# Functional Polymorphisms Associated with Human Muscle Size and Strength

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**ABSTRACT**

THOMPSON, P. D., N. MOYNA, R. SEIP, T. PRICE, P. CLARKSON, T. ANGELOPOULOS, P. GORDON, L. PESCATELLO, P. VISICH, R. ZOELLER, J. M. DEVANEY, H. GORDISH, S. BILBIE, and E. P. HOFFMAN. Functional Polymorphisms Associated with Human Muscle Size and Strength. *Med. Sci. Sports Exerc.*, Vol. 36, No. 7, pp. 1132–1139, 2004. **Introduction:** Skeletal muscle is critically important to human performance and health, but little is known of the genetic factors influencing muscle size, strength, and its response to exercise training. The Functional single nucleotide polymorphisms (SNP) Associated with Muscle Size and Strength, or FAMuSS, Study is a multicenter, NIH-funded program to examine the influence of gene polymorphisms on skeletal muscle size and strength before and after resistance exercise training. **Methods:** One thousand men and women, age 18–40 yr, will train their nondominant arm for 12 wk. Skeletal muscle size (magnetic resonance imaging) and isometric and dynamic strength will be measured before and after training. Individuals whose baseline values or response to training deviate $\geq$ 1.5 SD will be defined as outliers and examined for genetic variants. Initially candidate genes previously associated with muscle performance will be examined, but the study will ultimately attempt to identify genes associated with muscle performance. **Conclusion:** FAMuSS should help identify genetic factors associated with muscle performance and the response to exercise training. Such insight should contribute to our ability to predict the individual response to exercise training but may also contribute to understanding better muscle physiology, to identifying individuals who are susceptible to muscle loss with environmental challenge, and to developing pharmacologic agents capable of preserving muscle size and function. **Key Words:** SKELETAL MUSCLE, GENETICS, QUANTITATIVE TRAIT LOCI (QTL), HYPERTROPHY, GENOMICS

Muscle tissue comprises approximately 30% of body mass, and responds promptly and efficiently to environmental stimuli such as weight bearing and resistive exercise training. Muscle strength is also a primary determinant of an individual's functional capacity and influences the function of other tissues, such as the maintenance of bone density. Despite the critical importance of skeletal muscle, little is known of the genetic factors influencing muscle size and strength and the response of these parameters to environmental factors such as exercise training.

The genetic factors influencing muscle size and character in farm animals are well studied due to the economic importance of meat. The myostatin gene has been identified as affecting muscle size and quality in cattle (15,21,24), and two additional genes, the ryanodine receptor gene [calcium activated calcium release channel of the sarcoplasmic reticulum] (14) and the *IGF-2* gene (36), affect muscle size and quality in pigs. There is currently no evidence of similar genetic effects on muscle size or quality in humans. Indeed, to the contrary, we in preliminary data and others (13) found no association of the myostatin gene with muscle size or strength, and our preliminary data found no association of IGF-2 with human muscle phenotypes.

The Functional single nucleotide polymorphisms (SNP) Associated with Muscle Size and Strength, or FAMuSS, Study will examine the statistical relationship of gene polymorphisms with skeletal muscle size and strength before, and the increase in muscle size and strength after, 12 wk of supervised resistance exercise training. The identification of statistical associations between SNP and strength outcomes, if validated by other techniques, should have important implications for the study of how genetic variations affect the normal response to environmental stimuli. The present manuscript describes the rationale and methods of the FAMuSS Study.

## STUDY RATIONALE

Most studies examining the gene polymorphisms that contribute to human variation have focused on DNA

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changes associated with dysfunction and disease. More recent association studies, however, have examined the effect of genetic polymorphisms on normal human variation. Muscle is an ideal tissue in which to investigate the association of normal polymorphic variation and tissue function. Muscle strength and size vary tremendously among individuals, and there is considerable evidence that genetic variation is largely responsible for this functional polymorphism. Muscle is one of the most highly conserved and ordered organs, with most of the tissue composed of a single cell type, the myofiber. Muscle can be quickly and dramatically changed by a single environmental variable, exercise training. Our studies of patients with muscle disease have demonstrated that there is substantial amino acid polymorphism in muscle gene (cDNA) sequence, with many of the SNP causing amino acid changes in the encoded protein (nonsynonymous SNP).

There is evidence that muscle strength and size is heritable. Thomis et al. (34) examined the inheritance of arm strength and size before and after exercise training in 25 monozygotic and 16 dizygotic male twins. Strength measured as 1 repetition maximum (RM) showed a high degree of heritability (77% pre, 81% posttraining). Concentric and eccentric contraction measures at specific angles showed significant heritability before exercise training, but there was little change postexercise, and hence no heritability. Muscle girth measured by computerized tomographic scans showed high heritability before exercise, but percentage increase in girth after exercise was not heritable. Similarly hand-grip strength in 257 male (28) and 353 female (2) twins suggested that strength has a heritability of 0.65–0.30, respectively. Perusse et al. (27) used 1630 non-twin, French-Canadians from 375 families and path analysis to attribute 30% of the phenotypic variance in muscular strength to genetic factors. Thus, there is considerable evidence to suggest that muscle size and strength have a strong heritable component, but available studies are small and few have performed molecular genetic correlations. There are also few studies examining the interaction of genetic factors and the change in muscle size and strength with resistance exercise training.

FAMuSS selected to employ strength training of the nondominant arm because the study goal is to examine the statistical association of skeletal muscle polymorphisms with exercise induced changes in muscle size and strength, and because strength training is more likely to induce these changes than is aerobic exercise. Arm training was selected because the arms are used less in modern society than are the legs even among the physically inactive individuals to be recruited for study. Study of the arms, therefore, reduces some of the baseline differences in muscular strength produced by such daily physical activities as walking and stair climbing. Training the nondominant arm should further reduce variability in the training response caused by subjects' baseline physical activity because the nondominant arm is used less frequently and therefore less likely to be affected by habitual activity.

## METHODS

**Study overview.** This study is a cooperative effort of the Exercise and Genetics Collaborative Research Group, a consortium of investigators at nine institutions. After obtaining informed consent from all individuals, isometric and dynamic strength of the forearm flexors and cross-sectional diameter of the upper arm musculature will be measured before and after 12 wk of forearm flexor and extensor resistance training in 1000 subjects aged 18–40 yr. Subjects $>$ 40 yr old will be excluded to avoid studying men who have experienced the marked decrease in testosterone levels that occurs in older age groups (37). Pretraining strength measurements will be performed during three testing days (Fig. 1). Posttraining strength measurements will be performed on two testing days (Fig. 2). Maximal cross-sectional diameter of the upper arm musculature will be measured using magnetic resonance imaging (MRI). DNA samples will be obtained from subjects' white blood cells and the relationship between muscle genes and baseline muscle size and strength and their changes with training examined. Approximately 30 subjects per year will be tested and trained at each site.

**Study population, exercise training, and phenotyping (strength, size).** Subjects will sign an informed consent form, with the "master" Institutional Review Board application and consents designed by Children's National Medical Center genetic counselors, with revision and submission by each participating site. Men and women will be excluded if they: are $<$ 18 and $>$ 40 yr old; use medications known to affect skeletal muscle such as corticosteroids; have any restriction of activity; have chronic medical conditions such as diabetes; have metal implants in arms, eyes, head, brain, neck, or heart that would prohibit MRI testing; have performed strength training or employment requiring repetitive use of the arms within the prior 12 months; consume on average more than two alcoholic drinks daily; or have used dietary supplements reported to build muscle size/strength or to cause weight gain such as protein supplements, creatine, or androgenic precursors. Subjects will be reimbursed $100–150 depending on the site for their time and effort.

**Isometric biceps strength testing.** Isometric strength of the elbow flexor muscles of each arm will be determined before and after 12 wk of strength training using

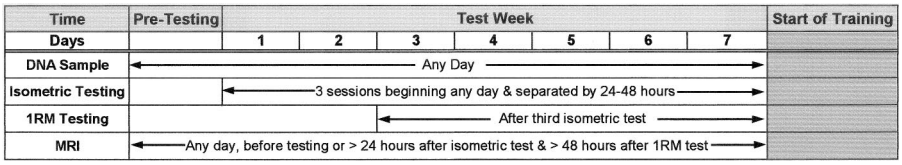


**FIGURE 1—FAMuSS pretraining testing protocol.**

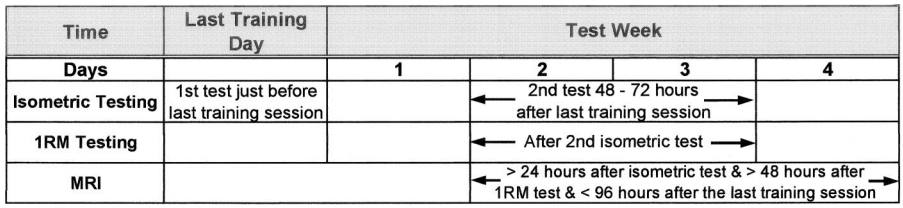


**FIGURE 2—FAMuSS posttraining testing protocol.**

a specially constructed, modified preacher bench and strain gauge (model 32628CTL, Lafayette Instrument Company, Lafayette, IN). Intraclass reliability coefficient (R) values for elbow flexor isometric strength at 90° elbow flexion range from 0.95 to 0.99. (7,8,30) The nine testing and training centers will each conduct reliability tests. Baseline measures of isometric strength will be assessed on three separate days, spaced no more than 2 d apart. Posttraining measures of isometric strength will be assessed immediately before the last training session and 48 h after the last training session. On each of the testing days, three maximal contractions will be performed with each arm. In order to obtain three consistent peak force values, up to two more contractions will be performed if a peak value deviates more than 5 lb from the other two peak values. The average of the results obtained on the second and third pretraining testing days will be used as the baseline criterion measurement and the results obtained 48 h after the last training session will be used for the posttraining criterion measurement.

For testing, the arm will be positioned on the preacher bench with the elbow fixed at 90°. The lever arm of the preacher bench will be aligned with the subject's forearm, the subject's wrist will be placed in a padded support, the subject's elbow will be aligned with the center of rotation of the lever arm, while the elbow joint is at a 90° angle and the upper arm rests against a padded upper arm support. The upper arm support will be stationary and produce a trunk/upper arm angle of approximately 45°. Subjects will pull maximally against a fixed resistance. The strain gauge will be attached between a fixed surface and the preacher bench lever arm. The elbow flexor muscles will be isolated by positioning the subject with his or her chest against the preacher bench chest support, legs straight with only his or her heels on the floor, and the opposite arm resting on the legs. Three maximal contractions will be assessed. Each contraction will last 3 s, and 1 min will be allowed between contractions. An average of the peak force produced during the three contractions will be used as the criterion score.

**One repetition maximum (1RM) biceps strength testing.** The dynamic strength of the elbow flexor muscles of each arm will be assessed by determining the maximum amount of weight with which a subject can perform one repetition of the standard preacher curl exercise. The 1RM testing will be performed before and after 12 wk of strength training. Unlike the isometric strength testing, baseline 1RM testing will be completed in 1 d, during visit 3. To prevent muscle fatigue from affecting the isometric results, 1RM testing will be performed only once after the final isometric test both before and after the training program.

**Assessment of biceps strength gain.** Because strength scores are not perfectly proportional to intersubject differences in body size, strength scores will be allometric scaled by dividing the recorded value by body mass$^{0.67}$ (20). Gain in strength will be determined as the normalized post-measurement − premeasurement. The approach will be applied separately to isometric strength and 1RM scores and is expected to produce lists of large and small strength gainers who differ little in body size. Because of gender-specific hormonal and body stature differences, large and small strength gainers will be determined separately for women and men.

**Measurement of muscle cross-sectional area.** MRI will be performed before and after exercise training to assess changes in the biceps brachialis cross-sectional area (CSA) as previously described (1,9,10,26,29). Because of concern that postexertion swelling can spuriously increase MRI measurements, pretraining and posttraining MRI will be performed before or 24 and 48 h after the isometric or 1RM tests, respectively, and posttraining MRI will be performed 48–96 h after the final training session. This ensures that temporary exercise effects, such as water shifts, are avoided, while also avoiding any reduction of muscle size from detraining. Posttraining CSA data will be compared with pretraining values to determine training-induced increases. Pre- and posttraining MR images will be obtained separately from both the dominant (untrained) and nondominant (trained) arms, thereby allowing the dominant arm to act as a control.

Because MR images will be collected before and after training, it is important that each subject's positioning within the MR magnet be reliably reproduced in order to avoid coregistration errors. To accomplish this, the maximum circumference of the upper arm (i.e., the belly of the muscle) will be measured with a vinyl, nonstretchable tape measure. The arm will be abducted 90° at the shoulder, flexed 90° at the elbow, and the biceps maximally contracted for this measurement. The location of the maximum circumference, or the point of measure (POM), will then be marked on the subject's skin using a radiographic bead (Beekley Spots, Beekley Corp., Bristol, CT). The radiographic bead will also be used to standardize MRI measurements by comparing its measured CSA with that of the MRI determined CSA. At each imaging site the onsite investigator will locate and mark the POM before each MRI measurement.

Subjects will have both arms scanned in the supine position with the arm of interest at their side and the center of the arm as close to the magnetic isocenter of the scanner as

possible, palms facing up. The hand will be supinated and taped in place on the scanner bed surface, and the POM centered to the alignment light of the MRI. A coronal scout image (6–9 slices) will be obtained to locate the long axis of the humerus, followed by a sagittal scout image (6–9 slices) to align the eighth slice of the axial/oblique image with the POM. Fifteen serial fast spoiled gradient images of each arm will then be obtained (TE = 1.9 s, TR = 200 ms, flow artifact suppression, 30° flip angle) using the POM as the center most point. These axial/oblique image slices (i.e., perpendicular to the humerus) will begin at the top of the arm and proceed toward the elbow such that the belly of the muscle occurs at slices 8 and 9. Individual image slices will be 16 mm thick with a 0-mm interslice gap, 256 × 192 matrix resolution, 22 cm × 22 cm field of view, NEX = 6. This method images a 24-cm length of each arm.

MR images from each investigational site will be transferred to the central MR imaging facility at Yale University via either Magneto Optical Disk or CD-ROM. Images will be analyzed using a custom design interactive processing and visualization program that operates in Matlab (The Math Works, Inc., Natick, MA) running on a Windows-based PC platform. This software enables the user to assign regions of interest (ROI) in an image set by tracing region borders with a mouse. Muscle is easily identifiable on MR images and its CSA will be measured using this computerized planimetry technique. Once the ROI is defined, the program reports the number of pixels contained in the selected ROI. Based upon the MR acquisition data (i.e., field of view and matrix resolution), the CSA ($cm^2$) of the defined ROI is then calculated. When the pretraining CSA is subtracted from the posttraining CSA, the training effect can be compared between subjects. In order to optimize the accuracy of the muscle size calculation, a subset of data will be analyzed by volumetric analysis. By analyzing the 15 successive slices throughout the scanned length of the upper arm each CSA can be multiplied by the known slice thickness (1.6 cm) to yield a slice volume ($cm^3$). Slice volumes will then be summed over the length of the anatomical structure of interest. Although volumetric analysis is generally considered more accurate than cross-sectional analysis (35), the amount of work required to perform volumetric analysis on 2000 studies (1000 subjects 2× each) is prohibitive. By monitoring volumetric changes in a subset of subjects and comparing volumetric and CSA results, the quality of MRI data will be ensured with reasonable effort.

**Exercise training program.** Subjects will undergo gradually progressive, supervised strength training of their nondominant arm in one of the collaborating facilities. The 1RM measured during pretraining testing will be used to estimate the weights that can be lifted for 12, 8, and 6 repetitions using standard formulas (3).

All exercises will be performed with the nondominant arm only. The exercises will consist of the biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension, and triceps kickback. All exercises will be performed with dumbbells (Powerblocks, Intellbell, Inc., Owatonna, MN), and some exercises will utilize a preacher curl bench. (Yukon International Inc., Cleveland, OH). Training sessions will bee supervised and last approximately 45–60 min. The exercise progression will use the following weekly training protocol: weeks 1–4: 3 sets with 12 repetitions of the 12RM weight; weeks 5–9: 3 sets with eight repetitions of the 8RM weight; weeks 10–12: 3 sets with 6 repetitions of the 6RM weight. This protocol is designed to increase both muscle size using high repetitions, low intensity early in training, and strength using low repetitions, high intensity as training progresses (32). The transition to fewer reps and higher intensity as training progresses activates more muscle mass through increased motor unit recruitment. The primary interest is to train the elbow flexors, but we will also train the elbow extensors to balance muscle strength across the joint.

**Dietary control procedures.** Subjects will maintain their habitual dietary intake over the course of the study. Individuals who have supplemented their diet with additional protein or taken any dietary supplement reported to build muscle or to cause weight gain (dietary supplements containing protein, creatine, or androgenic precursors) will not be recruited. Data in subjects who have lost significant amounts of weight during the study will not be analyzed.

**Phlebotomy.** Blood samples (21 cc) for DNA determinations will be obtained from subjects before beginning the exercise training portion of the study.

**Anthropometric measurements.** Body weight will be recorded before and after training using a balance beam scale. Height will be determined using a tape measure mounted on a wall. Arm circumferences of both upper arms will be measured on two separate days before and two separate days after 12 wk of training using a nonstretchable tape measure. All arm circumference measurements will be performed before the 1RM testing session to avoid the possibility that muscle swelling from the 1RM test could affect arm size. Lange skin-fold calipers (Cambridge Scientitic Industries, Cambridge, MD) and standard techniques (18,19) will be used to measure double thickness subcutaneous adipose tissue over the biceps and triceps muscles of both arms.

**Standardization between sites.** Adaptations to resistance training are highly specific to the training protocol. Therefore, to control for any difference among sites, each site will use an identical training protocol and identical exercise equipment purchased from the same manufacturers. The techniques for MRI, strength and anthropometrical measurements, and exercise training were videotaped at the start of the study, and each site's research personnel will be required to review the videotaped procedures before the start of each training group. All site principal investigators and personnel involved in testing will meet twice yearly, once to review the progress of the study and once to review standardization of the measurement techniques.

**SNP discovery and genotyping methods.** The initial phase of the genotyping section will choose a series of candidate genes (approximately 100 genes), based upon three criteria: 1) preliminary data for association with strength, size, or response to exercise in previous publica-

tions; 2) proteins known to be in biochemical pathways involved in muscle response to exercise; and 3) genes that we have shown to be strongly transcriptionally regulated during aerobic training (16) or eccentric exercise (6).

Given the prioritized list of "candidate genes," the second phase will be to identify polymorphisms within the candidate genes for subsequent genotyping in study participants. We will utilize two approaches to identify SNP within the candidates; database mining using existing databases (*dbSNP; jSNP; HGVbase; HGM; Celera*), and our independent "SNP discovery" using a panel of 96 ethnically diverse individuals (36 Caucasian, 29 African American, 26 Hispanic, 5 Asian). The logic for our own SNP discovery is that the large majority of existing SNP are in noncoding sequence. These noncoding SNP are useful for genetic linkage studies but not useful for the functional associations planned in our study. Also, the sensitivity and specificity of existing SNP resources is acknowledged to be quite poor, with as many as 40.7% artifactual SNP (sequencing and cloning errors) (5) and only 47% sensitivity for existing SNP in gene coding sequences (23).

SNP discovery will be done by amplification of all exons, exon/intron boundaries, 5' and 3' untranslated regions, and selected gene promoters. PCR products will then be screened for polymorphisms using one of two methods: direct automated sequencing of all PCR products in all 96 individuals with Phred/Phrap analysis of traces (11,12,25) or denaturing high pressure liquid chromatography (Transgenomic Wave system) and subsequent sequencing of heteroduplexes (17).

Genotyping of study subjects will be done using direct sequencing, Nanogen genotyping system, (33) restriction fragment digestions of PCR products, or fluorogenic 5' nuclease chemistry (Taqman assay).

We considered conducting our SNP discovery on phenotypic outliers drawn from the FAMuSS results (see below) rather than from the 96-person ethnically diverse screening panel (above). However, there was the pragmatic need to conduct the laboratory-based SNP discovery in parallel with the phenotyping arm of the study, both for budgetary reasons and to avoid delaying acquisition of association data. In addition, it is quite common to identify "private SNPs," where a polymorphism is seen in only one or a very few individuals. Use of phenotypic outliers from the initial FAMuSS results for the SNP discovery arm of our study could lead us to consider a "private SNP" as significantly associated with a specific phenotypic trait in a phenotypic outlier, when instead this would be a chance association. In our study design, SNP discovery is independent of phenotyping, eliminating the problem of private SNPs providing misleading the results.

**Genetic association studies.** There are likely dozens of genes and polymorphisms in those genes that contribute to muscle size, strength, and response to resistance training, the phenotypes scored in FAMuSS. Identification of these genes is a challenging task, particularly given the fact that each SNP can serve as a confounding variable for any different SNP under study, and that the outbred and ethnically diverse nature of the human populations under study makes the allele frequencies vary as a function of ethnicity. Our goal in the FAMuSS study is to prioritize the SNP association studies, where the dominant SNPs responsible for the largest proportion of variation are discovered first. In other words, those relatively few genes that contribute substantially to the phenotypes measured in a specific ethnic group. Toward this end, we will use an "outlier screening" approach to investigate genetic associations between any of the four muscle function variables (entry size and strength; percentage change in size and strength after training) and candidate gene SNPs. The experimental design will use the initial 350 individuals exercised in year 1 to identify the "outliers" of each of the four muscle function variables for both males and females. Outliers will be defined as those individuals falling 1.5 SD from the mean for each variable, with those $\geq$ mean + 1.5(SD) defined as "responders" and those $\leq$ mean $-$ 1.5(SD) defined as "nonresponders." These "outlier" groups will be limited to a single ethnicity containing the most individuals. This will result in approximately 10–30 individuals in both the "responder" and "nonresponder" outlier groups for each of the four variables (eight groups). Each of these groups will then be genotyped for each SNP in each candidate gene. Statistically significant associations between specific SNPs and any of the four phenotyped variables will then be determined by comparing genotype distributions of each SNP between "responders" and "nonresponders" using $\chi^2$ tests. Any SNP showing a statistically significant association with a trait will be further analyzed. First, we will use outliers from the second 350 subjects as a "confirmation" data set to validate the association in a separate subject population. Second, genotyping of the SNP will be done in the remaining subject population, those not defined as "responders" or "nonresponders." This will allow comparisons of the muscle function variables as continuous measures. For each SNP, means of each muscle function variable will be compared among genotypes using a generalized linear model, a method analogous to ANOVA that is appropriate for comparing means among groups of different sizes, with the Sidak method for multiple comparisons. This will allow statistically, significantly different means among genotypes to be determined. Lastly, we will investigate any significantly associated SNPs in other ethnic groups. We feel that it is important to avoid mixing ethnic groups in any statistical study, due to the greatly varying allele frequencies within any specific ethnic group, and also the potential for skewing ethnic contributions to the "responder" and "nonresponder" phenotype groups. In addition, we will take the SNPs discovered and analyze for haplotypes, a set of polymorphism alleles that co-occur on a chromosome (31). The different haplotypes that exist will be correlated with the different measurements in the study.

The "outlier screening" method balances our desire to identify genes showing the greatest effect on the phenotypes, with the high cost of genotyping the entire cohort for each SNP. There will then be three forms of validation. First, any SNP showing positive association in outliers will be genotyped in the remaining subject population. This will

allow comparisons of the muscle function variables as continuous measures. For each SNP, means of each muscle function variable will be compared among genotypes using a generalized linear model, a method analogous to ANOVA that is appropriate for comparing means among groups of different sizes, with the Sidak method for multiple comparisons. This will allow statistically, significantly different means among genotypes to be determined.

Second, we will investigate the effect of the same SNP in other ethnic groups. We feel that it is important to avoid mixing ethnic groups in any statistical study, due to the greatly varying allele frequencies within any specific ethnic group, and also the potential for skewing ethnic contributions to the "responder" and "nonresponder" phenotype groups. In addition, we will take the SNPs discovered and analyze these for haplotypes, a set of polymorphism alleles that co-occur on a chromosome. The different haplotypes will be correlated with the different measurements in the study.

Third, we will use outliers and/or the entire second 350 subject cohort as a "validation" data set to validate the association of the SNP in a separate subject population.

It should be noted that the outlier approach utilized here is not as sensitive as is genotyping the entire cohort for all SNPs. However, we are assuming that specific SNPs with a large effect on the variables under study would serve as confounding and uncontrolled variables when searching for SNPs with a less dramatic effect on the same phenotype. Thus, we plan to identify the dominant SNPs first, genotype the cohort for these, then conduct follow-up genetic association studies on subsets of our cohort that share the same genotype for the previously identified dominant SNPs. In this manner, we will be able to control for the additive or subtractive effect of major and minor quantitative trait loci, and thus provide greater sensitivity for minor loci.

**Data management.** All data will be maintained on web-accessible databases (Microsoft SQL Database). Each site will receive a username and password to the protected database, and each subject is automatically assigned a study number reflecting their site of recruitment. All tracking of exercise sessions, weight increments during training, anthropomorphic data, strength testing, and MRI endpoints will be entered into the web database at each site. The database will be maintained in a secure environment and subjected to daily backups.

All data will be screened for consistency between exercise sites, and any discordance in performance of subjects between sites will be discussed at semiannual meetings of all participating investigators. Data will be manually and statistically searched for unexpected values in any database fields, and data either discarded, or the originating site queried regarding accuracy.

## DISCUSSION

FAMuSS is to our knowledge the first large-scale study designed to examine the effect of genetic factors on the physiologic response to resistance exercise training. Other ongoing studies including HERITAGE (4) and STRIDE (16,22) are examining the effect of genetic factors on the physiologic response to aerobic exercise training. FAMuSS was funded by the National Institute of Neurological Disorders and Stroke, with cosponsorship by the National Institute of Aging, and National Institute of Arthritis and Musculoskeletal Disease in April 2001. Sites were activated in the Fall of 2001 and had completed data collection on 490 subjects by the beginning of Summer 2003.

**Ancillary studies.** In addition to the planned examination of genetic factors affecting baseline muscle size and strength change with exercise training, the investigators plan to examine other physiologic questions using this data set. These questions include the day-to-day variability in strength testing, correlations between muscle size and strength, the effect of exercise training on local adiposity, out a comparison of the effect of dynamic training on isometric and dynamic strength testing results, gender differences in the training response as well as an examination of the physiologic and genetic characteristics of those who do or do not complete the training regimen. There are also ancillary studies designed to examine how knowledge of one's possible genetic ability to increase muscle mass affects self-perception. Outside investigators with novel research proposals will be allowed to pursue research interests in collaboration with interested established members of the research group, once the initial data analysis is complete.

Identification of statistical association between a SNP and muscle phenotypic data does not confirm a functional relationship even if confirmed by the other statistical methods described above. Consequently, once SNPs showing a statistically significant association with one of the muscle phenotypes measured in FAMuSS is identified, we will seek to identify the functional consequences of the SNP on the gene or protein product. SNPs that change an amino acid may change the activity or half-life of the protein, while those in promoter regions or intronic sequences likely alter transcription or mRNA stability.

**Study limitations.** There are several limitations to the FAMuSS design. Most multicenter trials reduce measurement variability by utilizing a core laboratory to measure biological samples and to interpret images obtained at participating centers. The genetic and serum samples in FAMuSS as well as MRI results will be determined in core laboratories. Muscle strength measurements, however, will be performed at the research sites by the investigators, which will increase measurement variability. To minimize the effect of measurement variability on the primary aim to the study, the genetic analysis will initially be restricted to those individuals at the extremes of the size and strength response. Measurement variability will be reduced further by standardizing techniques in a study manual and by certifying researchers at each site. Research personnel also will meet twice yearly with one meeting dedicated to review and practice of the measurement techniques.

The dietary intake will not be monitored in the FAMuSS study because sufficient protein is provided in the usual American diet to support the metabolic demands for muscle growth expected in one arm exercise training. Subjects will

be asked to maintain their usual dietary intake, and subjects on unusual diets will not be recruited. To avoid the effect from special diets, subjects using nutritional supplements or ergogenic aids will not be recruited. Diet diaries were considered but not included in the final study design because of the additional subject and analysis burden. Body weight will be used to ensure that subjects are not restricting calories during the study, and outliers who lose significant amounts of weight during the study will not be included in the genetic analysis. Nevertheless, more subtle caloric or dietary manipulations not reported by the subjects or detected by weight loss could affect the results.

Volunteers in FAMuSS may not represent the general population. Subjects must be willing to engage in one arm exercise training for 12 wk, a criterion that may affect the study population. It is also conceivable that individuals with skeletal muscle genes that enhance muscle size or strength may not feel the need to engage in exercise training. This should not affect those outliers with low muscle size and strength at baseline but could restrict recruitment of individuals with high baseline parameters.

The initial data analysis will examine genotype in outliers whose baseline muscle strength, size or their response to resistance training is $>$ 1.5 SD from the mean response. This approach should identify SNPs with large effect sizes but has the risk of overlooking more subtle, but influential genetic variants. The ultimate goal of FAMuSS is to perform a genetic analysis of all muscle genes and to examine identified SNPs in all subjects. This will permit an examination of SNP distribution versus the distribution of muscle phenotypic and will provide sufficient sample size to examine the interaction of various SNPs.

## SUMMARY

FAMuSS is designed to determine skeletal muscle genes associated with muscle size and strength and the muscle response to strength training. The study will initially examine genetic variants previously associated with muscle size and response to resistance training. We anticipate that these same genes will show an effect on common yet variable clinical problems, such as extent of muscle loss during space travel, extent of rehabilitation after casting or immobilization, and the degree of muscle loss with aging. Although we are using a candidate gene approach, many of these candidate genes are derived from expression profiling, where the function of the gene may not be known. This "reverse physiology" in which genetic markers are used to identify previously unrecognized physiologic processes could contribute to our understanding of basic muscle physiology. Such knowledge may help identify pharmacologic agents capable of preserving muscle function. The genetic results may also prove useful in identifying individuals who are susceptible to muscle loss with such challenges as spaceflight and aging and thereby to identify people capable of prolonged inactivity without risk or in need of preventive therapies. Finally, much as pharmacogenomics is increasing our ability to predict individual patient response to medications, FAMuSS may contribute to exercise genomics or the ability to predict the individual response to exercise training.

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