

Original Article

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

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2q32.2, is a member of the TGF- β super-family and is important for the control and maintenance of skeletal muscle mass^[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^[6]. Most of *Myostatin*-null mice (*Mstn*^{-/-}) are 40%–100% larger than their wild-type littermates. This phenomenon is mainly caused by the hyperplasia and hypertrophy of myocytes^[7]. Schuelke *et al*^[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*^{-/-}) was significantly higher than that of wild-type mice^[5-11]. 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^[12]. In 2008, Zhang *et al*^[1] developed studies using QTDI of 401 nuclear families with female offspring consisting of 1260 subjects. Zhang *et al*^[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 information about 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 sequelae 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/cm²) 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 3 measurements each. The respective CV values for the BMD of the lumbar spine 1–4, total hip, femoral neck, and trochanter were 1.39%, 0.70%, 2.22%, and 1.41%^[1, 13-15]. The respective CV values for the fat mass measurements at the upper limbs, lower limbs, trunk, 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^[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^[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 (*ie*, 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^[1]. 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 rs7570532. Unfortunately, the primer and probe sequences of rs7570532 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, STRAT-AGENE, 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'-TTTTTTTTTTATTTTTTTTATTTTTTTTATGATGTTGATGCACTGATGTG-3' (forward); and 5'-GAA-TGCTAAGGCAGCTCAGAAA-3' (reverse). The PCR was performed using Taq DNA polymerase (Applied Biosystems) with the supplied buffer and a 55°C annealing temperature. The utilized restriction endonuclease was *Nla* III (Takara, Japan), and the RFLP sizes were 147, 99, and 48 bp. Random duplicate genotyping was routinely undertaken during the study: a mean genotyping error rate of less than 1% was found for all of the SNPs.

Tissue RNA extraction and mRNA expression analysis

Subcutaneous fat and muscle of the patients with fractures caused by traffic accident were collected for RNA extraction, altogether 2 fat and 6 muscle samples were acquired. Total RNA was extracted by conventional methods. After purity quotient, concentration detection and production assessment, reverse transcription was performed from RNA to cDNA. RT-PCR was performed in a 7300 Real-Time PCR System (Applied Biosystems, USA). The following primers were used: 5'-ACCTGTTTATGCTGATTGTTGCT-3' (forward) and 5'-GAGCTGTTCCAGACGAAGTTTA-3' (reverse). The *Myostatin* gene mRNA size is 177 bp. Human GAPDH was used as control. The following GAPDH-specific primers were used: 5'-GGTGGTCTCCTCIGACTTCAACA-3' (forward) and 5'-GTTGCTGTAGCCAAATTCGTTGT-3' (reverse).

LD and haplotype analyses

Haplotypes were constructed from the population genotype data using the Stephens algorithm and the Phase program version 2.0.2^[16]. The significance level of the LD between the markers for this gene was assessed based on the observed haplotypes and allele frequencies using the Haploview software

(version 3.2)^[17]. We examined Lewontin's D' and LD coefficient (r^2) between all pairs of biallelic loci. The frequencies of the genotypes and haplotypes were calculated using a group of 800 unrelated subjects (the parents from the 400 male-offspring nuclear families).

Statistical analysis

The allele frequencies were estimated by gene counting. The Hardy-Weinberg equilibrium was tested by a χ^2 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 χ^2 . 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

Table 1. The basic characteristics of the male-offspring nuclear families.

	Father (n=400)	Mother (n=400)	Son (n=415)
Age (years)	61.1±7.1	58.4±6.4	30.4±6.1
Height (cm)	166.7±6.0	155.8±5.5	173.0±5.9
Weight (kg)	69.6±9.5	58.3±8.2	70.6±10.7
Spine BMD (g/cm ²)	1.139±0.172	0.993±0.171	1.139±0.138
Femoral neck BMD (g/cm ²)	0.891±0.132	0.796±0.144	0.997±0.143
Total BMD (g/cm ²)	0.966±0.130	0.869±0.149	1.013±0.137
BMI (kg/m ²)	25.2±2.7	24.0±3.1	24.2±3.2
Trunk fat mass (kg)	-	-	9.341±4.37
Fat mass (kg)	-	-	16.31±7.56
Lean mass (kg)	-	-	51.43±5.76
PFM (%)	-	-	21.9±7.3

All data are presented as the mean±SD for the raw phenotype values without adjustment.

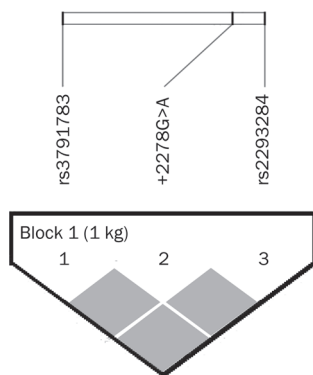


Figure 1. The linkage disequilibrium (LD) pattern in the *Myostatin* gene is depicted as an LD matrix. The classic D' measurement for all pairs of SNP markers was calculated to construct the LD matrix. The D' value of each panel is substituted with different colors. Squares in red indicate a strong LD.

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.

Table 2. Information on the *Myostatin* polymorphisms used in this study.

SNP	Allele	Function	Position	Amino acid change	MAF in dbSNP (CHB)	MAF in dbSNP (CEU)	Typing method	MAF in this study
rs2293284	A/T	Intron1	41134662	-	-	T: 2%	FAM/VIC	T: 14.2%
+2278G>A	G/A	Exon2	41134494	Glu153 (GAG)>Lys (AAG)	0	G: 1.7%	FAM/VIC	A: 2.9%
rs3791783	C/T	Intron2	41133580	-	C: 21.1%	C: 28.3%	FAM/VIC	C: 24 %

MAF, minor-allele frequency; FAS, fluorescent allele-specific PCR (FAM/VIC); the chromosome position of the base pairs are obtained from dbSNP build 129.

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

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

	rs2293284	+2278G>A	rs3791783	Haplotype AGG	Haplotype AAA	Haplotype TGG
Tests of population stratification						
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).

length of *Myostatin* gene, 17 SNPs were identified. Of these 17 SNPs, 6 SNPs were novel. Each of these SNPs was in strong LD ($D'=1$), and the entire gene was in one block of LD in the Chinese Han population^[1]. The 3 selected SNPs, rs2293284, rs7570532, and +2278G>A, were found to have associations with BMD or BMI in female-offspring nuclear families. However, because the body composition in female-offspring nuclear families was not measured, the correlation between these SNPs and body composition could not be further analyzed. Although the molecular mechanism by which these SNPs influence *Myostatin* function has not been determined, our previous study hinted that genetic variants of *Myostatin* could play a role in achieving and maintaining peak bone mass in females. In addition, the correlation between +2278G>A and BMI in females requires further validation in a larger sample size and in male populations. It was known that mutations in the *Myostatin* gene could result in changes to the lean mass of the body and bone strength. Additionally, the femoral bone density of *Myostatin*-null mice (*Mstn*^{-/-}) was significantly higher than that of wild-type mice^[5-11]. However, whether the variation in BMD is independent of the lean mass change is not clear. Therefore, the correlation between *Myostatin* gene polymorphisms and bone mass, lean mass, and fat mass is worthy of further study.

Our present study utilized the complete BMD and body composition database, and we analyzed the relationship between the SNPs and haplotypes of the *Myostatin* gene and peak BMD, lean mass, and fat mass using the QTDT statistical method in 400 male-offspring nuclear families. The genotype frequencies of rs2293284, +2278G>A, and rs3791783 were in accordance with our previous study in female-offspring nuclear families^[1]. In the current study, we failed to find an association between any polymorphism or haplotype and peak BMD, which was quite different from the results observed in females. To further examine this discrepancy, we should first analyze whether the study sample size has suffi-

cient 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 loci (QTL) and can explain about 10% of the bone phenotype variation^[1, 13, 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 collagen1 α 2 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 4. Associations between the *Myostatin* polymorphisms and haplotypes and obesity-related phenotypes in the male-offspring nuclear families (using QTDT).

	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	0.8565	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
Tests of total association						
Arms FM	0.3257	0.9983	0.7203	0.2177	0.8140	0.4036
Legs FM	0.4830	0.7070	0.4520	0.1241	0.9067	0.6460
Trunk FM	0.7110	0.9008	9e-011	1e-010	1e-010	1e-010
Total FM	0.5192	0.7586	0.4999	0.1939	0.7371	0.5938
Arms LM	0.3248	0.4647	0.8148	0.5211	0.4268	0.2989
Legs LM	0.9976	0.6983	0.6973	0.9987	0.9875	0.9943
Trunk LM	0.9669	0.8866	0.2653	0.1344	0.8042	0.9428
Total LM	0.8674	0.7421	0.4502	0.3334	0.7260	0.8909
PFM	0.6581	0.7086	0.3939	0.1882	0.9170	0.7340
BMI	0.7354	0.5902	0.2277	0.0992	0.9181	0.8823
Tests of within-family association						
Arms FM	0.3723	0.8014	0.5204	0.4385	0.3696	0.4379
Legs FM	0.5077	0.7687	0.9215	0.8879	0.4126	0.8352
Trunk FM	0.9999	0.7539	9e-011	1e-010	8e-011	1e-010
Total FM	0.3393	0.8366	0.6002	0.4447	0.2328	0.4987
Arms LM	0.5708	0.7574	0.5859	0.1714	0.5647	0.4864
Legs LM	0.9889	0.6936	0.5266	0.6683	0.9856	0.9932
Trunk LM	0.6566	0.6374	0.8479	0.1633	0.9004	0.2888
Total LM	0.8131	0.6397	0.6402	0.1784	0.8205	0.4546
PFM	0.4922	0.9674	0.7037	0.6164	0.4471	0.5206
BMI	0.4514	0.9527	0.4671	0.1114	0.7364	0.8398
1000 permutations of within-family association						
Trunk FM	0.9463	0.7889	9e-011	1e-010	8e-011	1e-010

Obesity-related phenotype values are adjusted for age as a covariate. Bold values indicate significant *P* values ($P < 0.01$).

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

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 revised the manuscript. Song-hua WU designed the study and provided part of the research funds. Jin-wei HE guided the work of the genetic laboratory, guaranteed and confirmed the quality of the genetic data. Hao ZHANG, Chun WANG, Wei-wei HU, Jie-mei GU, and Yao-hua KE were involved in the collection of the nuclear families. Wen-zhen FU and Yu-juan LIU contributed to collect blood specimen and DNA database management. Yun-qiu HU and Miao LI were responsible for measuring bone mineral density and body composition.

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