Published in final edited form as:

Hum Genet. 2006 November ; 120(4): 561–570.

Is a gene important for bone resorption a candidate for obesity? An association and linkage study on the RANK (receptor activator of nuclear factor- κ B) gene in a large Caucasian sample

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Abstract

In light of findings that osteoporosis and obesity may share some common genetic determination and previous reports that RANK (receptor activator of nuclear factor- κ B) is expressed in skeletal muscles which are important for energy metabolism, we hypothesize that RANK, a gene essential for osteoclastogenesis, is also important for obesity. In order to test the hypothesis with solid data we first performed a linkage analysis around the RANK gene in 4,102 Caucasian subjects from 434 pedigrees, then we genotyped 19 SNPs in or around the RANK gene. A family-based association test (FBAT) was performed with both a quantitative measure of obesity [fat mass, lean mass, body mass index (BMI), and percentage fat mass (PFM)] and a dichotomously defined obesity phenotype—OB (OB if BMI $\geq$ 30 kg/m²). In the linkage analysis, an empirical $P = 0.004$ was achieved at the location of the RANK gene for BMI. Family-based association analysis revealed significant associations of eight SNPs with at least one obesity-related phenotype ($P < 0.05$). Evidence of association was obtained at SNP10 ($P = 0.002$) and SNP16 ($P = 0.001$) with OB; SNP1 with fat mass ($P = 0.003$); SNP1 ($P = 0.003$) and SNP7 ($P = 0.003$) with lean mass; SNP1 ($P = 0.002$) and SNP7 ($P = 0.002$) with BMI; SNP1 ($P = 0.003$), SNP4 ($P = 0.007$), and SNP7 ($P = 0.002$) with PFM. In order to deal with the complex multiple testing issues, we performed FBAT multi-marker test (FBAT-MM) to evaluate the association between all the 18 SNPs and each obesity phenotype. The P value is 0.126 for OB, 0.033 for fat mass, 0.021 for lean mass, 0.016 for BMI, and 0.006 for PFM. The haplotype data analyses provide further association evidence. In conclusion, for the first time, our results suggest that RANK is a novel candidate for determination of obesity.

Introduction

The genetic and phenotypic correlation between two complex diseases: obesity and osteoporosis have been established across different ethnic groups (Coin et al. 2000; Deng et al. 2006; Toth et al. 2005). We revealed that body mass index (BMI) and bone mineral density

(BMD) shared about 10–20% genetic determination in Chinese (Deng et al. 2006). Adipocytes and osteoblasts originate from a common progenitor, pluripotential mesenchymal stromal cells, and some common factors may regulate the differentiation of these two cell lines (Akune et al. 2004; Gregoire et al. 1998). Recent molecular genetic studies also provided some common candidate genes for both bone and obesity (Liu et al. 2005b, 2003; Perusse et al. 2005; Thomas and Burguera 2002).

Receptor activator of nuclear factor- κ B (RANK), a molecule of the tumor necrosis factor-related family, plays a key role in inducing osteoclastogenesis (Bell 2003). It interacts with RANK ligand (RANKL) to stimulate proliferation and differentiation of osteoclasts as well as inhibit osteoclast apoptosis (Bell 2003). In addition, the genomic region harboring the RANK gene has been replicated to be linked with obesity via multiple human and animal studies (Cheverud et al. 2004; Perusse et al. 2005; Snyder et al. 2004). Furthermore, RANK is demonstrated to be highly expressed in skeletal muscle (Anderson et al. 1997), a key anatomic site for burning energy. All of the above implicated that RANK could be a pleiotropic genetic factor influencing both osteoporosis and obesity phenotypes. Therefore, we hypothesize that RANK, a gene essential for bone resorption, is linked and/or associated with obesity-related phenotypes. In this exploratory analysis, we first conducted a linkage study on the genomic region containing the RANK gene; we then carried out a family-based association analysis of dense SNPs spanning the RANK gene in a large Caucasian sample.

Materials and methods

Subjects

The study was approved by the Creighton University Institutional Review Board. All the study subjects signed informed-consent documents before entering the project. The study subjects came from an expanding database created for ongoing studies in the Osteoporosis Research Center (ORC) of Creighton University to search for genes underlying common human complex traits, including obesity and osteoporosis, etc. The sampling scheme and exclusion criteria have been detailed elsewhere (Deng et al. 2002). Briefly, patients with chronic diseases and conditions which may potentially affect the development of human obesity as well as other studied traits were excluded from the study. All the study subjects were Caucasians of European origin.

In the linkage study, the sample contained a total of 4,102 phenotyped subjects from 434 pedigrees (see Table 1 for their basic characteristics), of whom 3,726 subjects were genotyped. The sample mainly consisted of pedigrees of median to large size and provided us an exceedingly large number of relative pairs (>150,000) informative for linkage analysis. The study design and recruitment procedures were published before (Xiong et al. 2006).

In the association study, as indicated before (Liu et al. 2004; Zhao et al. 2004), we selected a total of 405 unrelated nuclear families with 1,873 individuals from the whole sample used for the linkage study. Nuclear families were selected based on the following criteria: (1) families with two parents and at least two children have a priority to be selected; (2) if there is only one parent available, families with at least three children have priority to be selected; and (3) children's age are preferred to be less than 50 years or premenopause for female. Among the 405 nuclear families, 341 families were composed of both parents and at least one offspring. The remaining 64 families, with one or no parent, contained two or more children. There were 27.2, 22.7, 22.7 and 27.4% of nuclear families with one, two, three and more than three children, respectively, yielding 1,512 sibling pairs in our sample.

Phenotype measurement

Body mass index was calculated as body weight (in kilograms) divided by the square of height (in meters). Weight was measured in light indoor clothing without shoes, using a calibrated balance beam scale, and height was measured using a calibrated stadiometer. Fat mass and lean mass were measured by dual-energy X-ray absorptiometry using a Hologic 2,000+ or 4,500 scanner (Hologic, Bedford, MA). The percentage fat mass (PFM) is the ratio of fat mass divided by body weight (i.e., the sum of fat mass, lean mass, and bone mass). Both machines were calibrated daily. The measurement precision of BMI as reflected by the coefficient of variation was 0.2%. The coefficients of variation for fat mass, PFM and lean mass were 2.2, 2.2 and 1.0%, respectively, for measurements obtained on the Hologic 2,000+, and 1.2, 1.1 and 0.7%, respectively, for measurements obtained on the Hologic 4,500. Members of the same family were generally measured on the same type of machine.

Genotyping

For each subject, DNA was extracted from peripheral blood using the Puregene DNA isolation kit (Gentra systems, Minneapolis, MN, USA). DNA concentration was assessed by a DU530 UV/VIS spectrophotometer (Beckman Coulter, Inc, Fullerton, CA, USA).

In the linkage study, nine microsatellite markers—GATA11A06, GATA64H04, GATA13, GATA6D09, ATA23G05, ATA7D07, GATA7E12, ATA82B02N, and GATA177C03N—were genotyped. These markers were spaced across the ~90 cM region containing the RANK gene. They were selected from the Marshfield screening set 14 by Marshfield Center for Medical Genetics. The detailed genotyping protocol is available at http://research.marshfieldclinic.org/genetics/Lab_Methods/methods.html. A genetic database management system (GenoDB) (Li et al. 2001) was used to manage the phenotype and genotype data for linkage analyses. GenoDB was also used for allele binning (including setting up allele binning criteria and converting allele sizes to distinct allele numbers), data quality control, and data formatting for PedCheck (O'Connell and Weeks 1998) and linkage analysis.

In the association study, SNPs were selected on the basis of the following criteria: (1) validation status (validated experimentally in human populations), especially in Caucasians; (2) an average density of 1 SNP per 3 kb; (3) degree of heterozygosity, i.e., MAF > 0.05; (4) reported to dbSNP by various sources. A total of 19 SNPs within and around the RANK gene were selected and successfully genotyped using the high-throughput BeadArray SNP genotyping technology of Illumina Inc. (San Diego, CA, USA). For the 19 SNPs, the average rate of missing genotype data was ~0.05%. The reproducibility rate as revealed through blind duplicating was 100%. Among the 19 SNPs, one SNP (rs17069906) has minor allele frequency (MAF) less than 0.05 and were therefore removed for subsequent data analyses according to common practice (Newton-Cheh and Hirschhorn 2005).

For the linkage and association study, PedCheck (O'Connell and Weeks 1998) was performed to ensure that the genotype data conform to Mendelian inheritance pattern at all the marker loci. Any inconsistent genotypes were removed.

Statistical analyses

Linkage study—The bivariate variance component analyses for obesity-related quantitative traits (BMI, fat mass, lean mass, and PFM) (Almasy and Blangero 1998) were carried out using SOLAR (sequential oligogenic linkage analysis routines) (Almasy and Blangero 1998). Age and sex were tested for importance on obesity variation and significant factors were adjusted as covariates in further analyses. The bivariate quantitative genetic analysis estimated the genetic correlation ρ_G and environmental correlation ρ_E shared between obesity phenotypes. Phenotypic correlation is calculated according to the ρ_G , ρ_E , and the heritability of obesity

phenotypes (Lynch and Walsh 1998). Multi-point LOD (log of odds) scores were calculated by using the maximum likelihood methods. To estimate the empirical P values for observed LOD scores we carried out 10,000 simulations using the procedure “lodadj” implemented in SOLAR (Blangero et al. 2000). The LOD scores given in the text were empirically adjusted LOD scores. The empirical P value for adjusted LOD scores was calculated using the “empp” command in SOLAR.

Association study—In total 18 SNPs with MAF > 0.05 were used for the data analyses. LD (linkage disequilibrium) block structure of the RANK gene was examined by the program Haploview (Barrett et al. 2005). The D' values for all pairs of SNPs were calculated and the haplotype blocks were estimated using the confidence-interval method (Gabriel et al. 2002). SNPs with low MAF may inflate estimates of D' and the use of confidence-bound estimates for D' reduces this bias. The default settings were used in these analyses, which invoked a one-side upper 95% confidence bound of $D' > 0.98$ and a lower bound of > 0.7 to define SNP pairs in strong LD. A block is identified when at least 95% of SNP pairs in a region meet these criteria for strong LD. Haplotypes were reconstructed and their frequencies estimated using an accelerated expectation-maximization (EM) algorithm similar to the partition/ligation method (Qin et al. 2002) implemented in Haploview. Haplotype tag SNPs were selected by Haploview on a block-by-block basis.

Initially, allelic association was conducted for each SNP marker, by using family-based association test (FBAT) V1.7.2 (Horvath et al. 2001). The additive genetic model was applied for allelic association, which examines the transmission of the interested markers from parents to the affected offspring. The null hypothesis here is no linkage and no association between the marker and the underlying causal locus. The $-o$ flag was used to minimize the variance of the FBAT statistic. The haplotype version of FBAT (HBAT) (Horvath et al. 2004) was applied to test the association between phenotypes and within-block haplotypes. Both FBAT and HBAT are robust to population admixture, phenotype distribution misspecification, and ascertainment bias.

In order to maximize power in our correlated obesity-related phenotypes, Monte Carlo permutation procedures were implemented in HBAT (10,000 permutations were performed) to calculate the empirical P values for both single-SNP and haplotype markers. The reported empirical P values from HBAT program are adjusted for the phenotypes that do not fit the asymptotic tests. Since the SNP markers are in high LD and the phenotypes are all highly correlated, results for individual SNP may be highly correlated. Therefore, a simple Bonferroni-correction may be overly too conservative to adjust for the complicated multiple testing. In this study, for single-SNP test, FBAT multi-marker test (FBAT-MM) were used to deal with multiple comparisons. The FBAT-MM simultaneously tests H_0 : no linkage or association between any marker and any Disease Susceptibility Loci underlying the trait.

Family-based association test was conducted for both qualitative obesity phenotype and quantitative obesity-related phenotypes (fat mass, lean mass and BMI). Dichotomous obesity phenotype (denoted as “OB”) was defined according to the proposed cut-off points by a World Health Organization (WHO) expert committee (Word Health 2000) (OB if BMI ≥ 30 kg/m²). The reason of studying OB is that genes controlling qualitative obesity phenotypes may have no effect on quantitative obesity-related traits and therefore will be missed if only quantitative phenotypes were studied. Simultaneously testing associations using both quantitative and qualitative obesity-related traits will thus further our understanding of the effects the RANK gene has on obesity. Moreover, it becomes common in obesity research to study both major predicative quantitative trait and its related dichotomous qualitative phenotype (Jiang et al. 2004).

Results

Linkage study

Linkage analysis was conducted for the four obesity-related quantitative traits (BMI, fat mass, lean mass, and PFM) in 434 pedigrees, containing 4,102 study individuals (see Materials and methods). Descriptions of the study phenotypes are provided in Table 1. In both males and females, BMI and fat mass increased with age, peaked at about age 60, and such trends are consistent with those in previous studies (Caterson and Gill 2002; Erens and Primatesta 1999; Pi-Sunyer 2002). Lean mass is relatively stable before age 50 and decreases with aging. Table 2 summarized the genetic, environmental, and phenotypic correlations between obesity phenotypes. It can be seen that the obesity phenotypes share both genetic and environmental factors, and they are highly correlated.

In the region of the RANK gene, we detected linkage evidence for BMI in 18q21.33 with a multipoint LOD score of 1.54 (empirical P value of 0.004). The RANK gene is located at the peak LOD position, which is about 95 cM from the tip of the short arm of chromosome 18 (Fig. 1). We did not detect any significant results for fat mass, lean mass, and PFM at the locus harboring the RANK gene.

Association study

The phenotypes of the study samples of the association study were described in our previous publication (Liu et al. 2005a). Table 3 presents the information for the 18 SNPs used for data analyses. The SNPs are within or around the RANK gene, with average marker distance of ~3.9 kb. The SNP markers are presented according to their physical location. SNPs tested in RANK gene are all intronic ones, with average of MAFs 31% (ranging from 11 to 50%). Six haplotype blocks were identified for the RANK gene (see Fig. 2), ranging from 2 to 11 kb in length. Haplotype blocks 1 and 2 mainly spanned intron 1. Block 3 spanned from intron 2 to intron 3. Block 4 ranged from intron 3 to intron 4. Block 5 extended from intron 7 to intron 9. Block 6 spanned the 3'-downstream of the RANK gene. Figure 3 shows the haplotype structure and diversity. Among the 18 SNPs, 16 SNPs were identified as haplotype tag SNPs. One SNPs (SNP15) had low LD with any other SNPs and could not be assigned to any blocks.

The empirical P values for single-SNP analysis were presented in Table 4. Eight SNPs (SNP1, SNP2, SNP4, SNP7, SNP10, SNP16, SNP17, and SNP18) showed evidence of association with either quantitative measures of obesity adjusted for age and sex or the qualitative OB trait ($P < 0.05$). Evidence of association was obtained at SNP10 ($P = 0.002$) and SNP16 ($P = 0.001$) with OB; SNP1 with fat mass ($P = 0.003$); SNP1 ($P = 0.003$) and SNP7 ($P = 0.003$) with lean mass; SNP1 ($P = 0.002$) and SNP7 ($P = 0.002$) with BMI; SNP1 ($P = 0.003$) and SNP7 ($P = 0.002$) with PFM. Among the eight SNPs, SNP1 and SNP2 showed the most consistent associations across all phenotypes ($P < 0.05$). In addition, significant results were observed for marker SNP4 and SNP7 with all the quantitative traits ($P < 0.05$). It should be noted that the results in Table 4 has not been adjusted for multiple comparisons. In order to deal with the complex multiple testing issues, we used the FBAT multi-marker test (FBAT-MM) to test association between each obesity-related phenotype and all the 18 SNPs. In the FBAT-MM test, the P value is 0.126 for OB, 0.033 for fat mass, 0.021 for lean mass, 0.016 for BMI, and 0.006 for PFM. Given five phenotypes are tested, Bonferroni correction is further used to establish the significant threshold for the multi-marker test, which is 0.01 ($\alpha = 0.05/5$, totally five phenotypes). PFM met this stringent criterion, which indicated that some SNPs of the RANK gene are associated with PFM.

Based on the haplotypes constructed by the 18 SNPs, we conducted the haplotype analyses and the results further supported the association of the RANK gene with obesity (Table 5). The

P values for whole markers in a haplotype block were conducted using 10,000 Monte-Carlo permutations, which adjusted for the phenotypes that did not fit the asymptotic tests. Haplotypes containing the eight most important individual markers showed significant association with at least one obesity-related trait. The haplotype Block 1, representing SNP1, SNP2 and SNP3, was found to be significantly associated with BMI ($P = 0.008$). Haplotype block 3 was associated with fat mass ($P = 0.006$), lean mass ($P = 0.003$), BMI ($P = 0.001$), and PFM ($P = 0.002$). Haplotype block 5, containing SNP10, and haplotype block 6, containing SNP16, were associated with OB (with $P = 0.001$ and 0.01 , respectively). Some of the results remained significant even after adjusting for multiple testing using the most conservative Bonferroni correction method, by which the significance level for a single test is set as 0.002 ($\alpha = 0.05/(6 \times 5) \approx 0.002$; 6 haplotype blocks and five phenotypes) (Table 5).

Discussion

To the best of our knowledge, this study is the first attempt to test the importance of the RANK gene to obesity phenotypes. We first undertook a linkage study and an empirical $P = 0.004$ was achieved at the location of the RANK gene for BMI. Subsequently, both single marker and haplotype association analyses suggest that a number of common RANK variants are associated with different measures of obesity, even after controlling for multiple testing using the unduly conservative method. We suspect that the associations between the intronic SNPs and obesity may be caused by the strong LD of those SNPs with potential functional genetic variants. The significant correlation between haplotype block 1 with obesity may be due to the high LD between block 1 with some variants in the promoter region or exon 1. The importance of exon 1 region in the RANK gene have been reported elsewhere (Hughes et al. 2000; Whyte and Hughes 2002). Mutations in the 15, 18, and 27 bp tandem duplication in exon 1 of RANK gene lead to three seemingly different skeletal diseases, expansile skeletal hyperphosphatasia (ESH), familial expansile osteolysis (FEO), and a familial form of Paget disease of bone (PDB) (Hughes et al. 2000; Whyte and Hughes 2002). The associations of haplotype block 5 and 6 with obesity may be due to their LD with the potential 3' UTR causal variants. However, the knowledge of the regulatory elements in the 3' UTR is far from comprehensive. Future functional analysis may help to identify the causal variants in the RANK gene for obesity.

RANK is essential for osteoclast formation and action (Bell 2003). It is the only receptor for RANKL and capable of initiating osteoclastogenic signal transduction through binding with RANKL or anti-RANK agonists (Bell 2003). The importance of RANK to both osteoporosis and obesity may be explained by the fact that there are common candidate genes for these two complex diseases. The examples include IGF-I, IGF-II, Leptin receptor, Leptin, NPY, VDR, ER- α , AR, TGF- β 1, IL-6, TNF- α , TNFR2, ApoE, adiponectin, and PPAR- γ (Liu et al. 2005b, 2003; Perusse et al. 2005). Among them, some factors were first identified to influence the development of osteoporosis; further, it is demonstrated to have an effect on adiposity, such as the calciotropic hormone receptor VDR (Reis et al. 2005) and ER- α (Deroo and Korach 2006). On the other hand, adipokine leptin (Reseland et al. 2001; Thomas and Burguera 2002) and adiponectin (Luo et al. 2005) were first reported to be associated with obesity, and later identified as significant factors in the regulation of bone mass. Considering the role of RANK in osteoclast formation and the evidence of association with both BMD (Hsu et al. 2006) and obesity, we suggest that RANK is a pleiotropic gene for both osteoporosis and obesity.

There is a widely held belief that increasing body weight (and thus higher risk of obesity) is associated with lower risk of osteoporosis (Bell 2004; Coates et al. 2004; Guney et al. 2003; Radak 2004; Wardlaw 1996). However, we previously found that in quantitative genetic analyses that increasing fat mass (thus higher risk to obesity) is actually associated with decreased bone mass (thus higher risk of osteoporosis) when the latter is adjusted for body weight (Zhao et al. 2005). Driven by this finding, we further examined whether the genetic

variations in the RANK gene may reduce/ increase the risk of obesity and osteoporosis in the same direction. We analyzed the association between the RANK gene and the risk of osteoporosis (OP, defined by lowest 10th percentile of BMD Z-score or by BMD T-score less than -2.5). The results revealed that the allele *T* of marker SNP7 (rs11664594) is not only associated with higher fat mass ($P = 0.008$), higher lean mass ($P = 0.003$), higher BMI ($P = 0.002$), but also related with higher risk of OP at both the spine ($P = 0.0002$) and hip ($P = 0.0001$) (Xiong et al. 2006). Thus, the current study provides evidence that genetic variations in a specific gene may increase the risk of obesity and osteoporosis in the same direction. This result echoes our earlier quantitative genetic analyses (Zhao et al. 2005).

Although the importance of RANK to bone biology was widely acknowledged, its importance to obesity was seldom reported. The linkage studies provide the first clue that the RANK gene is linked to obesity. The obesity QTL (quantitative trait loci) harboring the RANK gene have been replicated by numerous independent studies (Moody de Fau-Pomp et al.; Norman et al. 1998; Ohman et al. 2000; Shmulewitz et al. 2006) in both mice and humans (Perusse et al. 2005). In mice, QTL harboring the RANK gene were found to be linked with obesity (Cheverud et al. 2004). Moody et al. (1999) found that the QTL containing the RANK gene contributed to variation in energy balance (LOD = 5.62). In a genome-wide scan of obesity in human, the area flanking the RANK demonstrated a two-point MLS (maximum LOD score) of 2.4 in a set of Finnish subjects with BMI ≥ 32 kg/m² (Ohman et al. 2000) and a LOD score of 2.3 for percent body fat in Pima Indians (Norman et al. 1998). Northern blot analysis of human tissue RNA revealed that RANK mRNA is ubiquitously expressed, at a high level in skeletal muscle (Anderson et al. 1997). The high expression of RANK in skeletal muscle may suggest some potential functions of RANK on lean mass (mostly skeletal muscle). Lean mass is a major component of body mass and a key site for energy metabolism. It plays a critical role in the consumption of carbohydrate and lipid, and may contribute substantially to the development and maintenance of the obese status (Comuzzie and Allison 1998; Kopelman and Stock 1998).

In this study, both linkage and association study were performed. These two statistical approaches have their own merits and limitations, and can complement each other. The genome-wide linkage study is free of any prior hypothesis regarding which genes are involved. Compared with linkage analysis, the power of association is generally higher (Risch and Merikangas 1996). That may be the reason that our association study revealed significant association of RANK SNPs with BMI as well as fat mass, lean mass, and PFM, while the linkage study did not show any signal at the RANK region for fat mass, lean mass, and PFM. In the current association study, we have samples comprising 1,873 subjects from 405 Caucasian nuclear families, which render a relatively high statistical power. Assuming a marker is in strong LD ($|D'| = 0.9$) with a functional mutation that accounts for about 2% of phenotypic variation, our simulation shows that the present study has 90% power to detect association via the TDT. In addition, the MAF of the eight SNPs showing evidence of association are fairly high, ensuring a sufficient number of nuclear families informative for the TDT tests. However, this power calculation is only a rough estimate.

In conclusion, for the first time, we have shown evidence of linkage and association of the RANK gene with obesity in a large sample size. Considering the role of the RANK gene on bone, it is reasonable to speculate that RANK is a pleiotropic gene for determining osteoporosis and obesity. Our results indicate that genetic variation in this gene may reduce/increase the risk of obesity and osteoporosis in the same direction. Thus, it serves as excellent support for our earlier quantitative genetic analyses (Zhao et al. 2005). It will be important to replicate and confirm these findings in other independent studies.

Acknowledgements

Investigators of this work were partially supported by grants from NIH (R01 AR050496, K01 AR02170-01, R01 AR45349-01, and R01 GM60402-01A1) and an LB595 grant from the State of Nebraska. The study also benefited from grants from National Science Foundation of China, Huo Ying Dong Education Foundation, HuNan Province, Xi'an Jiaotong University, and the Ministry of Education of China.

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Fig. 1.

Linkage analysis profile for a region of ~90 cM around the RANK gene. The *x*-axis shows the distance in centimorgans from pter of chromosome 18, the *y*-axis shows the LOD score. BMI *solid line*, fat mass *long-dashed line*, lean mass *dotted line*, percentage fat mass *dashed line*

Fig. 2.

Gene structure and LD patterns for the *RANK* gene. Exons are depicted as *filled boxes*. Positions of the 18 SNPs used in the association study are sketched. LD block structure, as depicted by Haploview, is shown in the *bottom frame*. The increasing degree of darkness from white to black represents the increasing strength of LD. Values for $D' = 1$ are *dark black boxes* and $D' < 1$ (indicated as original value $\times 100$) are shown in the cells

Fig. 3.

Haplotype structure and diversity. SNP numbers corresponding to Fig. 2 are listed above each column of alleles, and *filled inverted triangle* denotes the haplotype tag SNPs. Recombination rates (numbers at the bottom) between adjacent blocks are defined by a multiallelic value of D' . Haplotypes in adjacent blocks are connected by a *thick line* if they occur together with a frequency of >10% and by a *thin line* if they occur together with a frequency >1% but <10%. For each SNP, alleles (A, T, C, G) are indicated. The haplotype frequency is denoted besides each corresponding haplotype

Table 1

Characteristics of the obesity-related phenotypes in the subjects for the linkage analyses stratified by age and sex

Age group	Age (years)	Fat mass (kg)	Lean mass (kg)	BMI (kg/m ²)	Fat mass (%)
Male					
20–29(250)	24.24 ± 3.04	18.19 ± 8.30	67.11 ± 9.25	26.07 ± 4.38	20.58 ± 6.34
30–39(279)	35.58 ± 2.87	21.01 ± 7.87	67.47 ± 8.22	27.51 ± 4.31	23.23 ± 5.69
40–49(383)	45.12 ± 2.88	23.26 ± 7.99	68.16 ± 8.23	28.08 ± 3.99	24.93 ± 5.54
50–59(309)	54.6 ± 2.96	24.32 ± 7.58	67.12 ± 8.44	28.95 ± 4.03	26.16 ± 5.03
60–69(204)	64.94 ± 2.78	25.76 ± 8.07	65.95 ± 8.65	29.47 ± 4.53	27.58 ± 5.18
70–79(152)	73.9 ± 2.53	25.48 ± 7.95	62.76 ± 7.24	28.66 ± 4.10	28.30 ± 5.33
80+ (48)	83.27 ± 3.41	22.15 ± 6.60	57.51 ± 7.96	27.01 ± 3.88	27.33 ± 5.58
Female					
19–29(355)	24.66 ± 3.15	22.24 ± 9.31	45.79 ± 6.80	24.47 ± 5.06	31.78 ± 6.82
30–39(489)	35.44 ± 2.85	24.04 ± 9.85	46.35 ± 6.76	25.38 ± 5.30	33.01 ± 7.28
40–49(633)	45.00 ± 2.84	26.50 ± 9.71	46.54 ± 6.75	26.65 ± 5.51	35.29 ± 6.65
50–59(412)	54.24 ± 2.90	28.53 ± 10.00	46.07 ± 6.80	27.33 ± 5.55	37.20 ± 6.09
60–69(312)	64.91 ± 2.83	30.47 ± 10.76	45.40 ± 7.04	28.06 ± 6.29	39.26 ± 6.43
70–79(218)	74.34 ± 2.82	29.67 ± 8.38	43.77 ± 5.88	27.93 ± 3.88	39.68 ± 5.16
80+ (58)	83.99 ± 3.26	27.47 ± 9.46	42.01 ± 5.11	27.56 ± 5.91	38.47 ± 6.79

For each trait, data are presented as mean ± SD. The numbers in the brackets are the sample size in each age group. There are a total of 4,102 subjects from 434 pedigrees summarized for this table. Although only 3,726 subjects have direct genotype data, genotypes of the remaining subjects may be inferred from their relatives, and thus these subjects are informative in linkage analyses. Therefore, their phenotype data are also included in the summary for this table *BMI* body mass index

Table 2

The environmental correlations (ρ_E), genetic correlations (ρ_G), and phenotypic correlations (ρ_P) between obesity related phenotypes

	Fat mass	Lean mass	Percentage fat mass
Body mass index			
ρ_G	0.879 [*]	0.670 [*]	0.706 [*]
ρ_E	0.937 [*]	0.744 [*]	0.798 [*]
ρ_P	0.924 [*]	0.717 [*]	0.778 [*]
Fat mass			
ρ_G		0.611 [*]	0.891 [*]
ρ_E		0.623 [*]	0.916 [*]
ρ_P		0.612 [*]	0.911 [*]
Lean mass			
ρ_G			0.219 [*]
ρ_E			0.369 [*]
ρ_P			0.325 [*]

The obesity phenotypes were adjusted by age and sex

P values were estimated by comparison with the likelihood of a nested model in which either ρ_G or ρ_E was fixed at zero (for ρ_G and ρ_E , respectively), or both were fixed at zero (for ρ_P)

^{*} $P < 0.001$

Table 3
Information of the 18 SNPs for the association analyses

SNP marker	Name	Intermarker interval (bp)	Alleles ^a	Minor allele frequency
1	rs4941125		G/A	0.32
2	rs7235803	2,695	G/A	0.34
3	rs4436867	3,265	A/C	0.24
4	rs8086340	3,334	G/C	0.45
5	rs12956925	6,662	A/G	0.18
6	rs3826619	2,110	A/G	0.11
7	rs11664594	2,456	A/T	0.36
8	rs3826620	3,298	A/C	0.28
9	rs12969194	2,561	A/T	0.26
10	rs4303637	7,698	G/A	0.32
11	rs17069904	1,186	A/G	0.11
12	rs6567274	4,851	A/C	0.33
13	rs12959396	1,509	C/A	0.48
14	rs4426449	3,508	A/G	0.34
15	rs9646629	8,382	C/G	0.35
16	rs884205	3,658	A/C	0.25
17	rs2957127	4,508	A/G	0.44
18	rs3017365	3,976	A/G	0.50

^aThe first allele is a minor allele

Table 4

Association results for allelic association based on 10,000× Monte Carlo permutations

SNP marker	OB		Fat mass		Lean mass		Body mass index		Percentage fat mass	
	Z	P	Z	P	Z	P	Z	P	Z	P
SNP1	-2.245	0.025	-2.971	0.003	-2.937	0.003	-3.078	0.002	-2.929	0.003
SNP2	-2.524	0.011	-2.797	0.005	-2.501	0.012	-2.702	0.007	-2.531	0.011
SNP3	0.247	0.822	1.142	0.267	1.301	0.183	1.423	0.155	1.089	0.276
SNP4	-0.426	0.665	-2.330	0.017	-2.623	0.008	-2.692	0.007	-2.699	0.007
SNP5	-1.611	0.103	-0.819	0.400	0.034	0.974	-0.411	0.681	-0.434	0.664
SNP6	-0.403	0.732	-0.506	0.592	-0.321	0.761	-0.553	0.580	-0.442	0.658
SNP7	-1.070	0.281	-2.697	0.008	-2.953	0.003	-3.100	0.002	-3.045	0.002
SNP8	-0.242	0.847	-0.633	0.492	-0.799	0.384	-0.739	0.460	-0.491	0.623
SNP9	1.747	0.082	1.063	0.286	0.761	0.455	0.839	0.401	0.559	0.576
SNP10	3.088	0.002	1.791	0.079	1.718	0.092	1.662	0.118	1.135	0.256
SNP11	-0.745	0.411	-1.178	0.235	-1.141	0.254	-1.319	0.187	-1.100	0.271
SNP12	-0.206	0.828	0.639	0.500	0.861	0.389	0.857	0.391	0.963	0.335
SNP13	-1.083	0.318	-0.900	0.337	-0.547	0.584	-0.777	0.437	-0.651	0.514
SNP14	-0.257	0.784	0.695	0.511	0.937	0.349	0.852	0.394	1.045	0.295
SNP15	1.905	0.056	1.641	0.100	1.733	0.088	1.763	0.072	1.382	0.166
SNP16	3.210	0.001	2.462	0.015	2.131	0.029	2.234	0.025	1.861	0.062
SNP17	2.350	0.023	1.616	0.112	1.702	0.100	1.734	0.083	1.314	0.189
SNP18	-2.157	0.027	-1.125	0.228	-1.161	0.246	-1.179	0.238	-0.803	0.422

Empirical *P* values < 0.05 are in bold face

Z-score is for the minor allele

Table 5Haplotype association analysis for each block of RANK (haplotype blocks with any *P* value < 0.05 are shown)

Haplotype Block	OB	Fat mass	Lean mass	BMI	PFM
Block 1(SNP1–SNP3)	0.072	0.017	0.015	0.008	0.101
Block 2 (SNP4–SNP5)	0.155	0.021	0.031	0.015	0.011
Block 3 (SNP6–SNP7)	0.379	0.006	0.003	0.001^a	0.002^a
Block 5 (SNP10–SNP14)	0.001^a	0.025	0.036	0.030	0.023
Block 6 (SNP16–SNP18)	0.010	0.257	0.395	0.349	0.448

Empirical global *P* values < 0.05 are in bold face*PFM* percentage fat mass^aThe results remaining significant after Bonferroni correction