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## Influence of promoter region variants of insulin-like growth factor pathway genes on the strength-training response of muscle phenotypes in older adults

Brian D. Hand<sup>1,2</sup>, Matthew C. Kostek<sup>1</sup>, Robert E. Ferrell<sup>3</sup>, Matthew J. Delmonico<sup>1,4</sup>, Larry W. Douglass<sup>5</sup>, Stephen M. Roth<sup>1</sup>, James M. Hagberg<sup>1</sup>, and Ben F. Hurley<sup>1</sup>

<sup>1</sup>Department of Kinesiology, University of Maryland, College Park, Maryland <sup>2</sup>Department of Kinesiology, Towson University, Towson, Maryland <sup>3</sup>Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania <sup>4</sup>Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania <sup>5</sup>Department of Avian and Animal Sciences, University of Maryland, College Park, Maryland

### Abstract

To examine the influence of insulin-like growth factor (IGF) pathway gene polymorphisms on muscle mass and strength responses to strength training (ST), we studied 128 White and Black men and women before and after a 10-wk single-leg knee extension ST program. One-repetition maximum strength, muscle volume (MV) via computed tomography, and muscle quality (MQ) were assessed at baseline and after 10 wk of ST. There was a significant combined *IGF1* cytosine adenine (CA) repeat gene effect, which included both the *IGF1* CA repeat main effect and *IGF1* CA repeat × *PPP3R1* insertion-deletion (I/D) gene × gene interaction effect, on the changes in strength ( $P < 0.01$ ) and MQ ( $P < 0.05$ ) with ST. There was a trend for a significant gene × gene interaction between *IGF1* CA repeat and *PPP3R1* I/D for changes in strength ( $P = 0.07$ ) and MQ ( $P = 0.06$ ) with ST. The influence of the *PPP3R1* A-202C gene polymorphism on change in MV with ST approached significance ( $P = 0.06$ ). The *IGF1* CA repeat polymorphism had a significant influence on the change in strength and MV combined with ST ( $P < 0.05$ ), whereas the influence of the *PPP3R1* I/D polymorphism approached significance ( $P = 0.08$ ). There were no associations between the *IGFBP3* A-202C gene polymorphism and the muscle phenotypic responses to ST. These data suggest that two of the three IGF pathway gene polymorphisms identified in this study influence muscle phenotypic responses to ST in both Black and White older men and women.

### Keywords

genetics; muscle strength; muscle volume; muscle quality

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Losses of muscle mass and strength with aging, referred to as sarcopenia, have been well documented (25,27,28) and have been associated with many adverse health consequences, including increased mortality (29–31). Strength training (ST) has been shown to be an effective intervention for the prevention and treatment of the adverse consequences of sarcopenia with few side effects (17,18,42). Nevertheless, increases in muscle mass and strength are highly variable among individuals (20,26), suggesting a genetic influence. Further support for a

genetic influence on muscle phenotypes comes from twin studies, which have shown that up to 90% of the variance in baseline muscle mass and ~60% of the variance in baseline muscle strength are heritable (19). Although it can account for a smaller percentage of the variance, response of these muscle phenotypes to ST also appears to be heritable (51). However, there have been few candidate genes identified that influence muscle responses to ST (2,10,20,24, 39), and there are no reports, that we are aware of, on the influence of candidate genes that are linked in a common physiological pathway.

Insulin-like growth factor-I (IGF-I) is a potent mitogen and anabolic agent important in the growth of various body tissues, including skeletal muscle (9,48). The decline of circulating IGF-I levels with advancing age is related to the losses of muscle mass and strength that occur with age (52). ST increases skeletal muscle IGF-I protein and IGF-I mRNA levels, even in the elderly (8,12), and IGF-I produced in skeletal muscle can stimulate muscle hypertrophy through activation of satellite cells and increased protein synthesis rates (15,16). Nevertheless, significant variability in skeletal muscle IGF-I levels have been reported (8,13).

Some of this variability in response to ST may be accounted for by a cytosine adenine (CA) dinucleotide repeat polymorphism in the promoter region of the *IGF1* gene, which encodes for the IGF-I protein (24). It is plausible that other polymorphisms within the IGF pathway of genes could be involved in muscle hypertrophy and strength response to ST, but no reports are available, to our knowledge, on the influences of these genes on muscle phenotypic responses to ST. Two examples of such genes within the IGF pathway are insulin-like growth factor binding protein 3 (*IGFBP3*) and calcineurin B (*PPP3R1*).

Most circulating IGF-I is bound by IGFBP-3 (1), which can potentiate or inhibit the action of IGF-I on skeletal muscle (48). Although it is unclear whether IGFBP-3 is the primary carrier of IGF-I in skeletal muscle, there is evidence that it does exist in skeletal muscle (11,43,47) and that secretion of IGFBP-3 in primary adult human skeletal muscle cell models is stimulated by IGF-I (11). There have been several reports that the A-202C polymorphism in the promoter region of the *IGFBP3* gene can influence the levels of this protein in circulation (3,22,46), which may in turn modulate the activity of IGF-I. Deal et al. (3) showed that this polymorphism was directly related to promoter activity of the *IGFBP3* gene, suggesting a functional association that potentially affects IGFBP-3 protein levels.

IGF-I-induced muscle hypertrophy in skeletal muscle cells in culture is at least partially mediated by a  $Ca^{2+}$ -dependent calcineurin signaling pathway (35,44). Calcineurin is a  $Ca^{2+}$ /calmodulin-dependent protein phosphatase, which consists of a catalytic subunit, designated as calcineurin A, and a regulatory  $Ca^{2+}$ -binding subunit, designated as calcineurin B (55). Calcineurin plays a role in both cardiac (34,40) and skeletal muscle hypertrophy (6,7,35,44, 49). Tang et al. (50) reported that the 5-bp insertion-deletion (I/D) polymorphism in the promoter region of the *PPP3R1* gene was associated with the incidence of inappropriately high left ventricular mass in severely hypertensive individuals. Although conflicting results have been reported on the role of calcineurin on skeletal muscle hypertrophy (38,45), it is conceivable that the *PPP3R1* I/D polymorphism may influence hypertrophic responses of skeletal muscle, based on findings in cardiac muscle, given that these two tissues share common hypertrophic pathways (36).

The purpose of the present study was to test the hypothesis that polymorphisms in the promoter regions of the *IGF1*, *IGFBP3*, and *PPP3R1* genes, which may be linked in a common biological pathway, will significantly influence the changes in muscle volume (MV), strength, and muscle quality (MQ) with ST in older White and Black men and women. To test this association, the CA dinucleotide repeat polymorphism in the promoter region of the *IGF1* gene, the -202 locus

polymorphism in the promoter region of the *IGFBP3* gene, and the 5-bp I/D polymorphism in the promoter region of the *PPP3R1* gene were studied.

## Methods

### Subjects

One hundred twenty-eight previously physically inactive relatively healthy men ( $n = 58$ ) and women ( $n = 70$ ) between the ages of 50 and 85 yr volunteered to participate in this study. A small portion of the subjects ( $n = 10$ ) were from a previous study cohort in our laboratory who underwent the identical ST intervention program (20). Before participation, all subjects underwent a phone-screening interview, received medical clearance from their primary care physicians, and completed a detailed medical history. They were nonsmokers and free of significant cardiovascular disease and metabolic or musculoskeletal disorders that would affect their ability to safely perform heavy-resistance exercise. Subjects who were already taking medications for at least 3 wk before the start of the study were permitted into the study as long as they did not change their medications or dosages at any time throughout the study. After all methods and procedures were explained, subjects read and signed a written consent form that had been approved by the Institutional Review Board of the University of Maryland (College Park, MD). All subjects were continually reminded throughout the study not to alter physical activity levels or dietary habits for the duration of the study. Body weight was monitored weekly throughout the study to ensure compliance in maintaining a stable diet.

### Body composition assessment

Body composition was measured by dual-energy x-ray absorptiometry using fan-beam technology (model QDR 4500A, version 8.21 software; Hologic, Waltham, MA). A total body scan was performed at baseline and again after the ST program to assess total body and thigh fat-free mass (FFM), fat mass, and percent body fat as described in a previous study from our laboratory (20). The coefficients of variation (CV) for all dual-energy x-ray absorptiometry measures of body composition were calculated from repeated scans of 10 subjects who were scanned three consecutive times with repositioning. The CV was 0.6% for FFM and 1.0% for percent body fat.

### Strength testing

One-repetition maximum (1 RM) strength tests were performed before and after the ST program using an air-powered knee-extension resistance machine (Keiser A-300 leg extension machine) as described previously (20). At least two familiarization training sessions were performed before the 1 RM strength testing and the ST program, in which the exercises were performed with little or no resistance. These low-resistance training sessions were conducted to familiarize the subjects with the equipment, to help control for the large 1-RM strength gains that commonly result from skill (motor learning) acquisition during the initial stages of training, and to help prevent injuries and soreness after the ST program. The criterion for a successful attempt during the strength test was extending the lower leg to a knee joint extension of  $\sim 165^\circ$ , which was assessed on every trial by a system that turned on a red light each time the leg was extended to this knee joint angle.

### ST program

The ST program consisted of unilateral (one-legged) training of the knee extensors of the dominant leg, three times per week, for  $\sim 10$  wk on a Keiser A-300 air-powered leg extension machine as described previously (20). The training protocol was specifically designed to elicit near-maximal effort in an individualized manner on all repetitions after warm-up, while maintaining high volume, as we have described previously in detail (5,24). The untrained

control leg was kept in a relaxed position throughout the training program. One-on-one supervision by qualified exercise leaders was provided for all exercise sessions.

### MV and MQ

To quantify quadriceps MV, computed tomography imaging of the trained and untrained thighs was performed (GE Lightspeed Qxi; General Electric, Milwaukee, WI) at baseline and during the last week of the 10-wk unilateral ST program with Medical Image Processing, Analysis, and Visualization software (National Institutes of Health, Bethesda, MD) used to analyze all images, as previously described by our group (20). The final MV was calculated with the truncated cone formula, as described previously by our group (53). The changes in MV in the untrained leg (measured pre- and posttraining) were subtracted from the changes in the trained leg (measured before and after) to correct for those factors that were not being investigated in this study (i.e., seasonal variation). In addition, data were used in this analysis from 10 subjects from a previous cohort. All methods for testing and training these subjects were the same as for the cohort in the present study, except that MV was measured by MRI. However, Mitsiopoulos et al. (32) have shown a correlation of 0.99 between computed tomography and MRI for the measurement of skeletal MV. Investigators were blinded to subject identification, date of scan, and training status, for both baseline and after-training analysis. Repeated-measure CV was calculated for each investigator based on axial assessments on 2 separate days. Intrainvestigator CV averaged 1.7 and 2.3%, whereas, interinvestigator CV was <4.3%. MQ for the quadriceps was defined as strength per unit of muscle mass (MV).

### Genotyping

Genomic DNA was prepared from EDTA-anticoagulated whole blood samples by standard salting-out procedures (Gentra Systems). The CA microsatellite of *IGF1* (rs no. 10665874) was amplified by PCR of genomic DNA using fluorescence-tagged primers (24,41). The ABI 3100 DNA sequencer (PE Applied Biosystems) and ABI Genescan/Genotyper 2.5 software program (PE Applied Biosystems) were used to determine the genotype of the CA repeat microsatellite in the promoter region of the *IGF1* gene. Genotype assignment was based on the method described by Rosen et al. (41) (e.g., 19 CA repeats = 192 bp), in which these authors found the 192 allele to be the most common and thus compared it with other alleles for this microsatellite. Genotyping of the *IGFBP3* A-202C polymorphism (rs no. 2854744) was performed by PCR and -202 restriction digest of the PCR product with *Alw21I* as described by Deal et al. (3). The 5-bp I/D polymorphism located at the -1059 to -1063 loci relative to the transcription site of the calcineurin B gene (*PPP1R3*) (rs no. 3039851) was genotyped by standard PCR and enzyme digest with *AseI* as described by Tang et al. (50). Direct sequencing was used to confirm the accuracy of all genotyping methods.

### Statistical analyses

All statistical analyses were performed with SAS software (SAS version 9.1, SAS Institute, Cary, NC). Changes in body weight, percent body fat, and FFM with ST within each sex, race, and genotype group were tested by paired *t*-tests. Fixed-effect linear models were used to test differences in baseline muscle phenotypes (1 RM strength, MV, and MQ) among the categorical variables: sex and hormone replacement therapy status (male, female on hormone replacement therapy, or female not on hormone replacement therapy), race, and genotype groups and to test for differences in the change in muscle phenotypes with ST among sex and hormone replacement therapy status, race, and genotype groups. Initial linear models for each muscle phenotype (dependent variable) included the main effect of the following independent variables: the CA dinucleotide repeat polymorphism of *IGF1*, the -202 locus polymorphism in the promoter region of *IGFBP3*, and the 5-bp I/D polymorphism of calcineurin B (*PPP3R1*). The initial models also included their two-way interactions with each other, as well

as with race (White and Black). There were insufficient data ( $n < 5$ ) for certain genotype by sex and hormone replacement therapy status groups, so these gene by sex and hormone replacement therapy status interactions were not tested in the models.

Because sample sizes for each genotype were a function of different allelic frequencies, the experiment was unbalanced. Therefore, nonindependent sources of variation were removed using a backward elimination process similar to that previously described (14). The final statistical model for the change in strength with ST included baseline strength, age, height, body-mass index, race, a class variable that combined sex difference and hormone replacement therapy status, *IGF1* CA repeat polymorphism  $\times$  *PPP3R1* I/D polymorphism interaction, *IGFBP3* A-202C polymorphism  $\times$  race interaction, *IGF1* CA repeat polymorphism, *IGFBP3* A-202C polymorphism, and *PPP3R1* I/D polymorphism. The final statistical model for the change in MV with ST included baseline MV, age, height, body-mass index, race, a class variable that combined sex and hormone replacement therapy status, *IGF1* CA repeat polymorphism, *IGFBP3* A-202C polymorphism, and *PPP3R1* I/D polymorphism. The final statistical model for change in MQ with ST included baseline MQ, age, height, body-mass index, race, a class variable that combined sex and hormone replacement therapy status, *IGF1* CA repeat polymorphism  $\times$  *PPP3R1* I/D polymorphism interaction, *IGF1* CA repeat polymorphism, *IGFBP3* A-202C polymorphism, and *PPP3R1* I/D polymorphism. For those final models in which interaction terms were present, the significance of the contribution for the combined gene effect, which included both main effect and interaction effects, was tested by comparing the error term sums of squares for the full model (all gene effects and error term) with the error term sums of squares for the model in which the gene effects of interest were removed from the model. The combined gene effect for a particular gene included the main effect for that gene and its interactions with other genes that were tested. For example, the combined gene effect for *IGF1* CA repeat would include the *IGF1* CA repeat main effect and either the *IGF1* CA repeat  $\times$  *IGFBP3* A-202C interaction or the *IGF1* CA repeat  $\times$  *PPP3R1* I/D interaction. Levels of significance for combined gene effects were tested in the cases in which the gene  $\times$  gene interaction was significant ( $P < 0.05$ ) or approached significance ( $P < 0.10$ ). Results are presented as means (SD) for age, height, body weight, percent body fat, and FFM and as least-squares means  $\pm$  SE for muscle phenotypes.

For all analyses, the initial threshold of significance was set to  $P < 0.05$ . Mean comparisons were made by *t*-tests, with *P* values adjusted using a Bonferroni correction to reduce the chance of a type I error.

### Hardy-Weinberg equilibrium

Genotype distributions were evaluated for conformity to Hardy-Weinberg equilibrium using  $\chi^2$  tests for the *IGFBP3* A-202C and *PPP3R1* 5-bp I/D loci and using exact tests for the *IGF1* CA repeat microsatellite.

### Race by genotype

To determine whether data for change in muscle phenotypes with ST for Black and White subjects could be pooled, gene  $\times$  race interactions were tested in each linear model for the *IGF1*, *IGFBP3*, and *PPP3R1* gene polymorphisms. For the *IGF1* gene polymorphism, there was insufficient data for Black subjects homozygous for the *IGF1* 192 allele. Therefore, initially for each of the linear models, subjects homozygous for the *IGF1* 192 allele and subjects heterozygous for the 192 allele were combined so that sufficient data existed to test the *IGF1*  $\times$  race interaction. However, once this interaction term was no longer significant in the model and removed from the model, the *IGF1* gene effects were tested with all *IGF1* genotype groups (subjects homozygous for the 192 allele, subjects heterozygous for the 192 allele, and

noncarriers of the 192 allele). In addition, to control for the potential influence of race on muscle phenotype responses to ST, race was used as a covariate in all linear models.

### Percent variability explained by genotype

To estimate the percent variability for the change in strength, MV, and MQ with ST attributable to *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D genotypes and any relevant gene  $\times$  gene interactions, the sums of squares of the gene or gene  $\times$  gene interaction of interest were divided by the sums of squares of all gene effects present in the model and the error sums of squares. With this procedure, it was not possible to determine the contribution of each gene to the gene  $\times$  gene interaction. Therefore, it was assumed that each gene involved contributes an equal portion to the percent variability.

A multivariate analysis was used to test for the influence of *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D gene polymorphisms on the combined change of strength and MV with ST.

## Results

### Allele and genotype frequencies

Table 1 shows the allele and genotype frequencies for the *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D promoter polymorphisms. The allele and genotype frequencies for the *IGF1* CA repeat microsatellite, *IGFBP3* A-202C, and *PPP3R1* 5 bp I/D loci did not differ significantly from Hardy-Weinberg expectations, and these frequencies were similar to those reported previously (3,50). There was a significant difference between races in the allele frequencies for the *IGF1* CA repeat ( $P < 0.05$ ) and *PPP3R1* I/D variant ( $P < 0.001$ ). There was no significant difference between race in allele frequency for the *IGFBP3* A-202C variant. Data for the *PPP3R1* DD genotype group were combined with the *PPP3R1* I/D genotype group, as D allele carriers, and were compared with data from subjects homozygous for *PPP3R1* II because only one subject was a *PPP3R1* DD homozygote.

### Physical characteristics

Tables 2 and 3 show that there were no significant changes in body weight, percent body fat, or FFM with ST within sex or race groups. Similarly, there were no significant within genotype group differences for change in body weight, percent body fat, or FFM with ST, except those with MV data who were *IGF1* ca carriers of the 192 allele, who had a significant decrease in percent body fat with ST ( $P < 0.001$ ). However, these data are not reported in Tables 2 and 3. Men had greater mean values than women for baseline 1 RM strength ( $P < 0.001$ ), MV ( $P < 0.001$ ), and MQ ( $P < 0.01$ ), and Black subjects had greater MV results than White subjects at baseline ( $P < 0.001$ ).

### Genotype associations with muscle phenotypes at baseline

There were no significant differences in baseline muscle phenotypes among *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D genotype groups. The *IGFBP3* A-202C  $\times$  race interaction for baseline 1 RM strength approached significance ( $P = 0.06$ ), and there was also a significant *IGFBP3* A-202C  $\times$  race interaction for baseline MQ ( $P < 0.05$ ).

### Muscle phenotype responses to ST for sex and race groups

Men had significantly greater absolute ( $9.2 \pm 0.8$  vs.  $5.1 \pm 0.7$  kg;  $P < 0.001$ ) and relative (%) increases ( $37.0 \pm 3.4$  vs.  $27.0 \pm 3.1\%$ ;  $P < 0.05$ ) in knee extension 1 RM strength with ST than women. It was not possible to determine whether there was a significant difference among races for absolute and relative change in strength because of a trend for a significant *IGFBP3*  $\times$  race interaction (see Fig. 2). There was no significant difference between the

absolute or relative changes in MV or MQ with ST between men and women. In addition, there were no significant differences between Black and White individuals for absolute or relative changes in MV with ST. There was a significant difference in the absolute change in MQ in Black compared with White individuals ( $3.5 \pm 0.4$  vs.  $2.6 \pm 0.3$  kg/cm<sup>3</sup>  $\times 10^{-3}$ ;  $P \leq 0.04$ ). The relative difference in MQ between Black and White individuals could not be determined because of an *IGFBP3* A-202C  $\times$  race interaction for this phenotype.

### Race $\times$ sex interactions for change in muscle phenotypes with ST

There was a significant race  $\times$  sex interaction for change in muscle strength with ST ( $P < 0.05$ ). However, there were no significant race  $\times$  sex interactions for changes in MV ( $P = 0.29$ ) or MQ ( $P = 0.46$ ).

### Race $\times$ gene interaction for change in muscle phenotypes with ST

There were no significant gene  $\times$  race interactions for the changes in 1 RM strength, MV, or MQ with ST, except for a trend for a significant race  $\times$  *IGFBP3* A-202C interaction for change in strength. For this reason, data from Black and White subjects were combined for all other genotype analyses to improve statistical power.

### Genotype influence on 1 RM strength responses to ST

There was a significant combined gene effect, including both *IGF1* CA repeat main effect and *IGF1* CA repeat  $\times$  *PPP3R1* I/D gene  $\times$  gene interaction effect, on change in strength with ST ( $P < 0.01$ ). Table 4 shows that this total gene effect accounted for 3.5% of the variability in change in muscle strength with ST. Figure 1 shows that the gene  $\times$  gene interaction between *IGF1* CA repeat and *PPP3R1* I/D alone approached significance for change in strength with ST ( $P = 0.07$ ). In this case, individuals homozygous and heterozygous for *IGF1* 192 responded similarly, whereas *IGF1* noncarriers of the 192 allele responded differently with respect to *PPP3R1* genotype groups. In fact, individuals homozygous for *PPP3R1* II who were also heterozygous for 192 allele for *IGF1* had significantly greater increases in strength with ST than those homozygous for *PPP3R1* II, who were also noncarriers of the 192 allele for *IGF1* ( $8.4 \pm 0.7$  vs.  $4.7 \pm 0.9$  kg;  $P < 0.01$ ). However, for *PPP3R1* D allele carriers, there was no significant difference in change in strength with ST among *IGF1* genotype groups. There was no significant combined gene effect for *IGFBP3* A-202C, which included both the *IGFBP3* A-202C main effect and the *IGFBP3* A-202C  $\times$  race interaction effect, although the *IGFBP3* A-202C  $\times$  race interaction approached significance ( $P = 0.09$ ). However, Black individuals who were in the AA genotype group for the *IGFBP3* A-202C polymorphism had a significantly greater increase in strength with ST than White individuals who were also homozygous for *IGFBP3* AA ( $10.1 \pm 1.2$  vs.  $5.3 \pm 0.8$  kg;  $P < 0.01$ ), whereas there were no significant differences between races for change in strength for the *IGFBP3* AC and CC genotype groups (Fig. 2). There was no significant combined gene effect for *PPP3R1* I/D, including both *PPP3R1* I/D main effect and *PPP3R1* I/D  $\times$  *IGF1* CA repeat gene  $\times$  gene interaction effect, on the change in strength with ST. The *IGF1* CA repeat  $\times$  *IGFBP3* A-202C, *IGFBP3* A-202C  $\times$  *PPP3R1* I/D, *IGF1* CA repeat  $\times$  race, and *PPP3R1* I/D  $\times$  race interactions for change in strength with ST were not significant. Also, there was no significant influence of the *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D main effects on change in strength with ST.

### Genotype influence on MV responses to ST

Because of no significant gene  $\times$  gene or gene  $\times$  race interactions for change in MV with ST, combined gene effects were not determined. However, Fig. 3 shows that individuals homozygous for II of the I/D polymorphism in the promoter region of the *PPP3R1* gene approached significance for having a greater increase in MV with ST than D allele carriers

( $130 \pm 10$  vs.  $100 \pm 14$  cm<sup>3</sup>;  $P = 0.06$ ). There was no significant influence of the *IGF1* CA repeat and *IGFBP3* A-202C main effects on change in MV with ST.

### Genotype influence on MQ responses to ST

There was a significant combined gene effect for *IGF1* CA repeat, including both *IGF1* CA repeat main effect and *IGF1* CA repeat  $\times$  *PPP3R1* I/D gene  $\times$  gene interaction effect, for change in MQ with ST ( $P < 0.05$ ). Figure 4 shows that the *IGF1* CA repeat  $\times$  *PPP3R1* I/D gene  $\times$  gene interaction alone approached significance for change in MQ with ST ( $P = 0.06$ ). Because of this interaction, there was no consistent difference between *PPP3R1* I/D genotype groups in MQ response to ST among *IGF1* CA repeat genotype groups. Those who were both homozygous for *PPP3R1* II and heterozygous for *IGF1* 192 allele had a significantly greater increase in MQ with ST than those homozygous for *PPP3R1* II who were *IGF1* noncarriers of the 192 allele ( $3.7 \pm 0.4$  vs.  $1.8 \pm 0.5$  kg/cm<sup>3</sup>  $\times 10^{-3}$ ;  $P < 0.01$ ). However, for *PPP3R1* D allele carriers, there was no significant difference in the change in MQ with ST among *IGF1* CA repeat genotype groups. There was no significant combined gene effect for *PPP3R1* I/D, including both *PPP3R1* I/D main effect and *PPP3R1* I/D  $\times$  *IGF1* CA repeat gene  $\times$  gene interaction effect, for change in MQ with ST. Also, the *IGF1* CA repeat  $\times$  *IGFBP3* A-202C, *IGFBP3* A-202C  $\times$  *PPP3R1* I/D, *IGF1* CA repeat  $\times$  race, *IGFBP3* A-202C  $\times$  race, and *PPP3R1* A-202C  $\times$  race interactions for change in MQ with ST were not significant. Finally, there was no significant influence of the *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D main effects on change in MQ with ST.

### Gene polymorphism contribution to each muscle phenotype

Table 4 shows the estimated percent variability attributable to *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D genotypes and to each relevant gene  $\times$  gene interaction for changes in strength, MV, and MQ with ST. The contributions to the percent variability for change in strength and MQ with ST for the *IGF1* CA repeat and *PPP3R1* I/D gene  $\times$  gene interactions was  $\sim 4.5$  and  $5.5\%$ , respectively. The single gene contributions of *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D to percent variability for change in strength and MQ were  $\sim 2$ – $5\%$  and  $1$ – $4\%$ , respectively. For change in MV with ST, the single gene contributions were  $2$ – $3\%$  for *IGF1* CA repeat and *PPP3R1* I/D, whereas the contribution of *IGFBP3* A-202C was  $< 1\%$ .

A correlation matrix analysis resulted in a correlation between change in strength and MV with ST of  $r = 0.25$ . Results from a multivariate analysis showed that there was a significant influence of *IGF1* CA repeat ( $P < 0.05$ ) and a nonsignificant trend for an influence of *PPP3R1* I/D ( $P = 0.08$ ) on the combined change in strength and MV with ST.

## Discussion

To our knowledge, this is the first report that has investigated the influence of genes linked in a common biological pathway on muscle phenotypic responses to ST. The results offer partial support to the hypothesis that IGF pathway gene polymorphisms, as operationally defined for this study, influence changes in muscle phenotypes with ST and suggest that the *IGF1* and *PPP3R1* genes may be linked to influence muscle strength and MQ responses to ST.

The major finding of this study was that change in strength with ST was influenced by the combination of gene polymorphisms for *IGF1* CA repeat main effects and *IGF1* CA repeat  $\times$  *PPP3R1* I/D interaction effects. This combination also influenced change in MQ. This finding was expected, given that the change in MQ is the change in strength per change in MV and that there was only a marginal main effect for *PPP3R1* I/D on change in MV. The findings of this study complement those of a previous study from our laboratory (24), which used some of the same subjects who were enrolled in this investigation and found a significant *IGF1* CA



repeat main effect on the strength response to ST. However, the present study extends these findings by showing that a combined gene effect for *IGF1* CA repeat, which includes both *IGF1* CA repeat main effect and *IGF1* CA repeat  $\times$  *PPP3R1* I/D interaction effect, influenced the strength and MQ responses to ST. In addition, our data support the need for further study to investigate interactions of genes in common biological pathways that may influence skeletal muscle phenotypic responses to ST.

Two previous studies, which investigated individual genes of this biological pathway, have shown an individual influence of the *IGF1* dinucleotide repeat polymorphism (24) and the *PPP3R1* 5-bp I/D polymorphism (50) on muscle phenotypes. In addition, other studies have shown an influence of IGF-I on calcineurin to promote skeletal muscle cell hypertrophy in culture (35,44). However, these studies did not test the influence of IGF pathway genes on responses to ST. The muscle phenotypes that we investigated in the present study are complex phenotypes and would likely be influenced by several genes in several different pathways. However, we found that the contribution to percent variability attributable to the *IGF1* CA repeat  $\times$  *PPP3R1* I/D interaction was  $\sim$ 4.5% and 5.5%, respectively, for the change in strength and MQ with ST. These contributions are larger than the contributions of single genes ( $\sim$ 2%) reported to influence other muscle phenotypic responses to ST (2).

The fact that we observed no significant combined gene effects for *PPP3R1* I/D, including both *PPP3R1* I/D main effects and *PPP3R1* I/D  $\times$  *IGF1* CA repeat interaction effects, on changes in strength and MQ with ST was unexpected. However, the influence of calcineurin on the muscle phenotypic response to ST may be less than that of IGF-I because of the presence of other IGF-I-linked signaling pathways influencing muscle phenotypic responses to ST. In addition, the role of calcineurin in the muscle phenotypic response to ST remains unclear. Although there have been studies that have suggested calcineurin plays a role in hypertrophy, other findings suggest calcineurin plays an important role in fiber-type switching (33,38). Nonetheless, in the present study, there was a significant difference between *PPP3R1* I/D genotype groups that approached significance for change in MV with ST. Tang et al. (50) reported that the 5-bp I/D polymorphism of the *PPP3R1* gene influenced left ventricular muscle mass in White and Black individuals who were severely hypertensive (50). These authors reported that those individuals possessing at least one D allele had a greater risk of developing inappropriately high left ventricular mass than those with two copies of the I allele. The functional significance of the variant (D) allele of the *PPP3R1* I/D polymorphism is unknown. However, these authors suggested that this variant eliminates a transcription factor binding site, and they hypothesized that this is an important binding site for a repressor or inhibitor of *PPP3R1* transcription. Our results differed from those of Tang et al. (50) in that II homozygotes tended to increase their skeletal muscle mass with overload more than D allele carriers. These discrepancies could be due to 1) differences in the function of calcineurin B, especially for the variant allele, in different tissues, 2) the nature of the load (ST vs. hemodynamic overload) inducing hypertrophy, 3) differences in population being studied, or 4) a combination of two or more of these factors. We are unaware of any other studies that have compared variations at this locus to the response of interventions designed to change muscle mass or strength.

We hypothesized a significant influence of *IGFBP3* A-202C because previous studies have shown that levels of IGFBP-3, a major carrier of IGF-I in circulation (1), can be influenced by IGF-I (54), and several studies have shown IGFBP-3 to be present in skeletal muscle. In addition, Foulstone et al. (11) have shown that increased secretion of IGFBP-3 in primary adult human skeletal muscle cells is stimulated by IGF-I (11). However, our results showed no significant influence of the *IGFBP3* A-202C gene polymorphism on muscle phenotypic responses to ST, although we did observe a trend for a significant race  $\times$  *IGFBP3* A-202C interaction to influence change in strength. Previous studies have shown that the -202 polymorphism in the promoter region of the *IGFBP3* gene influences levels of the IGFBP-3

protein (3,22,46), although these studies have shown that several factors can interact with this polymorphism to influence protein levels, including female hormone levels, height, and body-mass index (3,22,46). In an in vitro study, Deal et al. (3) showed that the -202 polymorphism influenced the promoter activity of the gene, suggesting the possibility that this polymorphism could influence the levels of the protein in skeletal muscle. However, it is also possible that the isoforms of IGF-I in skeletal muscle may be carried by a different binding protein than IGFBP-3. Therefore, although the -202 locus of the *IGFBP3* gene may influence the levels of IGFBP-3 in skeletal muscle, this protein may not be the major potentiator of IGF-I action in skeletal muscle.

In light of the trend for a significant *IGFBP3* A-202C  $\times$  race interaction for influencing change in strength with ST, the influence of IGF pathway gene polymorphisms on responses to ST should be studied more extensively in Black individuals. On the basis of the different frequencies for the *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D genes between Black and White individuals, it is possible that race effects may have played a greater role than genotype effects for the findings that we observed. For example, Black individuals had a higher frequency of the non-192 allele for the *IGF1* CA repeat gene polymorphism than White individuals. In contrast, White individuals had a higher frequency of the variant (C) allele for the *IGFBP3* A-202C gene polymorphism than Black individuals. Finally, Black individuals had a higher frequency of the deletion allele for the *PPP3R1* I/D gene polymorphism than White individuals. Similar differences between Black and White individuals in allele frequencies for the *IGF1* CA repeat and *PPP3R1* I/D gene polymorphisms have been observed in previous studies (4,21,23,50). There are no reports that we are aware of on the frequency difference between races for the *IGFBP3* A-202C polymorphism.

There are several limitations to the present study. One limitation, due to the sophisticated genetic analysis that is being performed, is low statistical power from small sample size for some genotype groups, particularly for MV and MQ assessments. The lower statistical power for detecting differences among genotype groups for changes in MV and MQ with ST was, in part, due to smaller effect sizes projected for these phenotypes compared with changes in muscle strength. Because of small sample sizes in the groups compared in this study, the possibility that positive false findings could have occurred cannot be ruled out. Additionally, the use of an untrained control leg in the design of the present study may have reduced the effect size needed for MV. However, the use of a control leg allowed for a better assurance that the results represent the independent effects of ST by controlling for variation due to methodological, biological, or seasonal factors. Thus future studies should consider changes in MV and MQ with larger sample sizes to test for gene  $\times$  gene interactions, as well as to investigate other genes in this biological pathway. Also, the present study is limited in that there is insufficient data to test for sex  $\times$  gene interactions. Thus the possibility exists that the polymorphisms tested may have a different impact on the muscle phenotypic responses in men and women.

Future studies should be performed with larger sample sizes to better determine the influence of *IGF1* CA repeat, *IGFBP3* A-202C, and *PPP3R1* I/D, especially for gene  $\times$  gene interactions, on muscle phenotypic responses and to investigate whether other polymorphisms in the IGF pathway play a larger role in influencing muscle phenotypes. For example, it is possible that other polymorphisms in the *PPP3R1* gene or a polymorphism in the catalytic subunit of calcineurin may be more responsible for influencing muscle phenotypic responses to ST. Second, there is a need to investigate other IGF-I-dependent mechanical signaling pathways that influence muscle phenotypic responses to ST. For example, it is conceivable that the IGF1-PI3K/Akt/mTOR pathway (37) may compensate for some of the effects of a potentially detrimental allele for the *PPP3R1* I/D gene polymorphism. Finally, measurements should be made on transcription and protein levels of the IGF pathway gene polymorphisms investigated

in the present study to better understand how they may influence muscle phenotypic responses to ST.

In conclusion, this is the first study to examine the effects of IGF pathway gene polymorphisms on muscle phenotypic responses to ST in older adults. The results suggest that combined *IGF1* CA repeat effects, i.e., the main effect for *IGF1* CA repeat plus the interaction effect with *PPP3R1* I/D, significantly influence muscle phenotypic responses. Although the results from *IGF1* CA repeat  $\times$  *PPP3R1* I/D interactions should be interpreted with caution because of the limited sample size for some of the combined genotype groups, they do provide support for the generation of new hypotheses involving *IGF1* CA repeat  $\times$  *PPP3R1* I/D interactions that should be tested in future studies. Such studies will provide a better understanding of the role of gene polymorphisms on the responses to ST.

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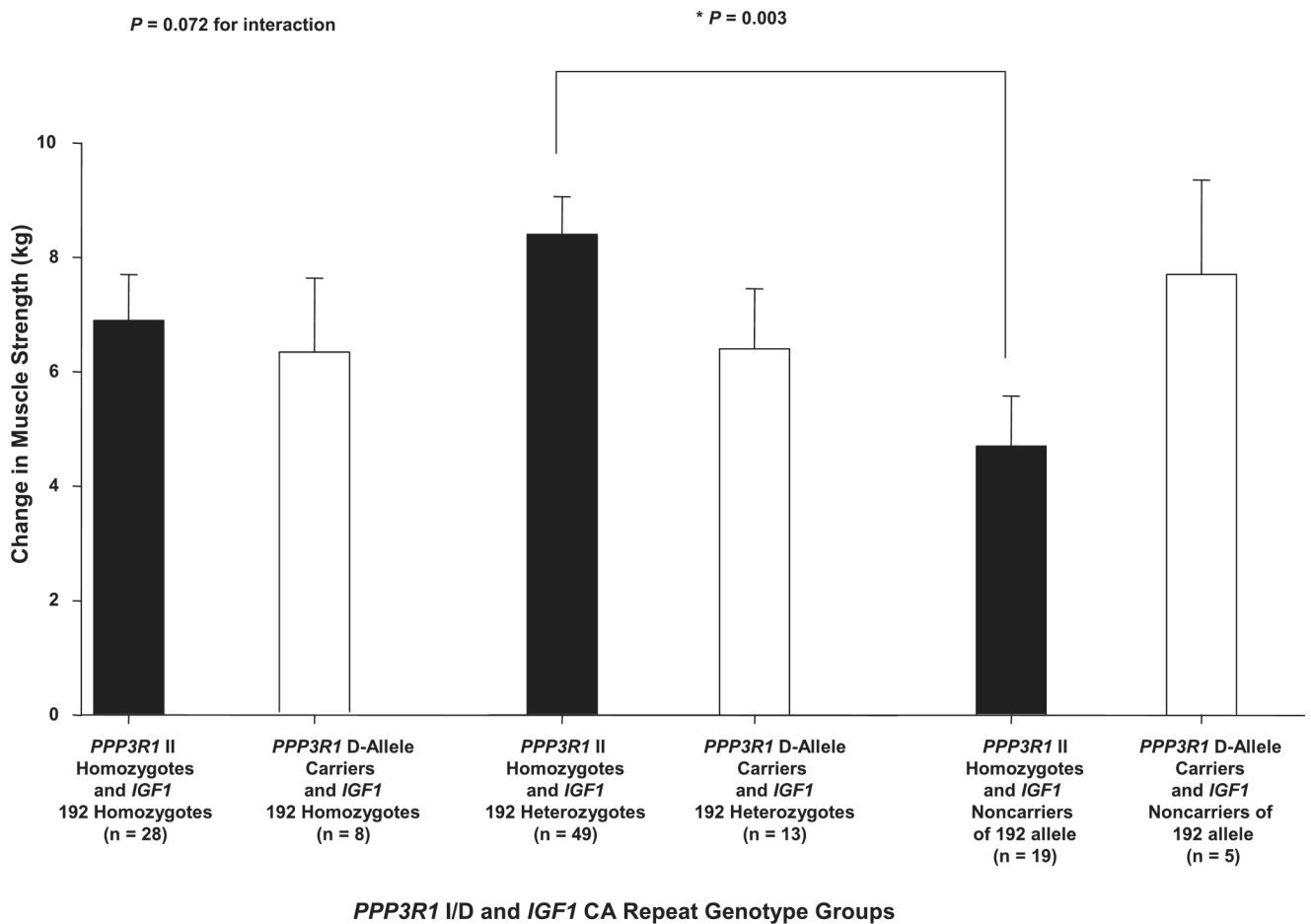
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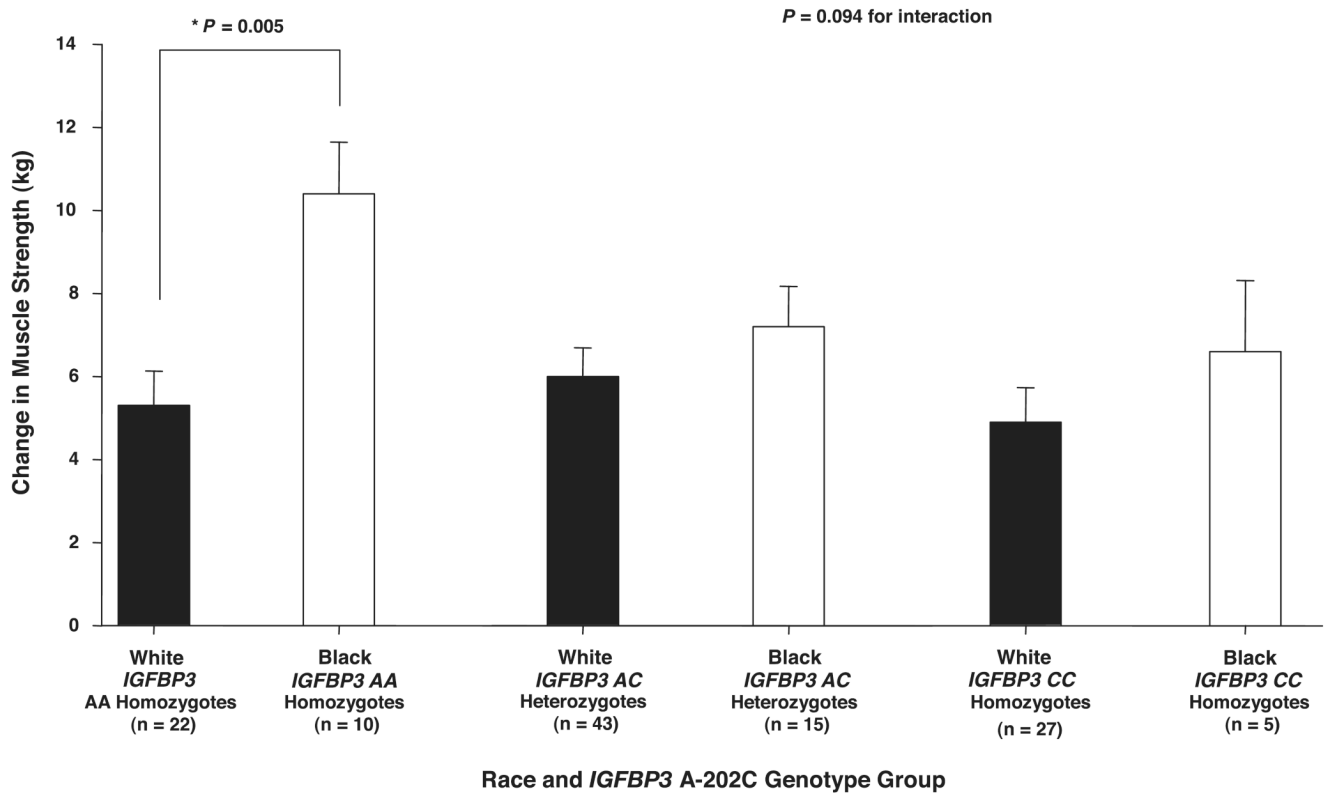
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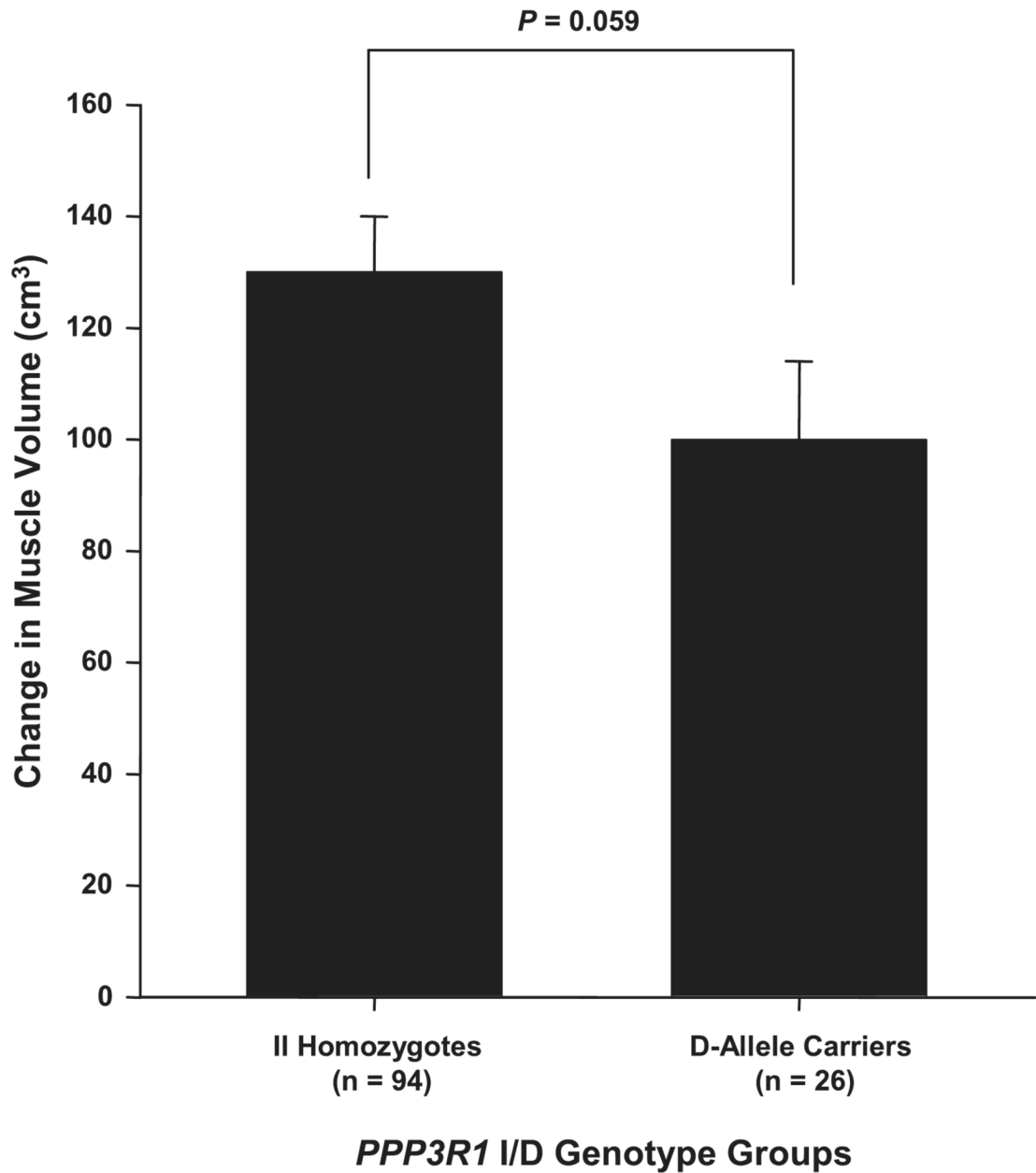
**Fig. 1.**

Influence of calcineurin B (*PPP3R1*) insertion-deletion (I/D)  $\times$  insulin-like growth factor 1 (*IGF1*) cytosine adenine (CA) repeat genotype groups on change in 1-repetition maximum (1 RM) strength with strength training (ST). There was a trend for a significant gene  $\times$  gene interaction between *IGF1* CA repeat and *PPP3R1* I/D ( $P = 0.072$ ). Individuals homozygous for *PPP3R1* II who were also heterozygous for *IGF1* 192 allele had significantly greater increases in 1 RM strength with ST than individuals homozygous for *PPP3R1* II who were also *IGF1* noncarriers of the 192 allele ( $*P = 0.003$ ). Values are covaried for age, hormone replacement therapy status and sex, race, height, body mass index, and baseline 1 RM strength. Values are means  $\pm$  SE.

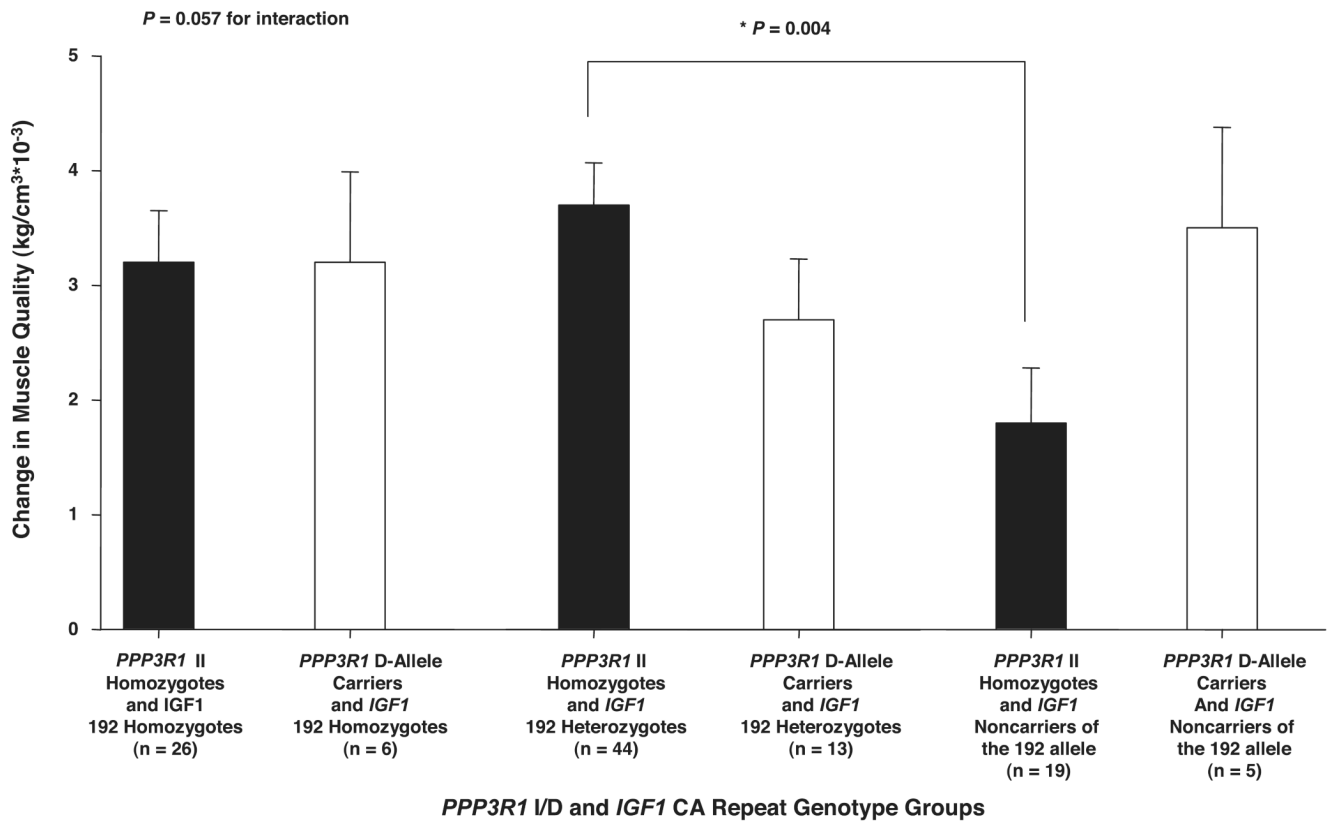


**Fig. 2.** Influence of insulin-like growth factor binding protein 3 (*IGFBP3*) A-202C genotype  $\times$  race groups on change in 1 RM strength with ST. There was a trend for a significant *IGFBP3* A-202C gene  $\times$  race interaction ( $P = 0.094$ ). Black subjects homozygous for *IGFBP3* AA had significantly greater increases in 1 RM strength with ST than White subjects homozygous for *IGFBP3* AA ( $*P = 0.005$ ). Values are covaried for age, hormone replacement therapy status and sex, race, height, body mass index, and baseline 1 RM strength. Values are means  $\pm$  SE.





**Fig. 3.** Influence of calcineurin B (*PPP3R1*) I/D genotype groups on change in muscle volume (MV) with ST. There was a trend for individuals homozygous for *PPP3R1* II to have greater increases in MV with ST than the *PPP3R1* D allele carriers ( $P = 0.059$ ). Values are covaried for age, hormone replacement therapy status and sex, race, height, body mass index, and baseline MV. Values are means  $\pm$  SE.



**Fig. 4.** Influence of calcineurin B (*PPP3R1*) ID  $\times$  *IGF1* CA repeat genotype groups on change in muscle quality (MQ) with ST. The gene  $\times$  gene interaction between *IGF1* CA repeat and *PPP3R1* ID approached significance ( $P=0.057$ ). Individuals homozygous for *PPP3R1* II who were also heterozygous for *IGF1* 192 allele had significantly greater increases in 1 RM strength with ST than *PPP3R1* II homozygote individuals who were also *IGF1* noncarriers of the 192 allele ( $*P = 0.004$ ). Values are covaried for age, hormone replacement therapy status and sex, race, height, body mass index, and baseline MQ. Values are means  $\pm$  SE.

**Table 1**  
**IGF1 CA promoter, IGFBP3 A-202C promoter, and PPP3R1 5-base pair I/D promoter allele**  
**and genotype frequencies for all subjects**

<i>IGF1</i> CA Promoter Gene Polymorphism			
	Total No. (%)	Total No. for White Subjects (%)	Total No. for Black Subjects (%)
<i>Allele</i>			
192	141 (55)	115 (61)	26 (39)
-	115 (45)	75 (39)	40 (61)
<i>Genotype</i>			
192/192	39 (30)	34 (36)	5 (15)
192/-	63 (49)	47 (49)	16 (48)
-/-	26 (20)	14 (15)	12 (36)
<i>IGFBP3</i> A-202C Promoter Gene Polymorphism			
	Total No. (%)	Total No. for White Subjects (%)	Total No. for Black Subjects (%)
<i>Allele</i>			
A	128 (50)	90 (47)	38 (58)
C	128 (50)	100 (53)	28 (42)
<i>Genotype</i>			
A/A	33 (26)	22 (23)	11 (33)
A/C	62 (48)	46 (48)	16 (48)
C/C	33 (26)	27 (28)	6 (18)
<i>PPP3R1</i> 5-bp I/D Promoter Gene Polymorphism			
	Total No. (%)	Total No. for White Subjects (%)	Total No. for Black Subjects (%)
<i>Allele</i>			
I	227 (89)	177 (93)	50 (76)
D	29 (11)	13 (7)	16 (24)
<i>Genotype</i>			
I/I	100 (78)	82 (86)	18 (55)
I/D	27 (21)	13 (14)	14 (42)
D/D	1 (1)	0 (0)	1 (3)

*IGF1*, insulin-like growth factor 1 gene; *IGFBP3*, insulin-like growth factor binding protein 3 gene; *PPP3R1*, calcineurin B gene; CA, cytosine adenine; I/D, insertion-deletion; 192 allele is equivalent to 19 CA repeats; - represents non-192 allele.

**Table 2**  
**Physical characteristics for all men (n = 58) and women (n = 70) at baseline and after ST**

	Men (n = 53–58)		Women (n = 61–70)	
	Baseline	After ST	Baseline	After ST
Age, yr	65 (8)		63 (9)	
Height, cm	174 (7)		162 (7)	
Weight, kg	85.8 (13.5)	86.0 (13.4)	72.1 (12.6)	72.3 (13.2)
Body fat, %	28.0 (4.9)	27.6 (4.6)	38.6 (5.7)	38.1 (5.7)
Fat-free mass, kg	61.2 (8.1)	61.7 (7.9)	43.8 (5.7)	44.2 (5.9)
1 RM, kg	33±1.0 <sup>†</sup>	41±1.2 <sup>‡</sup>	22±1.0	27±1.2
MV, cm <sup>3</sup>	1,770±34 <sup>†</sup>	1,930±38	1,330±38	1,430±43
MQ, kg/cm <sup>3</sup> × 10 <sup>-3</sup>	18.8±0.56 <sup>*</sup>	21.6±0.53	16.2±0.63	19.2±0.59
Blacks/Whites	12/46		21/49	

Values are means (SD). Values for knee extension one-repetition maximum (1 RM), muscle volume (MV), and muscle quality (MQ) are least square means ± SE. Sample size variability in number of subjects was because of missing data for muscle phenotypes. MQ is strength (1 RM) per unit of muscle mass (MV). ST, strength training.

\* Significantly greater than for women ( $P < 0.01$ );

<sup>†</sup> significantly greater than for women ( $P < 0.001$ );

<sup>‡</sup> significantly greater increase than the increase in women ( $P < 0.05$ ).

**Table 3**  
**Physical characteristics for all White subjects (n = 95) and Black subjects (n = 33) at baseline and after ST**

	White (n = 85-95)		Black (n = 29-33)	
	Baseline	After ST	Baseline	After ST
Age, yr	65 (8)		62 (8)	
Height, cm	168 (9)		166 (7)	
Weight, kg	78.2 (15.3)	78.4 (15.6)	78.5 (12.7)	78.9 (12.8)
Body fat, %	34.1 (7.6)	33.7 (7.5)	33.0 (7.3)	32.5 (7.2)
Fat-free mass, kg	51.4 (11.3)	51.8 (11.3)	52.6 (10.5)	53.3 (10.6)
1 RM, kg	24±0.9	29±1.0	27±1.2	35±1.4
MV, cm <sup>3</sup>	1,380±31	1,490±35	1,580±39 <sup>†</sup>	1,700±48
MQ, kg/cm <sup>3</sup> × 10 <sup>-3</sup>	16.9±0.49	19.4±0.46	17.1 ±0.66	20.7±0.62 <sup>‡</sup>
Men/women	46/49		12/21	

Values are means (SD). Values for 1 RM, MV, and MQ are least square means ± SE. Sample size variability in number of subjects was because of missing data for muscle phenotypes.

<sup>†</sup>Significantly greater than for White subjects ( $P < 0.001$ );

<sup>‡</sup>significantly greater increase than the increase in White subjects ( $P < 0.05$ ).

**Table 4**  
**Percent variability for muscle phenotypes attributable to IGF1 CA repeat, IGFBP3 A-202C, and PPP3R1 I/D gene polymorphisms**

Genotype	Individual Source	Combined Gene Effect	P Value
<i>Percent variability for genotype for change in muscle strength with ST</i>			
<i>IGF1</i>	1.22	$3.46 = 1.22 + \frac{1}{2} (4.48)$	<0.01
<i>IGFBP3</i>	2.83	$4.84 = 2.83 + \frac{1}{2} (4.02)$	>0.05
<i>PPP3R1</i>	0.03	$2.27 = 0.03 + \frac{1}{2} (4.48)$	>0.05
<i>IGF1</i> × <i>PPP3R1</i>	4.48		0.07
<i>IGFBP3</i> × race	4.02		0.09
<i>Percent variability for genotype for change in MV with ST</i>			
<i>IGF1</i>	2.04	N/A	0.32
<i>IGFBP3</i>	0.16	N/A	0.91
<i>PPP3R1</i>	3.23	N/A	0.06
<i>Percent variability for genotype for change in MQ with ST</i>			
<i>IGF1</i>	0.95	$3.71 = 0.95 + \frac{1}{2} (5.54)$	<0.05
<i>IGFBP3</i>	1.28	N/A	0.51
<i>PPP3R1</i>	0.22	$2.99 = 0.22 + \frac{1}{2} (5.54)$	>0.05
<i>IGF1</i> × <i>PPP3R1</i>	5.54		0.06

“Combined gene effect” was computed as the main effect plus one-half of any gene × gene interaction or gene × race interaction. For example for *IGF1*, combined gene effect is the *IGF1* main effect (1.22) plus one-half of the *IGF1* × *PPP3R1* gene × gene interaction [ $1/2(4.48)$ ]. The other half of the gene × gene interaction is credited to *PPP3R1*. N/A, combined gene effect could not be determined because there was not at least a trend for a significant gene × gene or gene × race interaction.