Contribution of Myostatin gene polymorphisms to normal variation in lean mass, fat mass and peak BMD in Chinese male offspring

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Aim: Myostatin gene is a member of the transforming growth factor-β (TGF-β) family that negatively regulates skeletal muscle growth. Genetic polymorphisms in Myostatin were found to be associated with the peak bone mineral density (BMD) in Chinese women. The purpose of this study was to investigate whether Myostatin played a role in the normal variation in peak BMD, lean mass (LM), and fat mass (FM) of Chinese men.

Methods: Four hundred male-offspring nuclear families of Chinese Han ethnic group were recruited. Anthropometric measurements, including the peak BMD, body LM and FM were measured using dual-energy X-ray absorptiometry (DXA). The single nucleotide polymorphisms (SNPs) studied were tag-SNPs selected by sequencing. Both rs2293284 and +2278G＞A were genotyped using TaqMan assay, and rs3791783 was genotyped with PCR-restriction fragment length polymorphism (RFLP) analysis. The associations of the SNPs with anthropometric variations were analyzed using the quantitative transmission disequilibrium test (QTDT).

Results: Using QTDT to detect within-family associations, neither single SNP nor haplotype was found to be associated with peak BMD at any bone site. However, rs3791783 was found to be significantly associated with fat mass of the trunk ($P<0.001$). Moreover, for within-family associations, haplotypes AGG, AAA, and TGG were found to be significantly associated with the trunk fat mass (all $P<0.001$).

Conclusion: Our results suggest that genetic variation within Myostatin may play a role in regulating the variation in fat mass in Chinese males. Additionally, the Myostatin gene may be a candidate that determines body fat mass in Chinese men.

Keywords: Myostatin; single nucleotide polymorphism (SNPs); bone mineral density; lean mass; fat mass; within-family association; quantitative transmission disequilibrium test (QTDT); Chinese male

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Introduction
Myostatin is a member of the transforming growth factor-beta (TGF-β) family, and it acts as a negative regulator of skeletal muscle growth. Currently, there is only one study, reported by our institute, on variation in the Myostatin gene and its role in the bone mineral density (BMD) and body mass index (BMI) of Chinese females[1]. However, the multiple regulatory mechanisms of the Myostatin gene in BMD and body composition have not yet been elucidated.

The TGF-β super-family encompasses a large number of growth and differentiation factors that play important roles in regulating embryonic development and in maintaining tissue homeostasis in adult animals. The TGF-β signaling pathway interacts with the PPAR-γ and Wnt signaling pathway, which has complex effects on marrow stromal cell differentiation[2]. High expression of TGF-β in bone suppresses adipocyte differentiation and promotes the proliferation and differentiation of osteoblasts[3]. TGF-β can repress the expression of PPAR-γ in marrow stromal cells and down-regulate the target genes of PPAR-γ[4]. In addition, TGF-β has an effect on the Wnt signaling pathway, which is responsible for the regulated expression of Wnt and LRP5 and the stabilization of β-Catenin. TGF-β stimulates the differentiation of chondrocytes and restrains the differentiation of marrow stromal cells to adipocytes[2, 5].

Myostatin, or GDF-8, which is situated on chromosome...
2q32.2, is a member of the TGF-β super-family and is important for the control and maintenance of skeletal muscle mass\cite{6}. Myostatin is a negative regulator of skeletal muscle growth in mammals, and loss-of-function mutations are associated with increased skeletal muscle mass in mice, cattle, and humans\cite{6}. Most of Myostatin-null mice (Mstn<sup>−/−</sup>) are 40%–100% larger than their wild-type littermates. This phenomenon is mainly caused by the hyperplasia and hypertrophy of myocytes\cite{7}. Schuelke et al\cite{8} described a boy with protruding muscles at birth who had a mutation in the Myostatin gene. Further study indicated that the femoral bone density of Myostatin-null mice (Mstn<sup>−/−</sup>) was significantly higher than that of wild-type mice\cite{8-11}. A recent study showed that Myostatin-null mice that performed physical exercise had a greater increase in bone strength relative to the wild-type mice with physical exercise and the Myostatin-null mice without exercise. This finding illustrated that physical exercise combined with increased muscle mass has a greater influence on bone strength than either increased muscle mass or strengthening physical exercise alone\cite{12}. In 2008, Zhang et al\cite{1} developed studies using QTDT of 401 nuclear families with female offspring consisting of 1260 subjects. Zhang et al\cite{1} detected that rs2293284 was significantly associated with total hip, neck, and trochanter BMD. Total hip and trochanter BMD was significantly associated with rs7570532, and +2278G>A was significantly associated with BMI. Therefore, these findings indicate that the Myostatin gene plays a role in regulating bone mass and muscle mass. A correlation between genetic variation in Myostatin and peak BMD or body composition has not been reported in males. Thus, the aim of this study was to investigate the associations between genetic variants in Myostatin with peak BMD, fat mass, lean mass, and BMI variation among 400 male-offspring nuclear families. Furthermore, we sought to observe the expression of the Myostatin gene in muscular tissues and adipose tissues and quantitate discrepancies in Myostatin expression. These data will help to establish a foundation upon which further study may elucidate the roles of Myostatin in bone, muscle, and fat tissues.

**Materials and methods**

**Subjects**

The 400 male-offspring nuclear families were recruited from 2004 to 2007. The group of subjects consisted of 1215 individuals with at least one healthy male child aged 18–44 years old (mean age 30.4±6.1 years). The average family size was 3.03. 385 families had 1 child, and 15 families had 2 children. All of the study subjects belonged to the Chinese Han ethnic group. For each study subject, we recorded age and sex and collected medical history, family history, marital status, physical activity, alcohol use, diet habits, and smoking history. We also collected information on menses, obstetrical history, and history of hormonal contraceptive use in the female subjects. The following exclusion criteria were used: (1) serious sequela of cerebrovascular disease; (2) diabetes mellitus; (3) chronic kidney disease; (4) serious chronic liver disease or alcoholism; (5) significant chronic lung disease; (6) corticosteroid therapy at pharmacologic levels for >6 months duration; (7) anticonvulsant therapy for >6 months duration; (8) evidence of other metabolic or inherited bone disorders, such as hyper- or hypo-parathyroidism, Paget’s disease of the bone, osteomalacia, or osteogenesis imperfecta; (9) rheumatoid arthritis or collagen disease; (10) recent (within the past year) major gastrointestinal disease, such as peptic ulcer, malabsorption syndromes, chronic ulcerative colitis, regional enteritis, or any significant chronic diarrhea state; (11) significant disease of any endocrine organ that would affect bone mass; (12) hyperthyroidism; and (13) any neurological or musculoskeletal condition that would be a non-genetic cause of low bone mass.

The study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People’s Hospital. All of the subjects involved in this study signed written informed consent before entering this study and were recruited by the osteoporosis center from a local population in Shanghai City, which is located in the middle of the east coast of China.

**Anthropometric measurements**

BMD (g/cm<sup>2</sup>) of the anteroposterior lumbar vertebrae 1–4 and the left proximal femur (including total hip, femoral neck, and trochanter) were measured by dual-energy X-ray absorptiometry (DXA). Fat mass (kg) and lean mass (kg) (including arms, legs, trunk, and total body) were also measured by DXA, using the same method. The DXA measurements were made using a Lunar Prodigy scanner (GE Lunar Corp, Madison, WI, USA), and the scanner was used on the fan-beam mode. The machine was calibrated daily. The coefficient of variability (CV) values were obtained from 15 volunteers with measurements each. The respective CV values for the BMI of the lumbar spine 1–4, total hip, femoral neck, and trochanter were 1.39%, 0.70%, 2.22%, and 1.41%\cite{1, 13-15}. The respective CV values for the fat mass measurements at the upper limbs, lower limbs, and total body were 7.58%, 3.28%, 2.52%, and 3.72%; the CV values for the lean mass at these sites were 1.18%, 1.59%, 1.12%, and 1.18%, respectively\cite{15}. The long-term precision (expressed as the CV of our DXA instrument, as was determined by daily measurements of a phantom) was 0.45% during the study period\cite{1, 14, 15}.

The data were analyzed with Prodigy Encore software (version 6.70, standard-array mode). Height was measured to the nearest centimeter on a wall-mounted stadiometer, and body weight was measured to the nearest 0.1 kg on a standard balance beam scale, with subjects wearing light indoor clothing and no shoes. Both the stadiometer and the scale were regularly calibrated during the study. BMI was calculated by dividing the weight in kilograms by the square of the height in meters, and the percentage fat mass (PFM) was calculated as the ratio of the fat mass to body weight (i.e., the sum of fat mass, lean mass, and bone mass). The percentage of lean mass (PLM) was calculated as the ratio of lean mass to body weight.
SNP selection and genotyping

The studied SNPs were selected by direct sequencing, which was performed in our previous study of Chinese women[16]. The SNPs from our previous work were selected for further study in our male-offspring nuclear families. These three tag-SNPs included rs2293284, +2278G>A, and rs750532. Unfortunately, the primer and probe sequences of rs750532 could not be synthesized by Applied Biosystems, and the polymorphism rs3791783 was selected as an alternative because of the strong LD (D'=1) between the two SNPs.

Genomic DNA was extracted from the peripheral blood samples by routine methods. The TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA) was used for the genotyping of rs2293284 and +2278G>A. The primer and probe sequences were optimized using the SNP assay-by-design service of Applied Biosystems. The SNPs, rs2293284 and +2278G>A, were submitted for custom Taqman SNP genotyping assay design (Applied Biosystems) and typed on a thermal cycler (Mx3000P Real-Time PCR System, STRATEGENE, CA, USA). The final SNP, rs3791783, was genotyped by PCR-restriction fragment length polymorphism (RFLP) analysis and was identified by electrophoresis through 2% agarose gels. The oligonucleotides used to amplify rs3791783 were as follows: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTYe H et al

Statistical analysis

The allele frequencies were estimated by gene counting. The Hardy-Weinberg equilibrium was tested by a χ² goodness-of-fit statistic. The QTDT program and the orthogonal model were used to test for population stratification, linkage, and within-family association between the SNPs and haplotypes of BMD and obesity-related phenotypes. The QTDT software package is available on the internet at the following address: http://www.sph.umich.edu/csg/abecasis/QTDT/. This method, as implemented in the QTDT software, extends the trios-based TDT to quantitative trait data and uses the genotype data from the available siblings and parents. In our male-offspring nuclear families, all of the children were sons, and the effects of the parent’s phenotypes were excluded in the QTDT. Thus, sex was not used as a covariate by which to adjust the son’s phenotype variations. Therefore, the BMD values were adjusted by age, height, and weight as covariates, and the obesity phenotypes were adjusted by age. Owing to the possibility of false-positives in multiple tests, 1000 permutations of the data set were performed to obtain the empirical P values and assess the reliability of the results. The QTDT program generates P values for various tests using a distribution that is asymptotically χ². A P value threshold of 0.05 was considered significant for all of the analyses.

In addition, one son from each of the 400 families was randomly selected to form a new group for testing the population-based association hypothesis. A general linear model-ANOVA (GLM-ANOVA) was used to compare the mean values of the phenotypic variables across the genotype combinations while adjusting for covariates (age, height, and weight). The statistical analyses were performed using the SPSS software package, version 11.0 (SPSS, Chicago, IL, USA). Significance was defined as P<0.05.

Results

Characteristics of the study population

Overall, 1215 individuals from the 400 male-offspring nuclear families were recruited for this study. The study population was composed of 400 pairs of parents and 415 sons. The basic characteristics of the study subjects are shown in Table 1.

SNP genotyping and linkage disequilibrium

A total of 1215 subjects from the 400 families were successfully genotyped. All of the 3 polymorphisms met the expectations of the Hardy-Weinberg equilibrium (HWE). Detailed information on the Myostatin SNPs and on the MAFs in dbSNP is listed in Table 2.

Based on the D' values, we found that the 3 SNPs were in strong LD and had D' values=1 in each pairwise comparison in the male-offspring nuclear families (Figure 1). Based on the
strong LD among the polymorphisms, 5 haplotypes that had frequencies of >3% were inferred in the block, using the likelihood method from the PHASE software. The respective frequencies of the haplotypes AGA, AGG, AAA, TGA, and TGG were 68.5%, 7.46%, 3.15%, 3.18%, and 17.6%. Together, the 5 haplotypes accounted for 99.38% of the total population.

Association between peak BMD and SNPs and haplotypes in the male-offspring nuclear families

There were 238, 53, and 264 informative nuclear families for the TDT analysis at rs2293284, +2278G›A, and rs3791783, respectively. At the haplotypes AGA, AGG, AAA, TGA, and TGG, there were 286, 106, 52, 44, and 217 informative nuclear families, respectively. Population stratification was detected for +2278G›A and lumbar spine BMD (P=0.0271). We failed to find a relationship between any polymorphism or haplotype and peak BMD in the male-offspring nuclear families (Table 3).

Association between obesity-related phenotypes and SNPs and haplotypes in the male-offspring nuclear families

There were 226, 50, and 249 informative nuclear families for the TDT analysis at rs2293284, +2278G›A, and rs3791783, respectively. At the haplotypes AGA, AGG, AAA, TGA, and TGG, there were 269, 99, 49, 42, and 206 informative nuclear families, respectively. Population stratification was detected for the haplotype AGG and fat mass in the arms (P=0.0380) and total fat mass (P=0.0366). A significant within-family association was found between rs3791783 and fat mass variation at the trunk (P<0.001). One thousand permutation tests were performed to improve the fidelity and further conform the above finding (P<0.001). The other two SNPs had no significant within-family association with any obesity-related phenotype. However, significant within-family associations were found between haplotypes AGG, AAA, and TGG and the fat mass of the trunk (all P<0.001) (Table 4).

No significant linkages between SNPs or haplotypes and peak BMD or obesity-related phenotypes were observed in the male-offspring nuclear families using linkage tests and linkage tests with modeling association (data not shown).

Gene expression of Myostatin in skeletal muscle and fat tissues

The mRNA transcribed from the Myostatin gene was examined with reverse transcription polymerase chain reaction (RT-PCR) in 8 samples of skeletal muscle and fat tissues. We found that Myostatin gene mRNA expressed in all eight samples, but no statistical significance was detected in different tissues (data not shown).

Discussion

In our previous study involving the sequencing of the full
We should first analyze whether the study sample size has sufficient power to detect positive results. Our sample size in this study is in line with the sample size of the female-offspring nuclear families in our previous study. The latter has more than 80% power to test a candidate gene as a quantitative trait locus (QTL) and can explain about 10% of the bone phenotype variation[1, 18, 19]. Using the female-offspring nuclear families, we not only detected an association between Myostatin polymorphisms and BMD variation, but we also successfully observed that genetic polymorphisms in estrogen receptor α and collagen1a2 likely influenced the attainment of peak BMD in Chinese females[1, 18, 20]. In addition, our latest study of genetic variants in the vitamin D receptor in relation to peak BMD in our male-offspring nuclear families detected an association[15]. In summary, the sample size of our male-offspring nuclear families had sufficient power to detect a candidate gene as a QTL. Taaffe et al[21] indicated that bone geometry and density of the femoral diaphysis differed by sex more than by race. Sex- and compartment-specific regulatory QTLs have been found in mice in some studies[22, 23]. Studies in humans have also revealed a gender difference in the degree of heritability of BMD at specific skeletal sites[24–26]. Ralston et al[27] provided evidence for gender-specific, site-specific and age-specific QTLs that regulate BMD in humans. However, exactly which gene is responsible for the differences observed between the males and females is still unclear. Therefore, our conclusion should be cautiously interpreted.

In recent years, multiple studies have focused on the role of brown adipocytes in regulating obesity. One recent study demonstrated that Myostatin is a potent negative regulator of brown adipogenic differentiation by the modulation of Smad3-induced β-catenin stabilization[28]. A recent animal study showed that Myostatin plays an important role in myogenic and adipogenic cells, and that the gene had different roles in the adipogenesis of pig adipose-derived stem cells (ADSCs) and muscle satellite cells (MSCs)[29]. Another animal study showed that the resistance of Myostatin-null mice to

### Table 3. Associations between the Myostatin polymorphisms and haplotypes and BMD in the male-offspring nuclear families (using QTDT).

| Tests of population stratification | rs2293284 | +2278G>A | rs3791783 | Haplotype AGG | Haplotype AAA | Haplotype TGG |
|----------------------------------|-----------|----------|-----------|---------------|---------------|---------------|
| Lumbar spine BMD                 | 0.0873    | 0.0271   | 0.7146    | 0.2085        | 0.0691        | 0.4929        |
| Femoral neck BMD                 | 0.9257    | 0.4524   | 0.5155    | 0.7640        | 0.2639        | 0.4894        |
| Total hip BMD                    | 0.4261    | 0.1967   | 0.9103    | 0.9678        | 0.1292        | 0.4697        |

| Tests of total association       |           |          |           |               |               |               |
| Lumbar spine BMD                 | 0.6352    | 0.1253   | 0.3089    | 0.2216        | 0.2117        | 0.6743        |
| Femoral neck BMD                 | 0.7324    | 0.5295   | 0.5475    | 0.3459        | 0.6428        | 0.9498        |
| Total hip BMD                    | 0.9155    | 0.9887   | 0.2569    | 0.1338        | 0.9090        | 0.5512        |

| Tests of within-family association|           |          |           |               |               |               |
| Lumbar spine BMD                 | 0.0879    | 0.2405   | 0.8404    | 0.5675        | 0.0880        | 0.4214        |
| Femoral neck BMD                 | 0.9245    | 0.7192   | 0.7859    | 0.8882        | 0.4567        | 0.5262        |
| Total hip BMD                    | 0.4643    | 0.2641   | 0.5012    | 0.4663        | 0.1710        | 0.3547        |

BMD values are adjusted for age, height and weight as covariates. Boldface type indicates significant P values (P<0.05).
diet-induced obesity, fat mass accumulation and metabolic dysfunction is not only a result of their large skeletal muscle mass, but it may also be a result of significant changes in the phenotype of white adipose tissue (WAT) [30]. Based on this research, we focused our study on the relationship between Myostatin and obesity-related phenotype in humans and we found that rs3791783 is significantly correlated with the trunk fat mass in the Chinese young males, which indicated that apart from the polymorphism itself is the functionally relevant locus or the polymorphism is in linkage disequilibrium with other functional variants in closely situated genes of Myostatin gene.

The SNP, rs3791783 is located in intron2 of the Myostatin gene, and further functional studies on rs3791783 are needed to determine whether this locus is functionally relevant. In this study, we also collected skeletal muscle and fat tissue from the patients with non-osteoporotic fractures to detect expression of the Myostatin gene using RT-PCR. The mRNA of Myostatin, which is primarily expressed in muscle, was found in both skeletal muscle and fat tissues. Further study in a larger sample of skeletal muscle and fat tissues is needed to measure the mRNA expression of the rs3791783 genotype in the above two tissues. This work may elucidate the molecular mechanisms of the Myostatin gene and its impact on lean mass and fat mass variation.

The DXA is generally accepted as a precise instrument to detect body composition. The fat mass of the trunk that we

### Table 4. Associations between the Myostatin polymorphisms and haplotypes and obesity-related phenotypes in the male-offspring nuclear families (using QTDT).

| SNP             | Tests of population stratification | Tests of total association | Tests of within-family association | 1000 permutations of within-family association |
|-----------------|------------------------------------|----------------------------|------------------------------------|-----------------------------------------------|
| rs2293284       |                                    |                            |                                    |                                               |
| +2278G>A        |                                    |                            |                                    |                                               |
| rs3791783       |                                    |                            |                                    |                                               |
| Haplotype AGG   |                                    |                            |                                    |                                               |
| Haplotype AAA   |                                    |                            |                                    |                                               |
| Haplotype TGG   |                                    |                            |                                    |                                               |
| Tests of population stratification | | | | |
| Arms FM         | 0.8369 | 0.8863 | 0.5231 | **0.0380** | 0.2941 | 0.8020 |
| Legs FM         | 0.8770 | 0.6429 | 0.2073 | 0.1628 | 0.3308 | 0.9252 |
| Trunk FM        | 0.8360 | 0.9887 | 0.4130 | 0.2095 | 0.9978 | 0.6234 |
| Total FM        | 0.6361 | 0.7558 | 0.3065 | **0.0366** | 0.1704 | 0.6735 |
| Arms LM         | 0.8434 | 0.3007 | 0.6973 | 0.2123 | 0.9965 | 0.9340 |
| Legs LM         | 0.8656 | 0.8388 | 0.3404 | 0.1911 | 0.3654 | 0.6376 |
| Trunk LM        | 0.9700 | 0.3212 | 0.5018 | 0.5890 | 0.6590 | 0.1432 |
| Total LM        | 0.8543 | 0.4315 | 0.6170 | 0.3543 | 0.4873 | 0.3508 |
| PFM             | 0.5920 | 0.7613 | 0.1565 | 0.0748 | 0.3228 | 0.5666 |
| BMI             | 0.4997 | 0.8251 | 0.8770 | 0.3337 | 0.6569 | 0.7534 |

Obesity-related phenotype values are adjusted for age as a covariate. Bold values indicate significant P values (P<0.01).
detected is mainly observed as fat accumulated in the abdomen. It is well known that fat accumulated in the abdomen is an important risk factor for type 2 diabetes and metabolic syndrome. Our finding of an association between rs3791783 and the fat mass of the trunk has great clinical significance, and we will screen for this SNP in patients with type 2 diabetes and metabolic syndrome in future experiments, to verify whether this polymorphism is a genetic risk factor of such diseases.

In conclusion, our study investigated the relationship between the Myostatin gene and obesity-related phenotype variation. The SNP rs3791783 and haplotypes AGG, AAA, and TGG had significant associations with the fat mass of the trunk in healthy young Chinese males aged 20–40 years. This result suggested that the Myostatin gene plays a role in regulating the variation in fat mass in males and that Myostatin may be a candidate gene for predicting body composition in males of the Chinese Han ethnic group. Further studies are required to elucidate the molecular mechanisms by which rs3791783 affects variations in fat mass and to verify whether this polymorphism is a genetic risk factor for type 2 diabetes and metabolic syndrome. In addition, our study did not detect a correlation between variants of the Myostatin gene and peak bone density variation, which indicates that the effect of Myostatin on peak bone mass variation may be gender specific.

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Author contribution
Hua YUE genotyped SNPs, extracted tissue RNA, carried out statistical analyses and drafted the manuscript. Zhen-lin ZHANG conceived and designed the study and provided part of the research funds. Jin-wei HE guided the work of the manuscript. Hao ZHANG, Chun WANG, Wei-wei HU, Jie-mei GU, and Yao-hua KE were involved in the collection of the genetic data. Yun-qiu HU and Miao LI were responsible for measuring bone mineral density and body composition. Hao ZHANG conceived and designed the study and revised the manuscript. Zhen-lin Hua YUE genotyped SNPs, extracted tissue RNA, carried out statistical analyses and drafted the manuscript. Jin-wei HE guided the work of the manuscript. Hao ZHANG, Chun WANG, Wei-wei HU, Jie-mei GU, and Yao-hua KE were involved in the collection of the genetic data. Yun-qiu HU and Miao LI were responsible for measuring bone mineral density and body composition.

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