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# Variants of the Ankyrin Repeat Domain 6 Gene (ANKRD6) and Muscle and Physical Activity Phenotypes Among European-Derived American Adults

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## Abstract

Ankyrin repeat domain 6 (ANKRD6) is a ubiquitous protein that associates with early development in mammals and is highly expressed in the brain, spinal cord, and heart of humans. We examined the role of 8 *ANKRD6* single-nucleotide polymorphisms (SNPs) on muscle performance and habitual physical activity (PA). Single-nucleotide polymorphisms were 545 T>A (rs9362667), 485 M>L (rs61736690), 233 T>M (rs2273238), 128 I>L (rs3748085), 631 P>L (rs61739327), 122 Q>E (rs16881983), 197805 G>A (rs9344950), and 710 L>X (NOVEL). This study consisted of 922 healthy, untrained, European-derived American men ($n$ = 376, 23.6 ± 0.3 years, 25.0 ± 0.2 kg·m$^{-2}$) and women ($n$ = 546, 23.2 ± 0.2 years, 24.0 ± 0.2 kg·m$^{-2}$). Muscle strength (maximum voluntary contraction [MVC] and 1 repetition maximum [1RM]) and size (cross-sectional area [CSA]) were assessed before and after 12 weeks of unilateral resistance training (RT). A subsample ($n$ = 536, 23.4 ± 0.2 years, 24.6 ± 0.2 kg·m$^{-2}$) completed the Paffenbarger Physical Activity Questionnaire. Associations among *ANKRD6* genotypes and muscle phenotypes were tested with repeated measure analysis of covariance (ANCOVA) and PA phenotypes with multivariate ANCOVA, with age and body mass index as covariates. *ANKRD6* 122 Q>E was associated with increased baseline biceps CSA. *ANKRD6* 545 A>T and *ANKRD6*



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710 L>X were associated with increased 1RM and MVC in response to RT, respectively. *ANKRD6* 631 P>L was associated with increased biceps CSA response to RT and time spent in moderate-intensity PA among the total sample and women. *ANKRD6* genetic variants were associated with the muscle size and strength response to RT and habitual PA levels. Further research is needed to validate our results and explore mechanisms for the associations we observed.

**Keywords**

diversin; exercise; resistance training; genetics

## Introduction

Ankyrin repeat domain 6 (ANKRD6) is a modular protein located on chromosome 6 (q14.2–q16.1). It is a member of the ankyrin repeat domain protein family that mediate physiologically important protein-protein interactions and act as adapters of signaling pathways (2). Tissir et al. (30) found that ANKRD6 is expressed prominently in zones of neuronal proliferation in the developing brain of mice. ANKRD6 also plays a role in signaling pathways that regulate crucial events in the development of vertebrates and invertebrates, including body axis formation in *Xenopus* and zebrafish embryos (24) and heart development in zebrafish embryogenesis (15). Thus, it appears biologically plausible that the *ANKRD6* gene regulating function of the ANKRD6 protein would also have an important role in neural development, axis formation, and cardiogenesis of humans, and subsequently, muscle performance and habitual physical activity (PA) participation through centrally and peripherally mediated pathways (15,24,30). However, to our knowledge, the influence of *ANKRD6* on neural, gastric, and heart development and its influence on muscle performance and habitual PA in humans have not been studied.

Our group (5) has documented considerable variability in the muscle strength and size response to a 12-week standardized resistance training (RT) program with muscle strength and size gains varying between 5–150% and 5–40%, respectively. In addition, it is estimated that 35–85% of RT strength gains are due to inheritance that appears to account for a significant portion of the variability in the muscle strength and size response to RT (19,27,28). Twin studies show habitual PA also have a significant genetic component, explaining 32–85% of the variation in adult PA levels (3,8,25). A recent advancement in the field of exercise genomics is the realization that the genetic basis of muscle performance and PA is accounted for by a large number of genes that play a small role rather than a small number of genes with large effects (3,25,26).

The purpose of our study was to explore the influence of *ANKRD6* genetic variants on the muscle size and strength response to a RT program and habitual PA among a large homogenous sample of healthy, European-derived American adults undergoing a 12-week, standardized, unilateral upper arm RT regimen from the Functional Single-Nucleotide Polymorphisms (SNPs) Associate with Muscle Size and Strength Study (FAMuSS) (13,21,29). Given the known role of ANKRD6 on neural development, axis formation, and

cardiogenesis, we hypothesized that *ANKRD6* genetic variants would influence these muscle and PA phenotypes.

## Methods

### Experimental Approach to the Problem

This study was a subset of a larger study designed to uncover novel nonsynonymous SNPs that associate with muscle size and strength phenotypes, that is, FAMuSS (13,21,29). The largest candidate gene association RT study conducted to date is FAMuSS. This multicenter study was conducted by the Exercise and Genetics Collaborative Research Group at 10 different institutions.

A detailed description of the experimental design of FAMuSS has been presented previously (9,13,21,29) and is described briefly for the reader here. Study volunteers were recruited from 8 of the 10 sites to complete a 12-week progressive RT program aimed at increasing strength and size of the elbow flexors and extensors of the nondominant arm only. Isometric (maximum voluntary contraction [MVC]) and dynamic (1 repetition maximum [1RM]) strength and cross-sectional area (CSA) by magnetic resonance imaging (MRI) were assessed pre- and post-RT. Blood samples for genotyping were taken before RT.

### Subjects

Participants were European-derived American adults aged 18 to 40 years or younger from the FAMuSS cohort who were genotyped for 8 *ANKRD6* genetic variants. FAMuSS exclusion criteria are described in detail elsewhere (9,29). The study protocol was approved by the institutional review board at each site, and all subjects gave written informed consent before the start of the study.

The FAMuSS subsample for the RT portion of this study consisted of 922 healthy, young (23.3 ± 0.2 years), normal weight (24.4 ± 0.2 $kg \cdot m^{-2}$) European-derived American men ($n$ = 376) and women ($n$ = 547). Age did not differ significantly by sex ($p$ = 0.267), yet men had greater body mass than women (25.0 ± 0.2 vs. 24.0 ± 0.2 $kg \cdot m^{-2}$, $p$ = 0.001). The FAMuSS subsample for the Paffenbarger Physical Activity Questionnaire (PPAQ) portion of this study ($n$ = 536; 23.4 ± 0.2 years, 24.6 ± 0.2 $kg \cdot m^{-2}$) consisted of 242 men and 294 women. Although age did not differ by sex ($p$ = 0.254), men were overweight and had greater body mass than women (25.3 ± 0.3 vs. 23.9 ± 0.3 $kg \cdot m^{-2}$, $p$ = 0.001).

## Procedures

### Physical Activity Determination

Habitual PA was determined through completion of the PPAQ, which has been validated in numerous studies as an accurate and reliable measure of adult leisure time PA (16). The PPAQ was completed by a subsample of FAMuSS subjects ($n$ = 536) during their initial visit. Physical activities with a metabolic equivalent (MET) value of >6 were classified as vigorous intensity, 3 to ≤6 METs as moderate intensity, and <3 METs as low intensity (18). The following PA phenotypes were derived from the PPAQ: distance walked (miles per week), PA index (kilocalories per week), and energy expended in vigorous, moderate, light-

intensity PA and sitting and sports and recreation (kilocalories per week) (16). Additional PA phenotypes included time (hours per week) spent in vigorous, moderate, and light-intensity PA and sitting (17).

### Anthropometric Measurements

Body weight (in pounds) and height (in inches) were assessed pre- and post-RT to calculate body mass index (BMI) (kilogram per square meter). Subjects were instructed to maintain their usual diet throughout the duration of the study. Body weight was measured every 3 weeks during the study to ensure weight stability (defined as ±5.0 lb pre-RT weight).

### Isometric Strength Testing (Maximum Voluntary Contraction)

Maximum voluntary contraction of the elbow flexors was assessed pre- and post-RT using a custom-made preacher curl bench and strain gauge (model 32628CTL; Lafayette Instrument Company, Lafayette, IN, USA). Each MVC attempt began with a verbal cue from the tester. Subjects gradually increased to a maximal effort sustained for 3 seconds with 1-minute rest between contractions. The test session was completed once 3 attempts were within 2.2 $ft \cdot kg^{-1}$ of each other or a maximum of 6 attempts had been made. The closest 3 measurements were averaged and recorded in kilograms. The investigator who administered the baseline MVC test also administered the post-RT MVC test.

### One Repetition Maximum Strength Testing

Dynamic strength of the elbow flexors of each arm was assessed pre- and post-RT. Participants were tested on a standard preacher curl bench (Yukon International, Inc., Cleveland, OH, USA) using Powerblock adjustable dumbbells (Intellbell, Inc., Owatonna, MN, USA) in increments of 1.1 and 2.2 kg. To start the assessment, the investigator verbally instructed the subject to perform one full repetition of full range of motion at 100% of estimated maximum weight. If the lift was unsuccessful, a 3-minute rest was given and the weight was decreased. If the lift was successful, a 3-minute rest was given and the weight was increased. This process was repeated until subjects failed to complete a full lift. Weights were used so that the 1RM could be completed in 3–5 attempts. The 1RM was recorded as the maximum weight lifted one time. The same investigator who administered the baseline 1RM test also administered the post-RT 1RM test.

### Muscle Cross-sectional Area Measurements

The MRI measurements to determine CSA have been described in detail elsewhere (29) and are briefly overviewed here. The CSA of each arm's biceps brachii muscle was determined using an MRI operated at 1.5 T. Measurements were taken before RT and within 48–96 hours of the final RT session. Subjects laid supine on the scanning bed with their arm aligned to the isocenter of the magnet and the point of measure centered to the alignment light of the MRI. Fifteen axial slices were taken over 24 cm beginning proximally and proceeding distally.

Images taken via MRI from each investigative site were saved via magnetic optical disk or CD-ROM in a DICOM format and sent to the central imaging facility for analysis. The same investigator analyzed the images using a custom- designed program created to function

within MATLAB (The MathWorks, Inc., Natick, MA, USA). Cross-sectional area was determined by multiplying the number of pixels within the defined area by a preset CSA value of 0.01 $cm^2$ determined from the MRI matrix and field of view.

## Resistance Training Program

A unilateral, 12-week upper arm RT program was chosen to minimize possible confounding effects of activities of daily living on the muscle size and strength response to RT (25). Subjects underwent 12 weeks of a gradually progressive supervised RT regimen of their nondominant arm only. Training sessions occurred twice weekly, with each session separated by a minimum of 48 hours. Exercises included biceps preacher curls, biceps concentration curls, standing biceps curls, overhead triceps extensions, and triceps kickbacks. Each RT session began with a warm-up consisting of 2 sets of 12 repetitions of the biceps preacher curls and overhead triceps extensions. A 3-minute rest followed each warm-up set. Subjects then performed 3 sets of 12 repetitions at 65–75% of their 1RM for each of the 5 exercises listed above. A 2-minute rest followed each set. At week 5, the number of repetitions was decreased to 8 and then to 6 at week 10. Thus, the exercise intensity at weeks 5 and 10 increased to 75–82% 1RM and 83–90% 1RM, respectively. All exercises were performed with Powerblocks, and some exercises also used the preacher curl bench. All training sessions were supervised and lasted approximately 45–60 minutes.

## Genotyping

Fasting venous blood samples were collected from all subjects at the start of the study. Samples were sent in EDTA-containing vacutainer tubes to the coordinating site (Children's National Medical Center, Washington, DC, USA) with all subject identification information removed. DNA was isolated from each blood sample using the Gentra Puregene Blood DNA Purification Kit (Qiagen, Valencia, CA, USA). Genotyping was performed using Applied Biosystem's TaqMan allele discrimination assay using standard thermal cycling conditions, with genotypes called by the 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). If available, the Applied Biosystem Assay ID for each SNPs is listed in Table 1.

Subjects were genotyped for each SNP listed in Table 2 using 2 separate polymerase chain reaction–based methods to assure accuracy with the novel TaqMan allelic discrimination and restriction enzyme assays (Table 3). A complete description of the genotyping methods used in this study can be found in previously published literature (13,21,29).

## Statistical Analyses

All analyses included only subjects who completed the study and were genotyped for *ANKRD6* genetic variants. Descriptive statistics and frequencies were calculated for study variables. The $\chi^2$ test was used to determine whether the *ANKRD6* genotype was in Hardy-Weinberg equilibrium for white populations (Table 4). All *ANKRD6* SNPs except *ANKRD6* 710 L>X were in linkage disequilibrium ($r^2 > 0.8$); thus, data for all SNPs are presented individually. Dependent variables included baseline and change in muscle strength and size (post- to pre-RT) for MVC, 1RM, and CSA in the trained (T) arm. Values are presented in absolute (no correction for MVC, 1RM, and CSA) and relative percent (post-RT − pre-RT/

pre-RT × 100% for MVC, 1RM, and CSA). For the PA analysis, dependent variables included the Paffenbarger PA phenotypes listed previously.

Associations among the *ANKRD6* genetic variants and muscle phenotypes were tested with repeated measure analysis of covariance (ANCOVA), with age and BMI as covariates and *ANKRD6* genotype and gender as between-genotype factors. Associations among the *ANKRD6* genetic variants and PA phenotypes were tested with multivariate ANCOVA by gender with age and BMI as covariates. No gender × *ANKRD6* genotype interactions were found for any of the muscle phenotypes examined. However, a gender × *ANKRD6* genotype interaction was found for one of the PA phenotypes examined, and thus results are presented for the total sample and by gender.

When significant main effects were found for the linear multivariate tests above (repeated measure ANCOVA and multivariate ANCOVA), post hoc analyses were performed, with Bonferroni adjustments applied for multiple comparisons. Significant findings for individual SNP cohorts are presented by genotype group (Tables 5 and 6). Statistical significance was set at $p < 0.05$, and all data were reported as mean ± SEM. Analyses were performed using SPSS 14.0 for Windows.

## Results

### Muscle Performance Phenotypes and ANKRD6 Genotype Associations

Muscle size and strength associations by *ANKRD6* genotypes among the total sample are presented in Table 5 for the *ANKRD6* SNPs that were found to have significant genotype main effects ($p < 0.05$). The Bonferroni post hoc genotype comparisons revealed that, for *ANKRD6* 122Q>E, subjects with the QE genotype tended to have higher baseline biceps CSA in the T arm than those with the QQ genotype ($p = 0.076$). For *ANKRD6* 631P>L, subjects with the PP genotype had a higher absolute increase in biceps CSA in the T arm post-RT than subjects with the PL genotype ($p = 0.062$). For *ANKRD6* 710L>X, subjects with the LL genotype tended to have higher absolute and relative gains in biceps MVC in the T arm post-RT than those with the LX genotype ($p = 0.074$). For *ANKRD6* 545A>T, subjects with the TA genotype tended to have greater absolute increases in biceps 1RM in the T arm post-RT than those with the TT genotype ($n = 454$) ($p = 0.069$). No significant associations among the muscle phenotypes and remaining 4 *ANKRD6* genetic variants were found among the total sample and by sex ($p > 0.05$) (data not shown).

### Physical Activity Phenotype and ANKRD6 Genotype Associations

Physical activity associations by *ANKRD6* 631 P>L genotype among the total sample are presented in Table 6 because this was the only *ANKRD6* SNP displaying a significant genotype main effect with the PA phenotypes obtained from the PPAQ ($p = 0.03$). Bonferroni post hoc genotype comparisons revealed that adults with the *ANKRD6* 631 LL genotype reported more time spent in moderate-intensity PA than those who were carriers of the P allele ($p = 0.030$). We further examined *ANKRD6* 631 P>L for the sex interactions we found (Table 6). Women with the *ANKRD6* 631 LL genotype reported more time spent in moderate-intensity PA than women who were carriers of the P allele ($p = 0.05$). There was

no significant associations among PA phenotypes and *ANKRD6* 631 P>L for men ($p > 0.05$). No other significant associations among PA phenotypes and the remaining 7 *ANKRD6* genetic variants were found among the total sample and by sex ($p > 0.05$) (data not shown).

## Discussion

We examined whether *ANKRD6* SNPs were associated with muscle performance in response to a 12-week, unilateral progressive RT intervention and habitual PA phenotypes in a large sample of healthy, untrained European-derived American adults from FAMuSS (29). The major findings were (a) *ANKRD6* 122 Q>E tended to be associated with baseline muscle size and *ANKRD6* 631 P>L with the muscle size response to RT (Table 5); (b) *ANKRD6* 710 L>X and *ANKRD6* 545 T>A tended to be associated with muscle strength response to RT (Table 5); and (c) *ANKRD6* 631 P>L was associated with moderate-intensity PA levels, and this association was sex dependent (Table 6).

The results of this exploratory study suggest that the *ANKRD6* gene appears to associate with the muscle size and strength response to RT and habitual PA levels, and these genotype differences may have important public health considerations. For example: (a) subjects with the *ANKRD6* 631 PP genotype gained approximately 10% more muscle size (i.e., CSA) in response to RT than those with the PL genotype; (b) subjects with the *ANKRD6* 710 LL genotype gained approximately 50% more isometric muscle strength (i.e., MVC) in response to RT than those with the LX genotype; and (c) subjects with *ANKRD6* 545 TA genotype gained approximately 15% more muscle strength (i.e., 1RM) in response to RT than those with the TT genotype. In addition, subjects with the *ANKRD6* 631 LL genotype spent approximately 60% more hours per week in moderate-intensity PA than those who were carriers of the P allele, whereas it appears that they spent approximately 50% less hours per week in vigorous-intensity PA, although these latter findings did not reach statistical significance. These findings suggest that *ANKRD6* genetic predispositions may be important to consider along with a growing number of genetic variants that have been reported to be associated with muscle performance (6,13,21,32) and habitual PA (7,8,14) when an individualized approach to PA prescription for health benefit based on genotype becomes more of a reality (22). For example, when recommending PA to adults for its overall health benefits, the *ANKRD6* 631 P>L and PA intensity-dependent genotype differences we found could be considered when encouraging people to become more physically active due to what appears to be genetic dispositions to prefer moderate over vigorous-intensity PA among those with the LL genotype. However, an individualized approach to PA prescription such as this remains a vision of the future rather than a reality of the present (20).

At this time, biological mechanisms by which *ANKRD6* SNPs would influence muscle performance and habitual PA levels remains unclear. To our knowledge, this is the first article investigating *ANKRD6* in humans and the first to report *ANKRD6* genotype associations with muscle performance and habitual PA phenotypes. Previous research in mice has shown that the ANKRD6 protein is expressed predominantly in the developing brain from embryonic day 12 (E12) to maturity, suggesting a role during brain development. Tissir et al. (30) found that the ANKRD6 signal was prominent in the embryonic central

nervous system and in dorsal root ganglia at E12. Within the central nervous system, expression was highest in ventricular zones of neuronal proliferation, particularly around the rhombencephalic and mesencephalic ventricles. At E15, ANKRD6 RNA concentration was also elevated in the spinal cord, dorsal root ganglia, and cranial ganglia. These findings provide evidence that ANKRD6 is highly expressed in the brain in a developmentally regulated manner, suggesting important functions that remain to be studied further.

Other research has demonstrated that ANKRD6 likely plays a role in human cardiogenesis. In zebrafish, ANKRD6 was found to control fusion of heart precursors, influence gastrulation movements during embryogenesis, and play a critical role in normal heart development. Additionally, ANKRD6 functions in Wnt signaling pathways, which regulate many developmental processes including cell proliferation, cell-fate specification, and morphogenesis in embryos (5). Within the Wnt pathway, ANKRD6 specifically binds with Dishevelled in the planar cell polarity pathway, creating a functional interaction essential for cardiogenesis and gastrulation in vertebrates (15).

Based on its physiology and function in developmental biology, ANKRD6 would appear to play an important role in human neural development, axis formation, and cardiogenesis. Additionally, according to Table 2, all SNPs we found phenotype associations with were located in exons, suggesting that these SNPs may influence the function of the protein. Thus, it is biologically plausible that this gene could influence muscle performance and habitual PA participation through centrally and peripherally mediated mechanisms, which may alter neural and cardiac tissue development, growth, and function. A centrally mediated mechanism for PA regulation has been suggested by studies that involve the candidate gene dopamine receptor 1 (*Drd1*) (14). Of note, Knab et al. (12) found that the brains of high physically active animals presented with down-regulated *Drd1* compared with low physically active animals for 7 different dopamine genes. Furthermore, Rhodes and Garland (23) showed that PA was altered through pharmacological manipulation of *Drd1*. Although mechanisms explaining how *Drd1* regulates PA are not yet known, the existent research suggests that the *Drd1* associations with PA in animals are centrally mediated. Thus, it is possible that the *ANKRD6* genetic variants may also associate with PA through central mechanisms. Our discussion regarding central and peripheral mechanistic explanations of how *ANKRD6* may influence PA and muscle performance are purely speculative, and further prospective studies are necessary to validate our preliminary findings and, if validated, investigate mechanisms for the associations we observed.

Strengths of our study include a large homogenous sample and a highly standardized training intervention. Additionally, although FAMuSS was a multicenter trial and measurements of muscle performance and habitual PA were taken at multiple sites by a variety of different investigators, a manual with standardized measurement techniques and investigator certification was required at each site to minimize measurement variability, and all sites used the same equipment.

One limitation of this study is that FAMuSS was not originally designed to assess habitual PA levels, which were determined using a self-reported questionnaire. However, the PPAQ has been validated in similar subject populations and is considered to provide an accurate

estimation of habitual PA in adults (1). Another limitation is that the study involved a young self-selected sample from university communities that may not represent the general population as a whole. However, the sample was an accurate representation of the general college-aged population from which it was studied. Similar to most candidate gene association studies, the significance we found in this study is limited by very low minor allele frequency values of the SNPs examined. However, cell sizes of the individual SNPs will never approach equality in this case because of the low prevalence of the minor allele in the general population. Despite such limitations, Urso (31) recently cited FAMuSS as one of the few initial studies in the field of exercise genomics that followed the model for a quality exercise genomics research study including a large sample size, rigorous exercise intervention, and diverse population. A final limitation of this study is that one of the SNPs, *ANKRD6* 710 L>X, was not in Hardy-Weinberg equilibrium.

In conclusion, the findings of this study support our hypothesis that *ANKRD6* genetic variants associate with muscle size and strength in response to RT and habitual PA. Literature identifying specific gene associations with habitual PA is scarce, and current data are preliminary. Furthermore, the ANKRD6 protein has never been investigated in humans. Additional research is needed to validate the results of this preliminary candidate gene association study and to explore the pathways through which *ANKRD6* genetic variants influence muscle performance and habitual PA.

## Practical Applications

Despite the many potential benefits of regular exercise, current PA levels do not measure up to the alarming increases in obesity and sedentary lifestyles across the nation. Findings such as ours regarding *ANKRD6* associations with muscle size and strength response to RT and PA may contribute to a better understanding of the significant role that genetics and individual physiological variability plays in muscle performance and participation in PA (4,11,22). Eventually, this may lead to the use of genetic information in developing individualized weight loss and training goals and personalized prescription to enhance PA participation and desired health outcomes. From a disease prevention perspective, improving PA levels in sedentary individuals has enormous potential for preventing cardiovascular disease and decreasing morbidity and mortality rates.

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**Table 1**

Applied Biosystem Assay ID.*

| SNP | ABI assay ID | Old SNP name |
|---|---|---|
| T233M | C____343850_1_ | PCR8SNP1 |
| P631L-X | | PCR15SNP2 |
| I128V | C__25754046_10 | PCR5SNP2 |
| Q122E-X | C__25754098_10 | PCR5SNP1 |
| K710X-X | | PCR15SNP3 |
| G197805A | C__25758613_10 | PCR15SNP4 |
| M458L-X | | PCR13SNP1 |
| T545A | C__30004426_10 | PCR15SNP1 |

*ID = Identification; SNP = single-nucleotide polymorphism; ABI = Applied Biosystem.

## Table 2

Information for SNPs: Gene, position, SNP ID, and location.*

| SNP position | Amino acid change | SNP ID | Location of SNP |
|---|---|---|---|
| C183431T | T233M | rs2273238 | Exon 8 |
| C197517T | P631L | rs61739327 | Exon 16 |
| C172840G | Q122E | rs16881983 | Exon 5 |
| C172858T | I128V | rs3748085 | Exon 5 |
| A197738T | K710X | NOVEL | Exon 16 |
| G197805A | N/A | rs9344950 | 3UTR |
| A194373C | M458L | rs61736690 | Exon 14 |
| G197258A | T545A | rs9362667 | Exon 16 |

*SNP = single-nucleotide polymorphisms; ID = identification.

**Table 3**

TaqMan primer sets for *ANKRD6* gene SNPs examined in this study.*

| SNP | RefSeq No. | Forward primer | Reverse primer | WT allele probe (5′ VIC) | MT allele probe (5′ FAM) |
|---|---|---|---|---|---|
| 233 T>M | rs2273238 | AAGGTGGCCAAAATCTTACTGGAA | TGGGAACCACTTTAGAAAGCAATGT | CAATGGTCGTATCTGC | CAATGGTCATATCTGC |
| 631 P>L | rs61739327 | GGGAAGAGTGGGCCAACAAG | GCCCACAGGTGCTGCT | CTGCGGGTTGCTG | CTGCGAGTTGCTG |
| 122 Q>E | rs16881983 | AGCCTTGCATGAAGCATCCT | CGTTGGCTCCTGCTTTAATGAG | CTGACTGGCTGAAAC | CTGACTCGCTGAAAC |
| 128 I>L | rs3748085 | GGCATGGTTTCAGCCAGTCA | CTTGTTCTTGGCAAGCACGTT | CAAGCTGCTCATTAAA | AAGCTGCTCGTTAAA |
| 710 L>X | NOVEL | CCCAGCAAGATAAGGCTACATTGAA | CCCTAGTCCTTAGTTTGGCAAGTT | CCTCTTCTAAACTTTTAATGTG | TCTTCTAAACTTTAAATGTG |
| 197805 G>A | rs9344950 | GGACTAGGGTGCAGAAGGAAAATTA | GCAAAACTGGAAATCTTCAAGAATCCT | CACCAATAAAGAGGAAAT | CACCAATAAAAAGGAAAT |
| 485 M>L | rs61736690 | GGGCTCACCCTGAGATCATCT | CTCAACCATCAGCTTGTCCAAAA | CTTCACTCCTAGATGCGTG | TTCACTCCTAGCTGCGTG |
| 545 T>A | rs9362667 | ACCAGGTGTGGACCAATTAGTG | CCTTCTCTTTGGGCCTAACCA | CAGCAGCAGCTTC | CAGCAACAGCTTC |

**ANKRD6* = Ankyrin Repeat Domain 6; SNP = single-nucleotide polymorphisms; VIC = VIC® (dye labeled probe); FAM = FAM™ (dye labeled probe).

**Table 4**

Chi-square ($\chi^2$) and allelic frequencies of *ANKRD6* SNPs examined in this study.*

| SNP | RefSeq No. | $\chi^2$ | *p* | *q* | *p* value for significance |
|---|---|---|---|---|---|
| ANKRD6 545 T>A | rs9362667 | 0.05 | 0.992 | 0.008 | 0.821 |
| ANKRD6 485 M>L | rs61736690 | 0.01 | 0.999 | 0.001 | 0.987 |
| ANKRD6 233 T>M | rs2273238 | 0.52 | 0.952 | 0.048 | 0.472 |
| ANKRD6 128 I>L | rs3748085 | 0.15 | 0.839 | 0.161 | 0.696 |
| ANKRD6 631 P>L | rs61739327 | 2.08 | 0.860 | 0.140 | 0.150 |
| ANKRD6 122 Q>E | rs16881983 | 0.38 | 0.979 | 0.021 | 0.534 |
| ANKRD6 198705 G>A | rs9344950 | 0.01 | 0.816 | 0.184 | 0.941 |
| ANKRD6 710 L>X | NOVEL | 21.1 | 0.986 | 0.014 | <0.001† |

*ANKRD6 = Ankyrin Repeat Domain 6 gene; SNPs = single-nucleotide polymorphisms. *df* = 1 for all analyses.

†Does not meet Hardy-Weinberg equilibrium.

## Table 5

Mean (± *SEM*) CSA, MVC, and 1RM pre- and pre- to post-RT of trained arm by *ANKRD6* genotype.*

| Phenotype | *ANKRD6* 122 Q>E | | |
|---|---|---|---|
| | QQ (*N* = 658) | QE (*N* = 27) | EE† (*N* = 0) |
| Pre-RT CSA ($cm^2$) | 16.4 ± 0.1‡ | 18.2 ± 1.0‡ | |
| Absolute CSA Δ ($cm^2$) | 3.1 ± 0.1 | 3.3 ± 0.4 | |
| Relative CSA Δ (%) | 19.4 ± 0.4 | 18.6 ± 2.0 | |
| | *ANKRD6* 631 P>L | | |
| | PP (*N* = 510) | PL (*N* = 166) | LL (*N* = 10) |
| Pre-RT CSA ($cm^2$) | 16.5 ± 0.2 | 16.2 ± 0.3 | 15.1 ± 1.6 |
| Absolute CSA Δ ($cm^2$) | 3.2 ± 0.1§ | 2.9 ± 0.1§ | 2.5 ± 0.4 |
| Relative CSA Δ (%) | 19.7 ± 0.4 | 18.4 ± 0.7 | 16.1 ± 1.7 |
| | *ANKRD6* 710 L>X | | |
| | LL (*N* = 721) | LX (*N* = 18) | XX (*N* = 2) |
| Pre-RT MVC (kg) | 45.4 ± 0.5 | 37.0 ± 1.6 | 32.5 ± n/a |
| Absolute MVC Δ (kg) | 8.1 ± 0.3// | 3.9 ± 1.0// | 9.6 ± n/a |
| Relative MVC Δ (%) | 21.5 ± 0.9¶ | 11.3 ± 4.1¶ | 29.5 ± n/a |
| | *ANKRD6* 545 T>A | | |
| | TT (*N* = 454) | TA (*N* = 10) | AA† (*N* = 0) |
| Pre-RT 1RM (kg) | 9.2 ± 0.1 | 9.5 ± 0.4 | |
| Absolute 1RM Δ (kg) | 4.1 ± 0.1# | 4.8 ± 0.6# | |
| Relative 1RM Δ (%) | 52.1 ± 1.5 | 57.6 ± 7.8 | |

*CSA = cross-sectional area; MVC = maximum voluntary contraction; IRM = 1 repetition maximum; RT = resistance training; *ANKRD6* = Ankyrin Repeat Domain 6. Muscle phenotype values displayed have been adjusted for age, body mass index, and gender. Genotype main effects by genotype: ANKRD6 122 Q>E ($p = 0.014$), ANKRD6 631 P>L ($p = 0.018$), ANKRD6 710 L>X ($p = 0.035$), ANKRD6 545 T>A ($p = 0.032$).

†The common allele frequency approached 1 for the common allele and 0 for the heterozygous minor allele (10).

‡QQ vs. QE, $p = 0.076$.

§PP vs. PL, $p = 0.062$.

//LL vs. LX, $p = 0.074$.

¶LL vs. LX, $p = 0.075$.

#TT vs. TA, $p = 0.069$; Δ = change.

## Table 6

Mean (± *SEM*) time spent in vigorous-, moderate-, and light-intensity physical activity (PA), and sitting among the total sample and women by *ANKRD6 631* P>L genotype.*

| Phenotype | | PP | PL | LL |
|---|---|---|---|---|
| (*N* = total sample) | | 392 | 125 | 10 |
| (*N* = women) | | 214 | 73 | 3 |
| Vigorous-intensity PA (h/wk) | Total sample | 8.1 ± 0.4 | 8.1 ± 0.7 | 4.4 ± 2.8 |
| | Women | 7.6 ± 0.5 | 8.3 ± 0.8 | 4.0 ± 4.0 |
| Moderate-intensity PA (h/wk) | Total sample | 20.8 ± 0.7 | 20.7 ± 1.2 | 33.4 ± 4.7† |
| | Women | 21.0 ± 0.9 | 21.3 ± 1.6 | 39.9 ± 7.8‡ |
| Light-intensity PA (h/wk) | Total sample | 36.2 ± 0.8 | 36.9 ± 1.4 | 33.7 ± 5.4 |
| | Women | 36.2 ± 1.0 | 39.9 ± 1.8 | 36.6 ± 8.7 |
| Sitting (h/wk) | Total sample | 44.9 ± 0.9 | 45.3 ± 1.7 | 43.4 ± 6.4 |
| | Women | 45.4 ± 1.2 | 42.0 ± 2.0 | 34.7 ± 9.9 |

**ANKRD6* = Ankyrin Repeat Domain 6; PA = physical activity; PL = heterozygous allele; PP = homozygous dominant allele; LL = homozygous recessive allele. Physical activity phenotype values displayed have been adjusted for age, body mass index, and gender. Genotype main effects by genotype: ANKRD6 631 P>L ($p = 0.03$).

†LL vs. PP, LL vs. PL, $p = 0.03$.

‡LL vs. PP, LL vs. PL; $p = 0.05$.