both shared genes and environment. Putative common environmental factors for lean and bone mass from one side and lean and fat mass from the other side are certainly of interest. Diet and physical activity seem obvious primary factors that may cause an association between the lean and fat mass, which in turn, interacts specifically physical activity, and may create a correlation between the lean and bone mass. This is well confirmed in a number of studies that showed that physical activity may increase BMD and bone strength by as much as 50%, especially if training was initiated before puberty (28). Therefore, in general terms, it is plausible that these and other behavioral factors may mostly determine the observed common r_E.

The major goal of this study was to evaluate the contribution of the putative genetic factors to interindividual differences in LEAN-tot and to identify specific chromosomal regions that can harbor corresponding genes. Present results suggest that lean mass in females is strongly determined by genes. About 65.2% (±4.6%) of the variation of LEAN-tot, adjusted for significant covariates, was attributable to genetic factors effect in this study. The impact of heritability on body lean mass has been evaluated in several previous studies (14–16). However, heritabilities are population specific, and some caution is needed when comparing the results obtained in ethnically different populations. Nevertheless, comparison of the heritability estimates for LEAN-tot obtained in various studies showed a relatively narrow range of the interpopulation variation, ranging from 0.56 ± 0.05 (9) to 0.60 ± 0.11 (14), perhaps because of the weak independent age effect and common environmental influence.

To our knowledge, our genome scan of 2162 DZ twins represents the first large-scale genome scan for lean mass and suggests reliable linkage of this phenotype to QTL on two chromosomal segments. The strongest signal was observed on chromosomes 12q24.32 (associated with marker D12S1675) and 14q22.3 (D14S276). The multipoint LOD scores were 4.49 and 3.62, respectively, with accompanied empirical P values = 0.0004 and 0.0013. These LOD scores correspond to nominal $P = 0.000005$ and 0.000042 (Table 3), and therefore achieved the P value 0.000049, significant for a genome-wide significance level of 0.05, according to guidelines proposed by Kruglyak and Lander (23), especially for the 12q segment. Both peaks were reasonably well supported by the bootstrapping of the data, which is important in light of the low reproducibility of many linkage results. They show that even for such a high LOD score as 4.49, observed in a large sample (n = 2162), a substantial proportion of the bootstrapped LOD scores (~28%) may be less than 3.0, and 12%, maybe even smaller than 1.9.

The additional marginally significant linkage peaks (LOD = 2.83 and 2.86) were observed on chromosome 7 (40cM from p terminus, D7S629 and D7S516, with an empirical P value = 0.0042) and on chromosome 8 (at 35cM, flanked by marker D8S258, with an empirical P value = 0.0044).

The best evidence for linkage in this study was detected at 12q24. Although, there are no prior linkage studies on total body lean mass, this region may certainly be of interest. First, recently our team found suggestive linkage for central fat mass to this chromosomal segment (29). In that study additional analysis of a number of available single nucleotide polymorphisms provided evidence for an association between central fat mass and two genes located on chromosome 12q24, PLA2G1B, and P2RX4 (12q23-q24.1; 119.24-.25Mb and 12q24.32; 120.13-.16Mb, respectively), with P values of 0.0067 and 0.017, respectively. However, of further interest in regard to LEAN-tot may be the fact that this region also harbors the acetyl-coenzyme A carboxylase-β (ACACB alias ACCB, 12q24.11; 108.06-.19Mb) gene. The product of this gene is primarily expressed in heart and skeletal muscles. In these tissues it is thought to be deeply involved in control of fatty acid oxidation and the regulation of energy homeostasis. Moreover, Schrauwen *et al.* (30) found that even a minimal amount of physical training tends to increase fat oxidation and leads to marked changes in the expression of ACACB. In their study ACACB mRNA expression was significantly decreased after training ($P = 0.005$), whereas lipoprotein lipase mRNA expression tended to increase ($P = 0.07$). It has been shown in animal models that mutations in this gene may strongly be associated with adipose tissue metabolism and can accumulate a lesser amount of fat than wild-type mice on the same diet. The knockout mice at this gene do not survive even embryonic development (31). Rel-

TABLE 3. Significant LOD scores observed in multipoint linkage analysis of LEAN-tot adjusted for all covariates in the DZ twins sample

Chromosomal segment	Nominal LOD score (*P* value)	Linkage peak location (cM)	Empirical *P* value	Closest DNA marker	Marker location (cM)
1p36.13	2.17 (0.0016)	45.0	0.0121	D1S199	47.0
2q31.1	2.26 (0.0012)	185.0	0.0105	D2S2257	184.3
7p15.3–7p15.1	2.86 (0.0003)	40.0	0.0042	D7S629; D7S516	38.5; 43.1
8p22	2.83 (0.003)	35.0	0.0044	D8S258	39.8
12q24.3	4.49 (5.43^{-6})	155.0	0.0004	D12S1675	155.2
14q22.3	3.62 (0.00004)	50.0	0.0013	D14S276	50.5

atively recently, Oh *et al.* (32) showed that the enzymatic product of this gene in humans is differently expressed in skeletal muscles and liver. They found that there are two promoters, P-I and P-II, controlling the transcription of the ACACB gene, of which P-I is active in the skeletal muscles and heart, but not in the liver. It would certainly be of importance for a future study to test whether the linkage we observed is associated with P-I.

As mentioned, currently there are very scarce published data on the genetics of total body lean mass. Two similar linkage studies to ours using a slightly different phenotype are the Quebec Family Study (33), which used 748 subjects genotyped for 292 microsatellite markers to find genes linked to fat-free mass (FFM), and the HERITAGE Study (34), which performed genome scan (with the use of 344 markers) for FFM at baseline and after 20 wk of endurance exercise in 364 sib pairs. However, their method implements underwater weighing, and it does not differentiate between bone and muscle mass. Therefore, their results are not directly comparable. Nevertheless, they reported among their significant linkage results chromosome 7p15.3 ($P = 0.0002$), observed also in the present study. The authors suggested that this linkage is probably related more to the skeletal muscle component of FFM than to BMD.

Because linkage peaks have wide confidence intervals (35, 36), which harbor hundreds of genes, the observed linkage signals are difficult to translate into obvious candidate genes with any confidence. However, within chromosome 7p15.1–15.3 mapped at least two genes of interest: the GSBS (7p15; 31.69-.71Mb) gene causing susceptibility to hypercholesterolemia (37), and DSMAV (7p15; 30.60-.64Mb) gene, mutations in which were associated with spinal muscular atrophy type V (38). However, there are no available data suggesting the association of these genes with lean mass, and ACACB gene looks, therefore, to be a much more promising candidate for follow-up.

There are some limitations to this study. The measurement method implemented in this project does not differentiate between the muscles and tendons from the one side and other soft tissues (viscera) on the other side. It is assumed that this method is inferior to computed tomography, although the recent comparison demonstrated good concordance between DXA and computed tomography for abdominal total tissue mass and fat mass (39). Moreover, in this study DXA also showed excellent reliability among three different operators to determine fat and lean body mass in the L1–L4 region of interest. Another limitation is that the obtained results may not be directly applicable to males having in general much more developed musculature and more massive bones. On the other side, using only females may not necessarily be a limitation but rather a possible advantage because it is not prone to potential genotype x sex interactions, which are likely the rule rather than exception (40). This is particularly important for body composition components, which are substantially different between the sexes due to fundamental differences in sex-related hormonal status.

There are additional major strengths of this study. These families were selected randomly from the relatively homogeneous ethnic background, and, therefore, are informative for genetic-mapping studies and representative of the general population. If there were substantial genetic heterogeneity, our study would have limited power to detect linkage signals. Age matching of twins and shared household decreased substantially random effects of environment, and made heritability estimates and linkage results more accurate than might be expected for the heterogeneous composition of the complex pedigree.

In summary, we have shown that LEAN-tot variation is a highly heritable trait. We have observed for the first time reliable linkage between LEAN-tot and DNA markers located on chromosome 12q and 14q, and good evidence of linkage on chromosome 7p and 8p. The linkage peaks on chromosomes 12q and 7p were confirmed in previous studies of related phenotypes. Of special interest is the linkage on 12q that may be caused by the physiologically important and relevant ACACB gene. Replication studies from other populations followed by fine mapping should be the next step in identifying the individual genes involved that could have

TABLE 4. Bootstrap results for main linkage peaks in the DZ twins sample

Chromosome	Observed LOD	Bootstrap results				
		Percentiles/LOD scores				Bias
		Mean	25%	50%	75%	
7 at 40 cM (7p15.3–15.1)	2.86	3.11	1.80	2.78	4.09	0.245
8 at 35 cM (8p22)	2.83	3.12	1.59	2.68	4.29	0.291
12 at 155 cM (12q24.3)	4.49	4.74	2.86	4.38	6.20	0.250
14 at 50 cM (14q22.3)	3.62	3.86	2.17	3.50	5.09	0.240

important physiological effects on bone and fat, as well as muscle.

Acknowledgments

Received February 23, 2007. Accepted May 30, 2007.

Address all correspondence and requests for reprints to: Tim D. Spector, Twin Research and Genetic Epidemiology Unit, St. Thomas' Hospital, Kings College London, London SE1 7EH, United Kingdom. E-mail: tim.spector@kcl.ac.uk.

This work was supported by Wellcome Trust, Arthritis Research Campaign, EU FW5 Genomeutwin project.

Disclosure Statement: The authors have nothing to declare.

References

1. **Daniels ED, Pettifor JM, Schnitzler CM, Russell SW, Patel DN** 1995 Ethnic differences in bone density in female South African nurses. J Bone Miner Res 10:359–367
2. **Reid IR** 2002 Relationships among body mass, its components, and bone. Bone 31:547–555
3. **Shaw DT, Rozeboom DW, Hill GM, Orth MW, Rosenstein DS, Link JE** 2006 Impact of supplement withdrawal and wheat middling inclusion on bone metabolism, bone strength, and the incidence of bone fractures occurring at slaughter in pigs. J Anim Sci 84:1138–1146
4. **Olszynski WP, Shawn Davison K, Adachi JD, Brown JP, Cummings SR, Hanley DA, Harris SP, Hodsman AB, Kendler D, McClung MR, Miller PD, Yuen CK** 2004 Osteoporosis in men: epidemiology, diagnosis, prevention, and treatment. Clin Ther 26:15–28
5. **Li S, Wagner R, Holm K, Lehotsky J, Zinaman MJ** 2004 Relationship between soft tissue body composition and bone mass in perimenopausal women. Maturitas 47:99–105
6. **Liu-Ambrose T, Kravetsky L, Bailey D, Sherar L, Mundt C, Baxter-Jones A, Khan KM, McKay HA** 2006 Change in lean body mass is a major determinant of change in areal bone mineral density of the proximal femur: a 12-year observational study. Calcif Tissue Int 79:145–151
7. **Nguyen TV, Howard GM, Kelly PJ, Eisman JA** 1998 Bone mass, lean mass, and fat mass: same genes or same environments? Am J Epidemiol 147:3–16
8. **Sun X, Lei SF, Deng FY, Wu S, Papacian C, Hamilton J, Recker RR, Deng HW** 2006 Genetic and environmental correlations between bone geometric parameters and body compositions. Calcif Tissue Int 79:43–49
9. **Danielson ME, Cauley JA, Baker CE, Newman AB, Dorman JS, Towers JD, Kuller LH** 1999 Familial resemblance of bone mineral density (BMD) and calcaneal ultrasound attenuation: the BMD in mothers and daughters study. J Bone Miner Res 14:102–110
10. **Jones G, Nguyen TV** 2000 Associations between maternal peak bone mass and bone mass in prepubertal male and female children. J Bone Miner Res 15: 1998–2004
11. **Perusse L, Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Snyder EE, Bouchard C** 2005 The human obesity gene map: the 2004 update. Obes Res 13:381–490
12. **Zmuda JM, Sheu YT, Moffett SP** 2006 The search for human osteoporosis genes. J Musculoskelet Neuronal Interact 6:3–15
13. **Arden NK, Spector TD** 1997 Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. J Bone Miner Res 12:2076–2081
14. **Hsu FC, Lenchik L, Nicklas BJ, Lohman K, Register TC, Mychaleckyj J, Langefeld CD, Freedman BI, Bowden DW, Carr JJ** 2005 Heritability of body composition measured by DXA in the diabetes heart study. Obes Res 13:312–319
15. **Blain H, Vuillemin A, Guillemin F, Jouanny P, Jeandel C, Le Bihan E** 2006 Lean mass plays a gender-specific role in familial resemblance for femoral neck bone mineral density in adult subjects. Osteoporos Int 17:897–907
16. **Jensen MD, Kanaley JA, Roust LR, O'Brien PC, Braun JS, Dunn WL, Wahner HW** 1993 Assessment of body composition with use of dual-energy x-ray absorptiometry: evaluation and comparison with other methods. Mayo Clin Proc 68:867–873
17. **Spector TD, MacGregor A** 2002 The St. Thomas' UK adult twin registry. Twin Res 5:440–443
18. **Pritchard LE, Kawaguchi Y, Reed PW, Copeman JB, Davies JL, Barnett AH, Bain SC, Todd JA** 1995 Analysis of the CD3 gene region and type 1 diabetes: application of fluorescence-based technology to linkage disequilibrium mapping. Hum Mol Genet 4:197–202
19. **Kong X, Murphy K, Raj T, He C, White PS, Matise TC** 2004 A combined linkage-physical map of the human genome. Am J Hum Genet 75:1143–1148
20. **Snieder H, MacGregor AJ, Spector TD** 1998 Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. J Clin Endocrinol Metab 83:1875–1880
21. **Malkin I, Ginsbirg E** 2006 MAN Program package for Mendelian analysis of pedigree data (MAN, version 7). Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Technical Report
22. **Barber MJ, Cordell HJ, MacGregor AJ, Andrew T** 2004 Gamma regression improves Haseman-Elston and variance components linkage analysis for sib-pairs. Genet Epidemiol 26:97–107
23. **Kruglyak L, Lander ES** 1995 Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454
24. **Sham PC, Purcell S** 2001 Equivalence between Haseman-Elston and variance component linkage analysis for sib pairs. Am J Hum Genet 68:1527–1532
25. **Iturria SJ, Williams JT, Almasy L, Dyer TD, Blangero J** 1999 An empirical test of the significance of an observed quantitative trait locus effect that preserves additive genetic variation. Genet Epidemiol 17(Suppl 1):S169–S173
26. **Liu BH** 1998 Bootstrap. In: Liu BH, ed. Statistical genomics: linkage, mapping, and Qtl analysis. Chap. 19.2.1. Boca Raton, FL: CRC Press; 546–551
27. **Sahin G, Polat G, Baethis S, Milcan A, Baethdatoethlu O, Erdoethan C, Camdeviren H** 2003 Body composition, bone mineral density, and circulating leptin levels in postmenopausal Turkish women. Rheumatol Int 23:87–91
28. **Karlsson MK, Gerdhem P, Ahlborg HG** 2005 The prevention of osteoporotic fractures. J Bone Joint Surg Br 87:1320–1327
29. **Wilson SG, Adam G, Langdown M, Reneland R, Braun A, Andrew T, Surdulescu GL, Norberg M, Dudbridge F, Reed PW, Sambrook PN, Kleyn PW, Spector TD** 2006 Linkage and potential association of obesity-related phenotypes with two genes on chromosome 12q24 in a female dizygous twin cohort. Eur J Hum Genet 14:340–348
30. **Schrauwen P, van Aggel-Leijssen DP, Hul G, Wagenmakers AJ, Vidal H, Saris WH, van Baak MA** 2002 The effect of a 3-month low-intensity endurance training program on fat oxidation and acetyl-CoA carboxylase-2 expression. Diabetes 51:2220–2226
31. **Abu-Elheiga L, Matzuk MM, Kordari P, Oh W, Shaikenov T, Gu Z, Wakil SJ** 2005 Mutant mice lacking acetyl-CoA carboxylase 1 are embryonically lethal. Proc Natl Acad Sci USA 102:12011–12016
32. **Oh SY, Lee MY, Kim JM, Yoon S, Shin S, Park YN, Ahn YH, Kim KS** 2005 Alternative usages of multiple promoters of the acetyl-CoA carboxylase β gene are related to differential transcriptional regulation in human and rodent tissues. J Biol Chem 280:5909–5916
33. **Chagnon YC, Borecki IB, Perusse L, Roy S, Lacaille M, Chagnon M, Ho-Kim MA, Rice T, Province MA, Rao DC, Bouchard C** 2000 Genome-wide search for genes related to the fat-free body mass in the Quebec family study. Metabolism 49:203–207
34. **Chagnon YC, Rice T, Perusse L, Borecki IB, Ho-Kim MA, Lacaille M, Pare C, Bouchard L, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C, HERITAGE Family Study** 2001 Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. J Appl Physiol 90:1777–1787
35. **Dupuis J, Siegmund D** 1999 Statistical methods for mapping quantitative trait loci from a dense set of markers. Genetics 151:373–386
36. **Manichaikul A, Dupuis J, Sen S, Broman KW** 2006 Poor performance of bootstrap confidence intervals for the location of a quantitative trait locus. Genetics 174:481–489
37. **Ono S, Ezura Y, Emi M, Fujita Y, Takada D, Sato K, Ishigami T, Umemura S, Takahashi K, Kamimura K, Bujo H, Saito Y** 2003 A promoter SNP (-1323T-C) in G-substrate gene (GSBS) correlates with hypercholesterolemia. J Hum Genet 48:447–450
38. **Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin SQ, Jordanova A, Kremensky I, Christodoulou K, Middleton LT, Sivakumar K, Ionasescu V, Funalot B, Vance JM, Goldfarb LG, Fischbeck KH, Green ED** 2003 Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. Am J Hum Genet 72:1293–1299
39. **Glickman SG, Marn CS, Supiano MA, Dengel DR** 2004 Validity and reliability of dual-energy X-ray absorptiometry for the assessment of abdominal adiposity. J Appl Physiol 97:509–514
40. **Weiss LA, Pan L, Abney M, Ober C** 2006 The sex-specific genetic architecture of quantitative traits in humans. Nat Genet 38:218–222

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.