Targeted genotype analyses of GWAS-derived lean body mass and handgrip strength-associated single nucleotide polymorphisms in elite masters athletes

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Abstract

Recent large genome-wide association studies (GWAS) have independently identified a set of genetic loci associated with lean body mass (LBM) and handgrip strength (HGS). Evaluation of these candidate single nucleotide polymorphisms (SNPs) may be useful to investigate genetic traits of populations at higher or lower risk of muscle dysfunction. As such, we investigated associations between six SNPs linked to LBM or HGS, in a population of elite master athletes (MA), and age-matched controls, as a representative population of older individuals with variable maintenance of muscle mass and function. Genomic DNA was isolated from buffy coat samples of 96 individuals (consisting of 48 MA (71±6yrs; age-graded performance 83±9%) and 48 older controls (75±6yrs)). SNP validation and sample genotyping was conducted using the tetra-primer amplification refractory mutation system (ARMS). For the 3 SNPs analysed that were previously associated with LBM (*FTO*, *IRS1* and *ADAMTSL3*), multinomial logistic regression revealed a significant association of the *ADAMTSL3* genotype with %LBM (P<0.01). For the three HGS-linked SNPs, neither *GBF1* nor *GLIS1* showed any association with HGS, but for *TGFA*, multinomial logistic regression revealed a significant association of genotype with HGS (P<0.05). For *ADAMTSL3*, there was an enrichment of the effect allele in the MA (P<0.05; Fisher's exact test). Collectively, of the six SNPs analysed, *ADAMTSL3* and *TGFA* showed significant associations with LBM and HGS, respectively. The functional relevance of the ADAMTSL3 SNP in body composition, and of TGFA in strength, may highlight a genetic component of the elite MA phenotype.

Key words: muscle; handgrip strength; lean mass; elite athletes

Introduction

Lean body mass (LBM) plays an important role in metabolic function, mobility and healthy ageing, where progressive declines in LBM and concurrent increases in lipid infiltration can have detrimental impacts related to functional impairments and disability (13, 14, 18, 37). Similarly, declines in muscle strength with ageing are associated with impaired quality-of-life in older adults and increased risk of frailty and hospitalizations (2, 34). Reflecting this, handgrip strength (HGS) is a widely used marker of frailty, and a strong predictor of morbidities and survival (21, 38). The heritability of muscle strength has been estimated to be between 30-65% (22, 35), with the heritability of the LBM phenotype estimated to be 52-60% (1, 12). To date, few studies have robustly identified candidate genes associated with LBM or HGS on a genome-wide level.

A recent study identified and replicated a set of five loci for total lean body mass (42). Three of these SNPs (near/in genes for *IRS1*, *ADAMTSL3* and *VCAN*) were also successfully replicated for appendicular lean mass. Further analyses reported that for a subset of these SNPs, LBM increasing alleles were associated with adverse metabolic profiles (such as the Alpha-Ketoglutarate Dependent Dioxygenase (*FTO*) SNP rs9936385), whereas some were associated with metabolic protection (e.g. the rs2287926 SNP associated with the versican (*VCAN*) gene) (17). Similarly, a number of recent GWAS have reported multiple loci associated with HGS (23, 39). Analyses by Matteini *et al.* (2016) identified one significant genome-wide association of an intergenic SNP located in a chromosomal region that regulates muscle repair and differentiation. In a study by Willems *et al*. (2017), a number of loci out of the 16 SNPs identified were related to genes involved in muscle structure/function; (*ACTG1*), neurotrophic regulation (*TGFA*) and excitation-contraction coupling (*SLC8A1*). Others were identified with less understood roles in muscle function, such as Golgi Brefeldin A Resistant Guanine Nucleotide Exchange Factor 1 (GBF1), a guanine nucleotide exchange factor, and GLIS Family Zinc Finger 1 (GLIS1), Kruppel-like zinc finger protein that regulates transcription. Thus, further investigation into understanding the roles of these genes in the context of genetic variability of muscle strength is required. Despite the growing number of GWAS linking candidate genetic loci to skeletal muscle-related traits in humans, further validation/replication of these SNPs in independent cohorts has not previously been evaluated, while issues surrounding their reproducibility have also been highlighted (11).

Heritable phenotypical traits such as strength and lean mass are undoubtedly associated with physical performance and thus contribute to elite athletic status (6). Specifically, elite master athletes (MA; >65yrs) represent a population in which the effects of age may be addressed independently of the often accompanying disuse (19), and in many cases have displayed greater neuromuscular function than their age-matched inactive counterparts (24, 27, 29). However, there are little data available relating genotype to phenotype in these unique cohorts. In the current study, we first aimed to determine whether associations of SNPs linked to either LBM or HGS in previous GWAS analyses could be replicated in a smaller cohort comprising of a mixed population of elite master athletes (MA; both sprint and endurance) and age-matched non-athlete controls. Secondly, we aimed to compare allele/genotype frequencies between these two populations in order to gain further insight into the aforementioned differences in muscular strength and mass between older elite athletes and their age-matched controls. We hypothesized that the population of MA would demonstrate greater enrichments in SNPs associated with higher LBM and/or HGS. To perform targeted genotyping, we used tetra-primer amplification refractory mutation system

(ARMS) PCR, which has been reported as a rapid, low-cost and reliable method for SNP genotyping (26, 40).

Materials and Methods

Participants and ethical approval

The study was conducted in accordance with the *Declaration of Helsinki*, except for registration in a database. The study was approved by the University Research Ethics Committee and the National Research Ethics Service Committee Northwest (14/NW0275) and (15/NW/0426). All participants provided written informed consent. The control group (n=48) were aged 75.3±6.0yrs and were recruited from the local community. The masters athletes (n=48) were aged 70.6±5.9yrs and were recruited from athletics clubs, from an advertisement placed in a national athletics magazine, and from two national masters athletics competitions as part of the wider Vertical Impact of Bone Health in Elderly (VIBE) multiple cohort study (5, 28). All masters athletes were actively competing in their respective disciplines, and all completed more than 5 hours of specific training per week at the time of testing. MAs were classified as sprinters (n=12) if competing in events less than 800 m in distance, or endurance athletes (n=36) if competing in events greater than or equal to 800 m in distance.

The age-graded performance (AGP) of a master athlete allows a comparison of current performance against world record performance in the same discipline, distance and age-group. Mean age-graded performance (AGP) was determined by taking the athlete's highest ranked performance within the last year and expressing it as a percentage of the world record for that age and distance. The mean AGP of this athletic cohort was 83.4 ± 8.6%. For example, a 21 min and 20 sec 5000m for a 70-year-old man gives an age-graded performance of 83%. All males were chosen for the current analysis in order to avoid influences of sex-specific hormones.

DXA Scans

Standing height was measured to the nearest millimeter and body mass was measured to the nearest 0.1 kg. Whole body, total hip and lumbar spine dual energy X-ray absorptiometry (DXA: Lunar Prodigy Advanced, GE Healthcare, encore version 10.50.086, London, UK) scans were performed while the participant lay supine wearing a light cotton t-shirt to reduce measurement errors due to clothing absorption. Lean mass was taken from results of total body scans and regional analysis of legs and arms. All measurements were recorded after manual adjustment of the regions of interest. Repeat total body scans were performed in 8 participants within one month of the first scan. Using these repeat scans, the short-term error for our laboratory was 0.01% for whole body lean mass.

Muscle function

The investigators provided verbal instructions and a physical demonstration of the muscle function tests. Participants were allowed one practice immediately before the actual assessed trials, which acted as a specific warm up and also confirmed that the instructions were understood. In all cases, the muscle function tests were completed between 10am and 3pm.

Hand grip strength was measured using the Jamar dynamometer handle (Sammons Preston Inc, Bolingbrook, IL, USA) as previously described (10). The width of the dynamometer was adjusted for each participant separately. Participants were instructed to stand upright with the arm fully extended along the body, maintaining approximately 5 cm gap between the wrist and the hip or upper leg (so that the hand was not rested against the body). Participants were instructed to squeeze against the handle as hard possible for three seconds. Grip strength was measured three times and recorded in kilograms to the nearest 0.1 kg. For the purpose of this study, the best of three attempts was included in further analysis.

A Leonardo Jump Mechanography Platform (Leonardo Software version 4.2: Novotiec Medical GmbH, Pforzheim, Germany) was used to assess lower limb muscle power during a countermovement vertical jump, as described previously (10). Results for both absolute (W) and relative (W/kg) power were recorded. Briefly, a two-footed countermovement jump was performed starting with feet approximately 30 cm apart (slightly narrower than shoulder width) and standing upright on the force plates. Force was sampled at 800 Hz. Participants flexed at the knees before extending as forcefully as possible to take off for the jump. Jumps were performed with a trained research assistant in close proximity to intervene in case of a trip or fall. Each participant repeated the jump sequence three times, with approximately 60 seconds rest between efforts. The jump with the highest value for power was used for statistical analysis.

Genomic DNA Extraction

Genomic DNA was extracted from buffy coat samples (200 µl) using the QIAamp blood mini DNA kit (Qiagen, UK), according to the manufacturer's instructions. Isolated DNA was quantified on the NanoDrop 2000 (Thermo Fisher Scientific, UK).

SNP selection and primer design

A set of SNPs were selected, chosen from SNPs previously linked with LBM (42) and HGS in humans (39). SNPs with very low/high effect allele frequencies (EAFs) in the original GWAS studies (e.g. *VCAN*, *KANSL1* and *POLD3*) were avoided due to expected difficulties in detecting them in relatively low sample sizes. Primer design was performed using the

PRIMER1 program: http://primer1.soton.ac.uk/primer1.html, using the default primer design settings. SNPs that yielded primers with very high GC content were avoided due to anticipated difficulties during amplification, as well as primer sets with very distinct melting temperatures. A total of 15 SNPs were initially tested for validation, however technical difficulties meant that a number could not be assessed with the tetra-primer ARMS PCR method, and the final set of six SNPs, three predicted to be associated with LBM and three with HGS, are presented in Table 1.

Tetra-primer ARMS PCR and gel electrophoresis

Validation of SNP primers and genotyping was performed using the tetra-primer ARMS PCR technique (40). The sequences of primers used for the genotyping of the selected SNPs are shown in Table 1. SNP primers were initially validated and optimised using the guidelines set out in (26). Initially, amplification was performed using the outer primers only, using a gradient annealing temperature PCR to determine the optimal annealing temperature for each primer set. Subsequent validation involved incorporating the inner primers in varying amounts to produce detectable bands for each allele-specific amplicon via agarose gel electrophoresis (see below). PCR reactions with a final volume of 18 µl including 30 ng genomic DNA, SYBR™ Select Master Mix (Applied Biosystems) and primers in ratios according to Table 1. Amplification was performed using a Viia™ 7 real-time PCR machine (Applied Biosystems), using the following cycling conditions: 1 cycle of initial denaturation at 95^{o}C, 2 min; 35 cycles of denaturation at 95^{o}C for 30s, annealing at 61-62^{o}C (see Table 1 for SNP-specific annealing temperature) for 45s and extension at 72^{o}C for 45s, with a final extension for 5 min at 72^{o}C on standard cycling conditions. PCR products were mixed with 4 µl gel loading buffer (Sigma-Aldrich, UK) and 10 µl was electrophoresed on 3% (w/v) agarose gels for 120 min at 80V.

Statistical analyses

Multinomial logistic regression was performed in R (version 3.6.1) using the nnet package (16) to examine associations between *GBF1*, *GLIS1* and *TGFA* genotypes and maximal grip strength, and to examine associations between *IRS1*, *FTO* and *ADAMTSL3* and total lean mass, appendicular lean mass, and percentage body lean mass. Strength of associations were assessed by p values calculated from z values provided from the regression model coefficients and standard errors for each predictor variable. Fisher's exact test was used for comparison of allele distributions and genotype distributions between MA and control groups, while one-way ANOVA was used for multi-group comparisons, with Tukey's test to correct for multiple comparisons. Comparisons between two groups were made using unpaired t tests. $P<0.05$ was taken to be statistically significant. Data were analysed using GraphPad Prism software version 7.0.

Results

Associations between genotype and functional parameters in MA and non-athletes

We first aimed to identify any associations of the selected SNP genotypes with lean mass or HGS in a mixed population of older MA and non-athletes (i.e., irrespective of groupings). Within this cohort, total LBM ranged from 36.6 to 69.4 kg, while HGS ranged from 20.6 to 54.7 kg. In relation to the SNPs previously linked to HGS (*GBF1* (rs2273555; effect allele A), *GLIS1* (rs4926611; effect allele C) and Transforming Growth Factor Alpha (*TGFA*; rs958685; effect allele A)), there was no significant association of either *GBF1* or *GLIS1* genotype with HGS (Figure 1), but with *TGFA*, there was a significant association between HGS and genotype (mean difference between AA and CC 6.32; 95% CI 0.43-12.1; $P<0.05$, Figure 1A), with the AA genotype (A being the effect allele) having higher HGS. In relation to SNPs that were previously associated with LBM, multinomial logistic regression showed no significant association of total lean mass, % LBM or appendicular lean mass with insulin receptor substrate 1 (*IRS1*; rs2943656; effect allele A), *FTO* (rs9936385; effect allele T) or A Disintegrin-Like And Metalloprotease Domain With Thrombospondin Type I Motifs-Like 3 (*ADAMTSL3*; rs4842924; effect allele T) genotypes (Figures 2-4). For *ADAMTSL3* however, there was a significant association with % LBM (mean difference between TT and CC 5.36; 95% CI 1.38-9.34; $P<0.01$; Figure 4A), where the TT genotype was associated with higher % LBM. Since LBM and HGS are biologically closely related, we also determined whether any of the LBM-associated SNPs were linked to HGS, and vice-versa. However, none of the HGS-associated SNPs were significantly associated with LBM (*TGFA*; β=-4.88, p=0.305 *GLIS1*; β=-18.64, p=0.641, *GBF1*; β=2.433, p=0.354), and none of the LBM-associated SNPs were associated with HGS (*FTO*; β=-1.716, p=0.354, *IRS1*; β=-3.242, p=0.059, *ADAMTSL3*; β=-1.432, p=0.378). There were also no genotype associations of any of the SNPs measured with muscle power measurements (maximum power relative to body weight, Pmax rel) (*TGFA*; β=-0.079, p=0.714, *GLIS1*; β=-0.002, p=0.911, *GBF1*; β=-0.006, p=0.891, *FTO*; β=-0.021, p=0.358, *IRS1*; β=-0.002, p=0.905, *ADAMTSL3*; β=0.015, p=0.423).

Allele frequencies in individuals grouped according to the highest and lowest quartile for % LBM or HGS.

Following on from this, we aimed to determine whether there were any differences in allele frequencies in individuals that had been grouped according to the highest and lowest quartiles for % LBM or HGS. Comparing the upper and lower quartiles for %LBM (irrespective of groupings) there was no difference in allele frequency for the *IRS1* or *FTO SNPs* (Table 2). For *ADAMTSL3*, comparing the upper and lower quartiles for %LBM (irrespective of groupings), there was an enrichment in the effect allele in the upper quartile for %LBM ($P<0.05$; Fisher's exact test) (Table 2). For *TGFA*, comparing the upper and lower quartiles for HGS (irrespective of groupings), there was an enrichment in the effect allele in the upper quartile for HGS ($P<0.05$; Fisher's exact test) (Table 2). There were no significant differences in either *GBF1* or *GLIS1* alleles between the upper and lower quartiles for HGS (Table 2).

Allele/genotype distributions for LBM or HGS-associated SNPs in MA versus non-athletes

In subsequent analyses, we sought to compare allele/genotype distributions for the LBM and HGS-associated SNPs between the elite MA and older non-athlete groups, first comparing participant muscle-related characteristics between MA and control groups. Since multiple group analyses were limited by the relatively low number of available samples from participants in the sprint category (n=12), sprint and endurance MA were grouped for the majority of our analyses. While total lean mass and appendicular lean mass (ALM) was not different across groups (Figure 5A & B), LBM as a percentage of total body weight (%LBM) was significantly lower in controls than MA (P<0.001 by unpaired t test; Figure 5C). Likewise, percentage fat mass was significantly higher (P<0.001 by unpaired t test) in controls than MA (Figure 5D). HGS and Pmax rel were no different between MA and controls (Figure 5E and 5F).

Genotype distributions for 3 SNPs that were previously associated with LBM (*IRS1*, *FTO* and *ADAMTSL3*) and 3 SNPs that were previously associated with HGS (*TGFA*, *GBF1* and *GLIS1*) were analysed in the 48 MA and 48 older controls. For the SNP associated with the *ADAMTLS3* gene, genotype distributions were significantly different between MA and controls (P<0.05; Fisher's exact test; Figure 6). For the SNPs associated with *IRS1*, *FTO*, *TGFA*, *GLIS1* and *GBF1*, there was no difference in genotype frequencies between MA and control groups (Figure 6). While analyses focused on the master athletes as a group, compared to non-athlete control, we also assessed allele distributions for the 6 SNPs between sprint and endurance MA relative to controls (Table 3). Similar to the genotype distributions between MA and controls, allele distributions for the SNPs associated with *IRS1*, *FTO*, *TGFA*, *GLIS1* and *GBF1* were not significantly different between groups, while for *ADAMTSL3*, there was an enrichment in the effect allele for both sprint and endurance athletes, relative to non-athlete controls (P<0.05 vs. Control (Fisher's exact test); Table 3).

Discussion

While LBM and HGS represent two highly heritable traits in humans (1, 22, 35), only recently have studies begun to explore the specific genes that contribute to the underlying inter-individual variability in skeletal muscle traits such as these (30, 39, 42). Evaluation of these candidate SNPs could prove useful in investigating underlying genetic traits of individuals at variable risk of muscle dysfunction. In the present study, our aim was to determine whether SNPs linked to either LBM or HGS in previous GWAS analyses could be replicated in a smaller cohort comprising of elite MA and age-matched controls. We also aimed to determine whether genotype/allele distributions for these SNPs were different between elite MA in comparison to age-matched non-exercising controls, as a representative population of older individuals with greater maintenance of muscle mass and function. By comparing allele/genotype frequencies between these two populations using the tetra-primer ARMS technique we aimed to gain greater insights into the underlying genetic component of the MA muscle phenotype.

We chose to use the tetra-primer ARMS technique as a rapid approach to SNP genotyping as it provides a cost-effective and accurate methodology, (40) but alternative methods are available. The restriction fragment length polymorphism (RFLP) typing method involves restriction endonuclease digestion of PCR products to discriminate between alleles (25), while microarray approaches (32) and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (8) allow high-throughput genotyping. We found the tetra-primer ARMS technique robust, but requiring substantial optimisation, and some primer sets for SNPs could not be validated; potentially due to the SNPs loci i.e. in a high

GC-rich region, giving rise to difficulties due to incomplete denaturation of DNA and less than optimal primer annealing (26).

We began by investigating associations between genotype and functional parameters in older MA and non-athletes as a collective cohort. In terms of their predicted associations with either LBM or HGS, out of the six SNPs analysed, four failed to show any significant association with LBM or HGS (even when analysing those individuals with the highest and lowest quartiles for %LBM or HGS). In contrast, the SNP associated with *TGFA* showed significant associations with HGS, while the SNP linked with *ADAMTSL3* was associated with LBM (independent of exercise discipline), as predicted by the original GWAS'. These findings provide further support to the previous data indicating the potential importance of the *TGFA* SNP in muscle strength, and of *ADAMTSL3* in body composition. Interestingly, we found that none of the HGS-associated SNPs were associated with LBM, and vice-versa, nor were there any significant associations with Pmax rel. The reason for this lack of overlap is not clear and requires further investigation of the potential roles of these genes in muscle function. For the SNP associated with *TGFA*, there was an association between HGS and genotype, with the AA genotype (A being the effect allele promoting increased HGS), having a significantly higher HGS. The consequence of the polymorphism with rs958685 is an intron variant. The potential functional relevance of the *TGFA* in muscular strength remains to be evaluated, but other intronic SNPs have been shown to be associated with functional elements, including intron splicing enhancers/silencers that regulate alternative splicing events as well as other transcriptional regulatory elements (4). The *TGFA* gene encodes a growth factor which plays a key role in cellular proliferation, differentiation and development (33). TGF-α also plays a neurotrophic role and promotes neuronal survival during acute injury of motor neurons (15, 20).

A further important finding was that for rs4842924, the SNP related to the *ADAMTSL3* gene, the TT genotype was associated with higher %LBM amongst all volunteers. Initial analyses aimed to replicate the original GWAS (42), which identified SNPs associated with total LBM, with subsequent analysis demonstrating higher associations when adjusting for total fat mass (17). We found instead that for *ADAMTSL3* (and other SNPs), there was no association to LBM in either unadjusted or after adjusting for fat mass or for height. We also found no associations of any of the SNPs to appendicular lean mass. There was, however, a significant association of the *ADAMTSL3* genotype to LBM as a percentage of whole-body mass, demonstrating it may have importance in terms of body composition. As with *TGFA*, the consequence of the *ADAMTSL3* SNP is an intron variant, and the functional effect (if any) on gene expression is not currently known. Little is understood about the biological functions of ADAMTSL3, but it is a glycoprotein that is related to the ADAMTS family of metalloproteases, that may have functions in extracellular matrix regulation (9). The *ADAMTSL3* gene has also consistently been linked to height (36) in genome-wide association analyses. Further *in vitro* experiments will be required to understand the mechanisms underlying *ADAMTSL3* gene variants in muscle physiology, and relation to LBM *in vivo*.

We next investigated allele/genotype distributions for LBM or HGS-associated SNPs in MA versus non-athletes. Elite MA represent a unique population of individuals that in general display greater maintenance of neuromuscular function than age-matched inactive populations (24), and while undoubtedly environmental factors play a large role in the MA phenotype (7), there are little conclusive data available related to any underlying genetic components. Whether the high-functioning characteristics of master athletes is more influenced by heritable factors regulating muscle composition/performance, or whether the environmental component (i.e. continued high levels of training over the years) is more important for the master athlete phenotype, remains to be fully understood. For the present group of individuals studied, while total LBM or ALM were not different between MA and controls, %LBM was significantly higher in the MA population. While HGS or Pmax Rel were not different between MA and non-athlete controls, this is likely due to the fact that the majority of the cohort were endurance athletes, which is in line with previous observations with regards to strength differences in endurance versus power MA (24). Although HGS does not always correlate with strength of other functionally important muscle groups such as the quadriceps (41), it is a useful predictor of a number of health outcomes in middle to older age (3), including all cause mortality (31). In the present study, of the six SNPs measured, five were not different between MA and control; however, for *ADAMTSL3*, there was an enrichment of the effect allele (T) in the group of MA. Further work investigating these candidate SNPs, and the mechanisms by which they may influence muscle function, could prove useful in understanding the genetic basis of populations with increased/decreased susceptibility of muscle dysfunction (such as frailty and sarcopenia).

Perspectives and Significance

While there are difficulties associated with studying a cohort such as that of the MA in terms of gaining sufficient sample numbers, clearly larger MA sample sizes will be needed to explore MA, on a genome-wide basis, or in a targeted fashion. Indeed, the lack of individuals with the GG genotype for *IRS1* in the present study is also a limitation in the context of the relatively small sample size of this study. There is also a potential that the lack of replication for some of the SNPs analysed in the present study was partly due to the the elite athletes having a different phenotype to those of the general population (as used in the original GWAS analyses). Additionally, effect sizes in the original analyses would be viewed as being small, with standardized beta of -0.12 - -0.14 for LBM and 0.13 – 0.16 for HGS. More work is required to determine the biological significance of these SNPs in LBM and/or muscular strength across different populations of individuals. Nonetheless, in a targeted fashion, we demonstrate that a SNP related to the *ADAMTSL3* gene was enriched in elite MA and had significant associations with % LBM. We also confirmed data from previous GWAS' of an association of the *TGFA* SNP with HGS. Future work elucidating the mechanisms by which these gene variants influence muscle mass and function are required to facilitate our understanding of the genetic basis of, not only the MA phenotype, but also the genetic basis underlying a range of conditions such as frailty and sarcopenia.

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Disclosures

The authors declare they have no competing interests.

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Figure Legends

Figure 1. Genotype versus Grip Strength for *TGFA* (rs958685; effect allele = A), *GLIS1* (rs4926611; effect allele = C) and *GBF1* (rs2273555; effect allele = A) in a mixed population of older elite athletes (sprint and endurance) and non-athletes. Grip strength according to genotype for (A) *TGFA*, (B) *GLIS1* and (C) *GBF1* (irrespective of groupings). *=P<0.05 versus AA (multinomial logistic regression analysis).

Figure 2. Genotype versus Total Lean Mass for *ADAMTSL3* (rs4842924; effect allele = T), *IRS1* (rs2943656; effect allele = A) and *FTO* (rs9936385; effect allele = T) in a mixed population of older elite athletes (sprint and endurance) and non-athletes. Total Lean Mass according to genotype for *ADAMTSL3* (A), *IRS1* (B) and *FTO* (C; irrespective of groupings).

Figure 3. Genotype versus Appendicular Lean Mass for *ADAMTSL3* (rs4842924; effect allele = T), *IRS1* (rs2943656; effect allele = A) and *FTO* (rs9936385; effect allele = T) in a mixed population of older elite athletes (sprint and endurance) and non-athletes. Appendicular Lean Mass according to genotype for *ADAMTSL3* (A), *IRS1* (B) and *FTO* (C; irrespective of groupings).

Figure 4. Genotype versus Percentage Lean Mass for *ADAMTSL3* (rs4842924; effect allele = T), *IRS1* (rs2943656; effect allele = A) and *FTO* (rs9936385; effect allele = T) in a mixed population of older elite athletes (sprint and endurance) and non-athletes. Percentage Lean Mass according to genotype for *ADAMTSL3* (A), *IRS1* (B) and *FTO* (C; irrespective of groupings). **=P<0.01 versus CC (multinomial logistic regression analysis).

Figure 5. Phenotype characteristics of older elite athlete (sprint and endurance) and non-athlete (Control) populations. Total lean mass (A), appendicular lean mass (ALM; B), % lean mass (C), % fat mass (D), grip strength (E) and maximum power relative to body weight (F) in master athletes and non-athlete controls. ***=P<0.001 (unpaired t test).

Figure 6. Genotype distributions of selected single nucleotide polymorphisms (SNPs) previously associated with lean body mass or grip strength in master athlete (MA) and non-athlete (Ctrl) populations. Balloon plot displaying frequencies of genotypes for three lean mass-associated SNPs (*IRS-1*, *FTO* and *ADAMTSL3*) and three grip strength-associated SNPs (*TGFA*, *GLIS1* and *GBF1*) between elite older athletes and non-athlete controls. *=P<0.05 (Fisher's exact test).

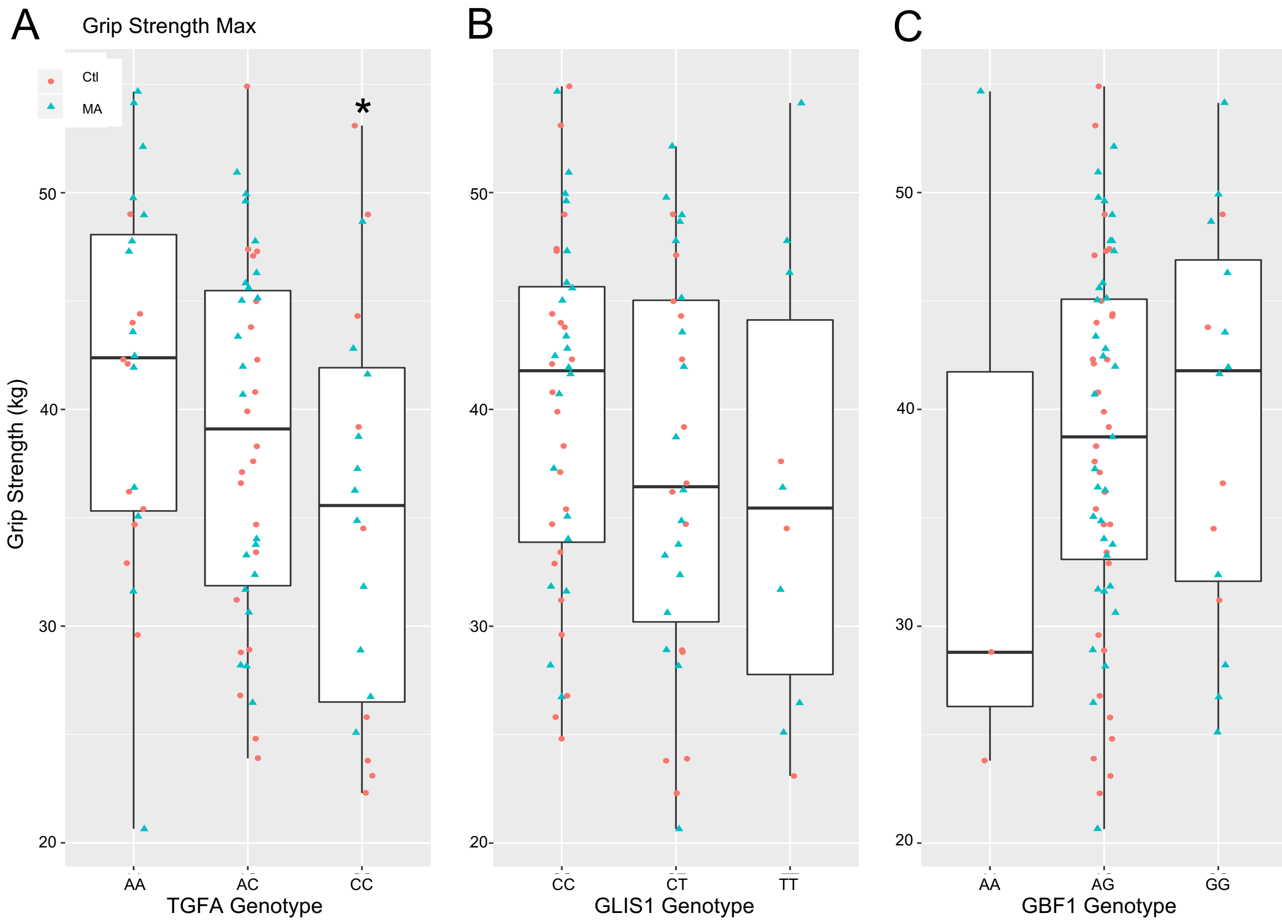

A
Grip Strength Max
Ctl
MA
Grip Strength (kg)
50
40
30
20
*
AA
AC
CC
TGFA Genotype
B
50
40
30
20
CC
CT
TT
GLIS1 Genotype
C
50
40
30
20
AA
AG
GG
GBF1 Genotype

Figure 1

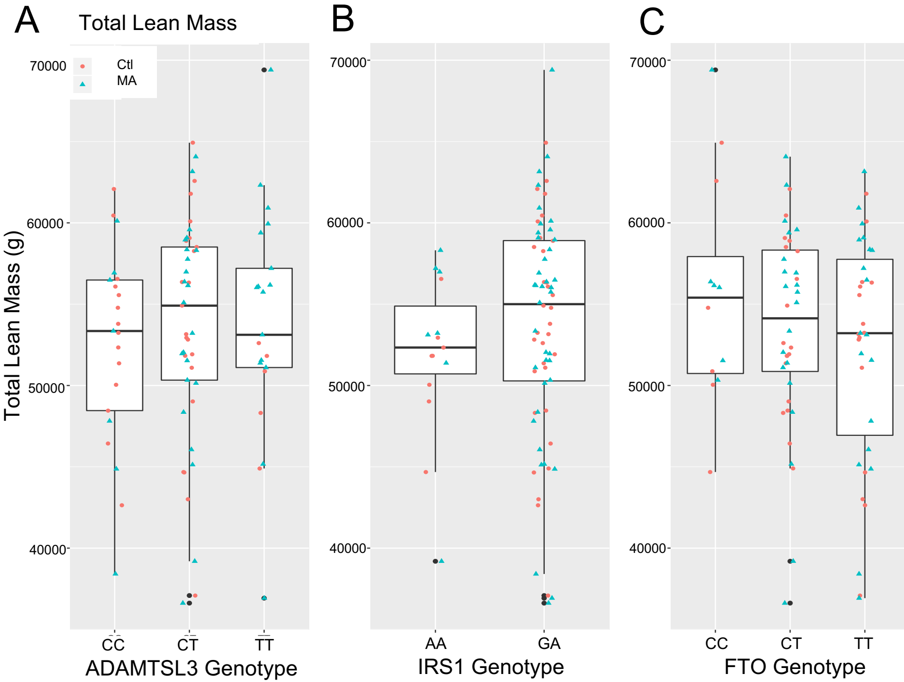
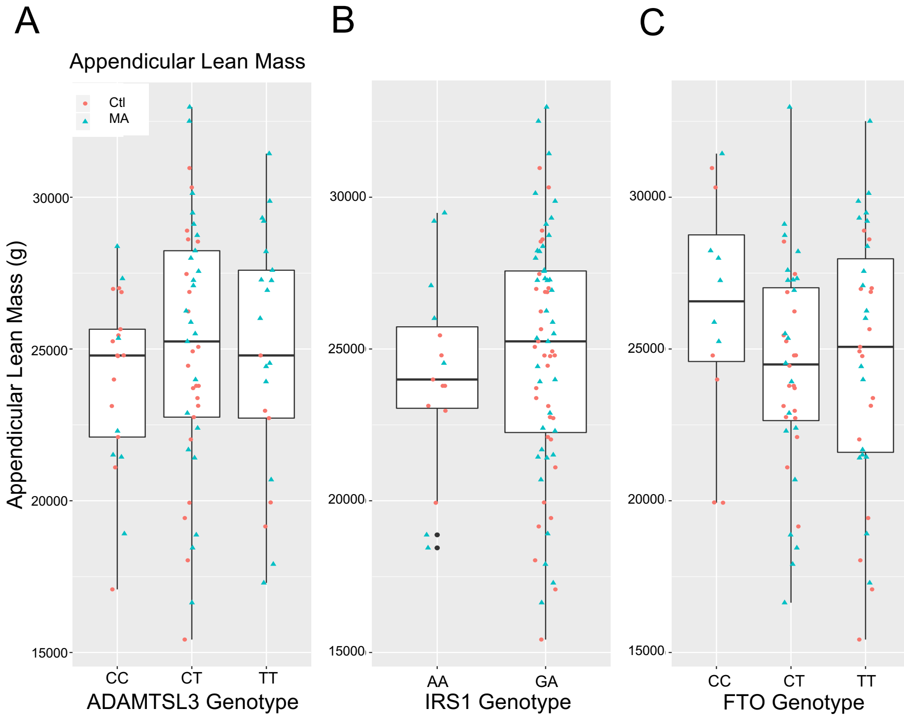
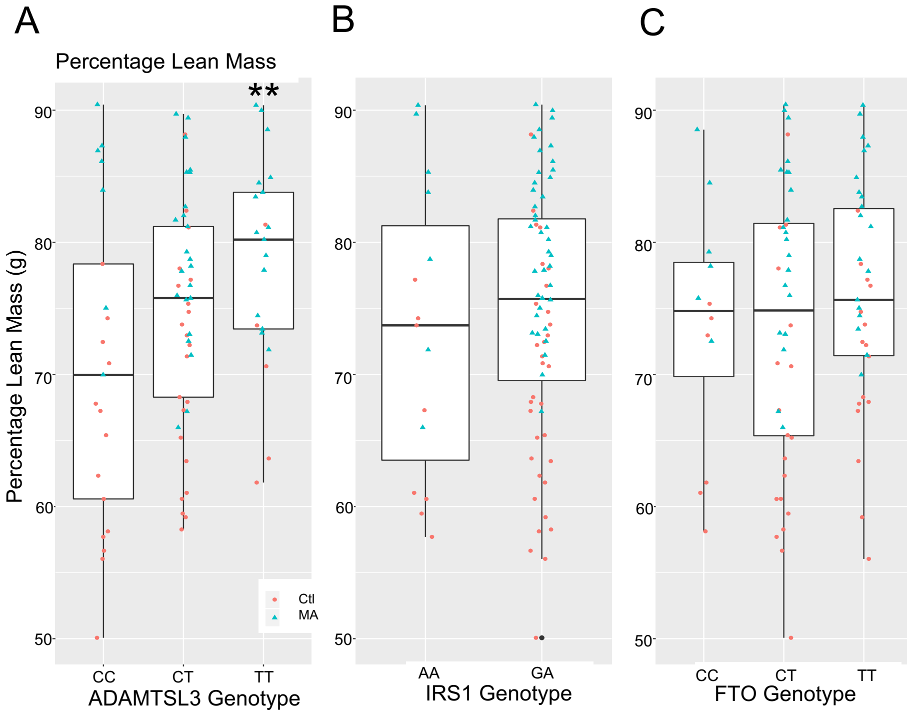

A
Total Lean Mass
Ctl
MA
70000
60000
50000
40000
Total Lean Mass (g)
CC
CT
TT
ADAMTSL3 Genotype
B
70000
60000
50000
40000
AA
GA
IRS1 Genotype
C
70000
60000
50000
40000
CC
CT
TT
FTO Genotype

Figure 2



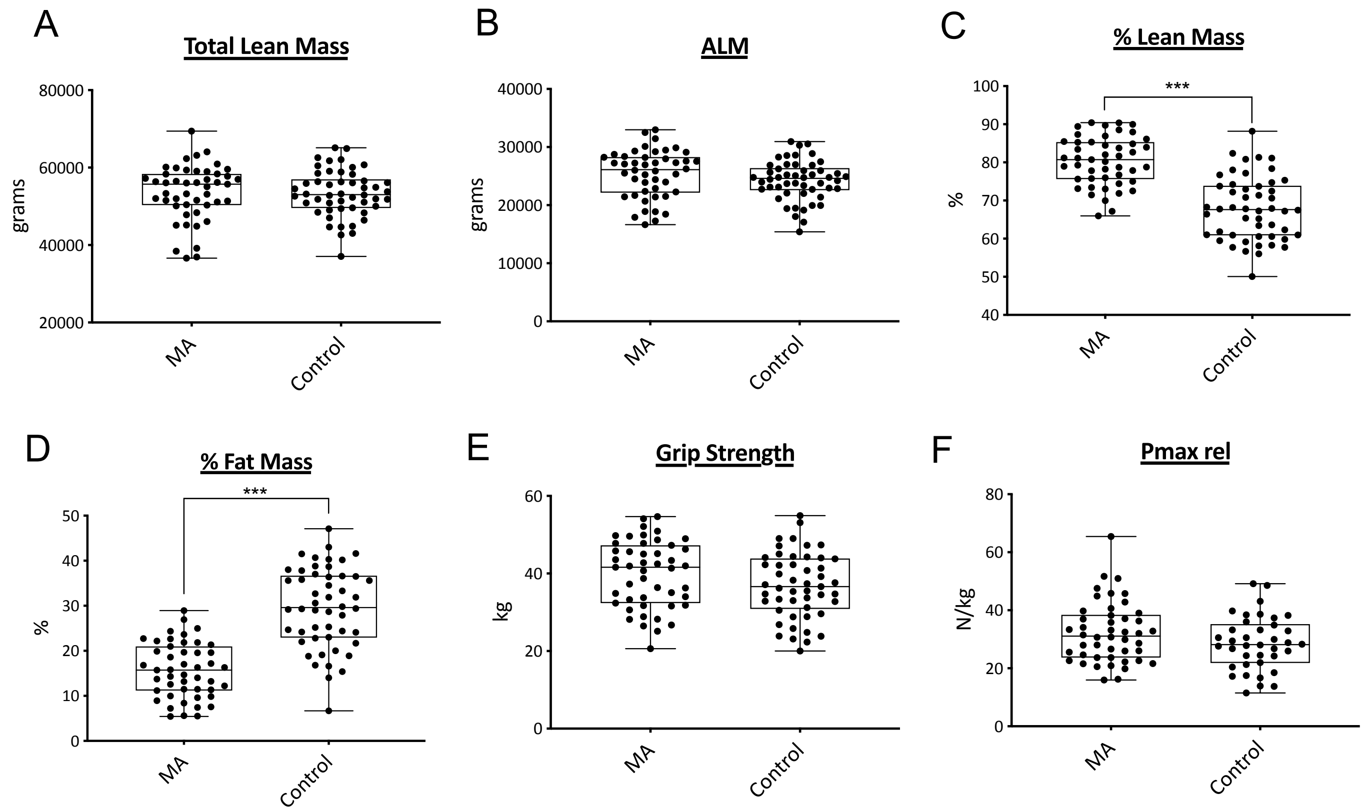
A
B
C
Appendicular Lean Mass
Ctl
MA
Appendicular Lean Mass (g)
30000
25000
20000
15000
CC
CT
TT
ADAMTSL3 Genotype
AA
GA
IRS1 Genotype
FTO Genotype

Figure 3



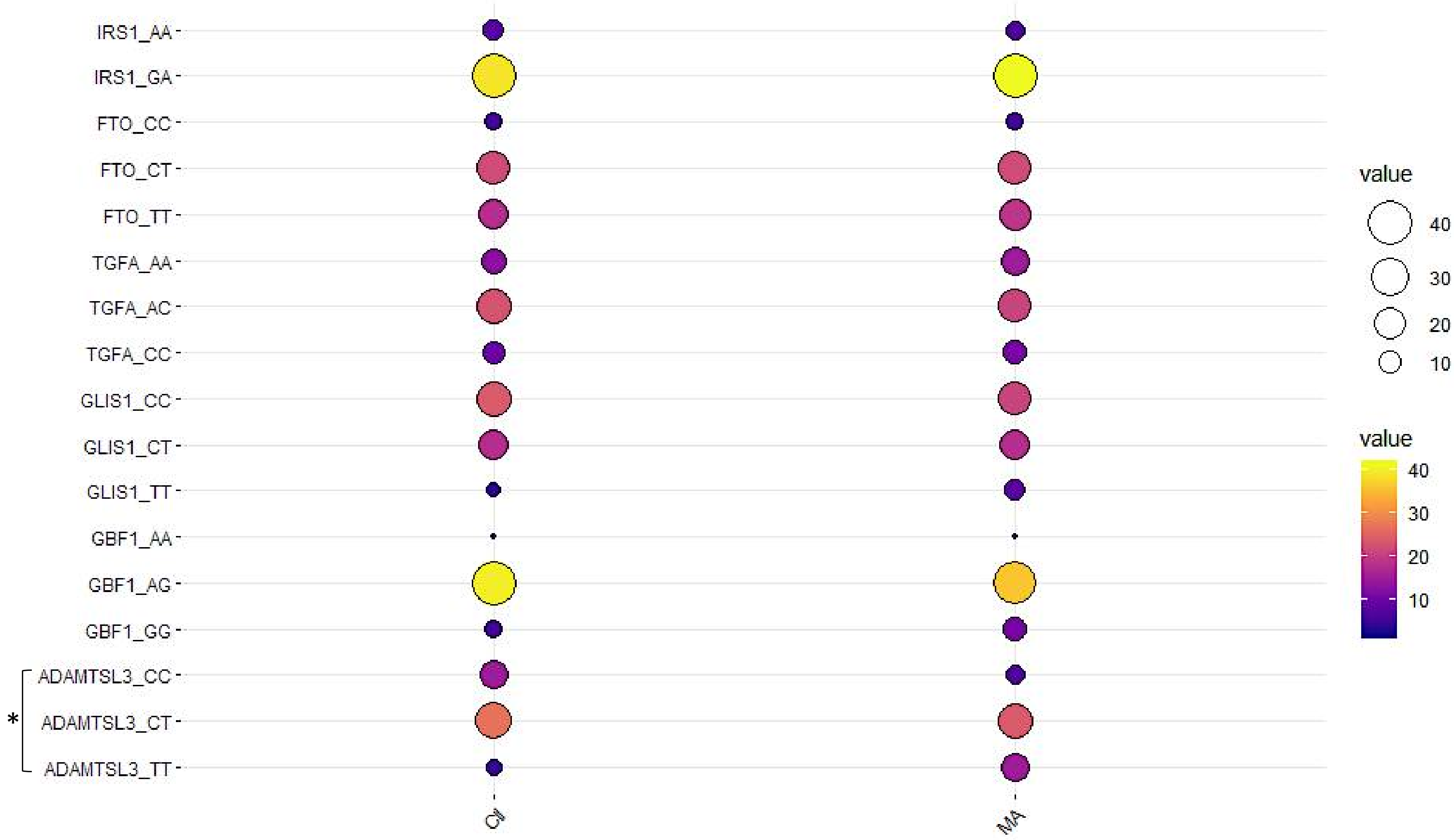
A
B
C
Percentage Lean Mass
**
Percentage Lean Mass (g)
90
80
70
60
50
Ctl
MA
CC
CT
TT
ADAMTSL3 Genotype
AA
GA
IRS1 Genotype
FTO Genotype

Figure 4



A
Total Lean Mass
grams
80000
60000
40000
20000
MA
Control
B
ALM
grams
40000
30000
20000
10000
0
MA
Control
C
% Lean Mass
%
***
100
90
80
70
60
50
40
MA
Control
D
% Fat Mass
%
***
50
40
30
20
10
0
MA
Control
E
Grip Strength
kg
60
40
20
0
MA
Control
F
Pmax rel
N/kg
80
60
40
20
0
MA
Control

Figure 5



IRS1_AA
IRS1_GA
FTO_CC
FTO_CT
FTO_TT
TGFA_AA
TGFA_AC
TGFA_CC
GLIS1_CC
GLIS1_CT
GLIS1_TT
GBF1_AA
GBF1_AG
GBF1_GG
ADAMTSL3_CC
ADAMTSL3_CT
ADAMTSL3_TT
*
Ctl
MA
value
40
30
20
10
value
40
30
20
10

Figure 6

Table 1: Single nucleotide polymorphism (SNP) and primer information for tetra-primer ARMS PCR.

SNP	Closest Gene	Allele 1/2	EAF	Primer sequences (5'-3')	Annealing temperature	Ratio of FO:RO:RI:FI
rs2943656	*IRS1*	A/G	0.38	FO: CTGAGAGCCTGCTCCTTACTCTTGTCTT RO: CGGCATGTTGGAGAGTTACTCTACATGT FI: TTCACCTAAAATTCTCCTCTAAAAACACAG RI: CTCTCTCCATCACCATGGCTTCACCT	$62^{o}C$	1:1:3:3
rs4842924	*ADAMTSL3*	T/C	0.52	FO: CAGTTGGAGTACTGAGAATGAGACAGGG RO: AGTCTTAGGACTCAGACTTGCCATCACA FI: GGAAAGGATAAGGATGTTGTGAGCGT RI: GAATAGGCAATAGCTTCCTATGTGAGCG	$61^{o}C$	2:1:6:2
rs9936385	*FTO*	T/C	0.61	FO: TGTGTGACCAGCCTCAATAGATTTTATTCA RO: CCATCCTATCAAAAACAGCACTCTCACC FI: TGCATATGAAGAGGGATTTTTTTGCATC RI: TACTGGGAATATGCAGTGAACCACGA	$62^{o}C$	1:1:3:3
rs958685	*TGFA*	A/C	0.52	FO: TCCACCCTTAGGAAAAAATGCTTCCTCT RO: TCACATCTTTGTCATGGGACATAGTCCC FI: TTTTTTCATCGGCAGTTTGCAGATACC RI: AGGAGTATCCTTCTTCCACCCACGCT	$62^{o}C$	1:2:2:6
rs2273555	*GBF1*	A/G	0.61	FO: CACAACCACAATGTTCGTAAACAGAATG RO: TCTAAAAACTGGGAAAGGAAGCAATGTG FI: TTTCCTAAGTCCTATTTACTGAAAACCAAG RI: ACACTGAAGCCCCACCTAAGGAACGCT	$61^{o}C$	1:1:3:3
rs4926611	*GLIS1*	C/T	0.64	FO: GCAGAGCTGGATTTTCAAGAGTCTACCT RO: TTCATCCCTGCTTACCCACTAGAGGTAA FI: TAGAGACACCTGCAACATCCAGCAAAAT RI: CTGAGATTTGCTTTTTAAATTCAGCAGTG	$61^{o}C$	1:2:3:6

Table 2. Allele frequencies of selected single nucleotide polymorphisms (SNPs) previously associated with lean body mass or grip strength in individuals grouped according to the highest and lowest quartile for % LBM or HGS.

SNP	Closest Gene	Allele 1/2	Lowest Quartile for %LBM		Highest Quartile for %LBM		SNP	Closest Gene	Allele 1/2	Lowest Quartile for HGS		Highest Quartile for HGS	
			Allele 1	Allele 2	Allele 1	Allele 2				Allele 1	Allele 2	Allele 1	Allele 2
rs2943656	*IRS1*	A/G	28	20	28	20	rs958685	*TGFA*	A/C	19	29	30	18*
rs4842924	*ADAMTSL3*	T/C	15	33	27	21*	rs2273555	*GBF1*	A/G	22	26	21	27
rs9936385	*FTO*	T/C	34	14	29	19	rs4926611	*GLIS1*	C/T	28	20	34	14

Frequencies of alleles for three lean mass-associated SNPs (IRS-1, FTO and ADAMTSL3) between the highest and lowest quartile for LBM (irrespective of groupings), and three grip strength-associated SNPs (TGFA, GLIS1 and GBF1) between the highest and lowest quartile for HGS (irrespective of groupings). *=P<0.05 vs. Lowest Quartile (Fisher's exact test).

Table 3. Allele frequencies of selected single nucleotide polymorphisms (SNPs) previously associated with lean body mass or grip strength in elite athletes (sprint and endurance) versus non-athlete controls.

SNP	Closest Gene	Allele 1/2	Control (n=48)		Sprint (n=12)		Endurance (n=36)	
			Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
rs2943656	*IRS1*	A/G	57 (59%)	39 (41%)	13 (54%)	11 (46%)	42 (58%)	30 (42%)
rs4842924	*ADAMTSL3*	T/C	38 (40%)	58 (60%)	15 (62%)*	9 (38%)*	42 (58%)*	30 (42%)*
rs9936385	*FTO*	T/C	60 (62%)	36 (38%)	10 (42%)	14 (58%)	51 (71%)	21 (29%)
rs958685	*TGFA*	A/C	51 (53%)	45 (47%)	13 (54%)	11 (46%)	39 (54%)	33 (46%)
rs2273555	*GBF1*	A/G	44 (46%)	52 (54%)	7 (29%)	17 (71%)	31 (43%)	41 (57%)
rs4926611	*GLIS1*	C/T	68 (71%)	28 (29%)	15 (62%)	9 (38%)	46 (64%)	26 (36%)

Frequencies of alleles for three lean mass-associated SNPs (IRS-1, FTO and ADAMTSL3) and three grip strength-associated SNPs (TGFA, GLIS1 and GBF1) between non-athlete controls and elite athletes (split into sprint and endurance types). *=P<0.05 vs. Control (Fisher's exact test).