Open access • Journal Article • DOI:10.1249/MSS.0B013E31828983F7

Genetic predisposition scores associate with muscular strength, size, and trainability.

Tom Thomaes, Martine Thomis, Steven Onkelinx, Kaatje Goetschalckx ...+3 more authors

Institutions: Katholieke Universiteit Leuven

Published on: 01 Aug 2013 - Medicine and Science in Sports and Exercise (Lippincott Williams & Wilkins)

Topics: Genetic predisposition

Medicine & Science IN Sports & Exercise
The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

Genetic Predisposition Scores Associate with Muscular Strength, Size, and Trainability

Tom Thomaes[1], Martine Thomis[2], Steven Onkelinx[1], Kaatje Goetschalckx[3], Robert Fagard[3], Diether Lambrechts[4], and Luc Vanhees[1]

[1]Research Group for Cardiovascular and Respiratory Rehabilitation, Department of Rehabilitation Sciences, KU Leuven, Belgium; [2]Physical Activity, Sports & Health Research Group, Department of Biomedical Kinesiology, KU Leuven, Belgium; [3]Department of Cardiovascular Diseases, University Hospital Leuven, Belgium; [4]Laboratory of Translational Genetics, Vesalius Research Center, VIB, Belgium

Accepted for Publication: 23 January 2013

Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0b013e31828983f7

Genetic Predisposition Scores Associate with Muscular Strength, Size, and Trainability

Tom THOMAES[1*], Martine THOMIS[2*], Steven ONKELINX[1], Kaatje GOETSCHALCKX[3], Robert FAGARD[3], Diether LAMBRECHTS[4], and Luc VANHEES[1]

[1] Research Group for Cardiovascular and Respiratory Rehabilitation, Department of Rehabilitation Sciences, KU Leuven, Belgium; [2] Physical Activity, Sports & Health Research Group, Department of Biomedical Kinesiology, KU Leuven, Belgium; [3] Department of Cardiovascular Diseases, University Hospital Leuven, Belgium; [4] Laboratory of Translational Genetics, Vesalius Research Center, VIB, Belgium

* equal contributing first authors

Professional relationships with companies or manufacturers who will benefit from the results of the present study:

Tom THOMAES: no conflict of interest

Martine THOMIS: no conflict of interest

Steven ONKELINX: no conflict of interest

Kaatje GOETSCHALCKX: no conflict of interest

Robert FAGARD: no conflict of interest

Diether LAMBRECHTS: no conflict of interest

Luc VANHEES: no conflict of interest

Address for correspondence:

Prof. Dr. Luc Vanhees

Department Rehabilitation Sciences

Tervuursevest 101 B1501

3001 Leuven, Belgium

Phone: 32-16-329158 Fax: 32-16-329197

E-mail: Luc.Vanhees@faber.kuleuven.be

Disclosure of funding

This study was supported by grants from the Fund for Scientific Research –Flanders 'Fonds voor Wetenschappelijk Onderzoek - Vlaanderen', Belgium (F.W.O. grant G.0624.08 and G.0124.02).

Running head

GPS associates with strength training response

ABSTRACT

Introduction: The number of studies trying to identify genetic sequence variation related to muscular phenotypes has increased enormously. The aim of this study was to identify the role of a genetic predisposition score (GPS-score) based on earlier identified gene variants for different muscular endophenotypes to explain the individual differences in muscular fitness characteristics and the response to training in patients with coronary artery disease (CAD).

Methods: 260 CAD patients followed a standard ambulatory, three month supervised training program for cardiac patients. Maximal knee extension strength (KES) and rectus femoris (RF) diameter were measured at baseline and after rehabilitation. 65 SNPs in 30 genes were selected based on genotype-phenotype association literature. Backward regression analysis revealed subsets of SNPs associated with the different phenotypes. GPS-scores were constructed for all sets of SNPs by adding up the strength-increasing alleles. General linear models and multiple stepwise regression analysis were used to test the explained variance of the GPS-score in baseline and strength responses. Receiver-operating characteristic (ROC) curve analyses were performed to discriminate between 'high and low responder' status.

Results: GPS-scores were significantly associated with RF diameter ($p<0.01$) and its response ($p<0.0001$), isometric KES ($p<0.05$) and its response ($p<0.01$), isokinetic KES at 60°/s ($p<0.05$) and 180°/s ($p<0.001$) and their responses to training ($p<0.0001$) and isokinetic KES endurance ($p<0.001$) and its change after training ($p<0.0001$). The GPS-score was shown as an independent determinant in baseline and response phenotypes with partial explained variance up to 23%. ROC analysis showed significant discriminating accuracy of the models including the GPS-scores for responses to training, with areas under the curve ranging from 0.62 to 0.85.

Conclusion: GPS-scores for muscular phenotypes showed to be associated with baseline KES, muscle diameter and the response to training in cardiac rehabilitation patients.

Keywords: Cardiac rehabilitation; polymorphisms; muscular phenotypes; genetic associations; exercise training

INTRODUCTION

Ageing is characterized by a decline in functionality due to progressive loss of muscle tissue associated with a decrease in strength and force output. Low skeletal muscle strength has been shown to be an important predictor of all-cause mortality in healthy as well as diseased individuals (19,20). The increasing age of coronary artery disease (CAD) patients accompanied by 'fear of moving' and hospitalization in these patients often results in a substantial loss of skeletal muscle mass and muscle strength. It has also been shown that CAD patients suffer from increased muscle fatigability (13). Regular physical activity in cardiac rehabilitation improves aerobic power and skeletal muscle strength and is associated with an increase in survival in these patients (15,18,37). However individual differences in the response to rehabilitation are large (38), which may be partly related to genetic characteristics (28).

Heritability studies in humans have found a genetic contribution up to 66% to fat free mass (FFM) (1) and up to 65% to muscle strength (21). Studies in older twins reported that heritability could explain 20% to 52% of the variance in handgrip strength, leg extensor power and maximal walking speed (2,7,12,21,32-35). Heritability for handgrip strength seems to decrease with increasing age, with a concurrent increase of the relative contribution of environmental effects (7). However, when excluding individuals with age-related chronic diseases, heritability estimates were found to increase (12). Muscle cross sectional area (CSA) of upper and lower extremities among young individuals, showed to have a heritability up to 85-95% (17,29,30). In female twins, genetic effects accounted for 52-84% of the explained variance in lean body mass (26). High heritability estimates have been found in different muscular phenotypes, with multivariate genetic studies showing a shared genetic effect for different

muscular characteristics (9,29,31-33). Linkage studies performed on different muscular characteristics have found evidence for shared chromosomal regions (10).

Over the last decade the number of studies trying to identify genetic sequence variation related to muscular phenotypes has increased enormously. In some candidate genes like *ACE, ACTN3, CNTF* and *MSTN*, specific variants have been repeatedly studied to test for associations in different population groups with different strength measures, albeit with varying success. In more recent years with the introduction of genome wide association studies (GWAS) (16) and genome wide linkage studies (GWLS) (11,31,34), novel genes for muscular strength-related phenotypes have emerged. However to date, most studies have focussed on associations between single SNPs in single genes and strength phenotypes, with only few studies evaluating haplotype-phenotype associations (reviewed in 6). Most significant associations represented only a small proportion of explained variance in the studied strength phenotypes.

To the best of our knowledge, no study has been executed combining strength-increasing alleles of multiple 'strength' genes into a genetic predisposition score (GPS) to search for possible associations with different muscular phenotypes in a non-athlete population. This approach has been performed previously in endurance phenotypes (23-25, 39) and in the prediction of elite power-related performance (24).

Therefore the aim of this study was to identify the role of a GPS-score based on earlier identified gene variants for different muscular endophenotypes (muscular structure, metabolism, cytokines, growth or differentiation factors, neurotropic factors and hormones) to explain the individual differences in muscle characteristics and the response to physical training in coronary artery disease (CAD) patients. Backward-regression based selection of phenotype-specific SNPs to be included in each GPS-score was applied in this study, as was introduced by Bouchard et al.

(5). A secondary aim of this study was to compare this approach with the construction of a total GPS-score based on all 54 genotyped SNPs (GPS_{54}). We hypothesized a significant contribution for the GPS score to explain -at least in part- the variability in strength gain in CAD patients following rehabilitation.

METHODS

Study Sample and training intervention

All patients with CAD (acute myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting and/or angina pectoris), who were submitted to the cardiac rehabilitation program at the University Hospital in Leuven, were invited to participate in the CAREGENE II study (CArdiac REhabilitation and GENetics of Exercise performance). Patients with valve disease, congenital heart disease, major arrhythmia, pacemaker or ICD implantation, heart transplantation or other cardiac diseases were excluded. Inclusions were held from October 2008 until January 2011. The study protocol was approved by the Ethical Committee of the Faculty of Medicine of the Catholic University of Leuven and written informed consent was obtained from each participant. In total, 260 CAD patients, who had performed a graded cycle ergometer test with respiratory gas analysis until exhaustion, were included at baseline. Data at peak exercise was collected before and after 3 months of rehabilitation. Patients followed a standard ambulatory supervised cardiac rehabilitation program, three times per week for three months with 90 minutes/session involving cycling, running, arm ergometry, rowing, predominantly isotonic calisthenics and relaxation. The average training frequency was 2.26 ± 0.03 times/week and each patient trained on average at an intensity of 80 ± 0.82% (training heart rate/peak heart rate) x100, where the mean exercise heart rates of the last three exercise sessions

and peak heart rates of the exercise testing after training were used. Two hundred and four patients completed the 3-month cardiac rehabilitation program and were included for the analyses of responses to training. The following tests were held before and after three months of rehabilitation.

Estimates for body composition

Six skinfolds located at the biceps, triceps, sub scapula, suprailiaca, mid-thigh, and medial-calf area were taken with a Harpenden-calliper. Stature and weight were measured and percentage of body fat and fat free mass (FFM) were estimated using an OMRON hand-held body fat monitor (Omron BF 300; OMRON, Matoukasa Co. Ltd, Japan).

Muscular strength

Isokinetic testing equipment (BIODEX System 3 Pro, Biodex Medical Systems, 20 Ramsay Road, Shirley, New York, USA) was used to determine the maximal knee flexion- and extension torque and muscular endurance. Isometric knee extension strength (KES) was measured at a 60° knee angle, isokinetic KES was measured at two contraction speeds (60°/s and 180°/s) and quadriceps muscle endurance was assessed by the total work delivered during a 25 repetition knee extension-flexion bout at a contraction speed of 180°/s. Patients were seated in an upright position with hips and knees 90° flexed. Straps were firmly fastened around the chest, hips and upper leg to stabilize the trunk and leg. Verbal encouragement was given to achieve maximal effort. Due to a technical problem with the BIODEX testing device, muscle strength of 14 patients at baseline and 15 patients post rehabilitation could not be tested. This led to a loss of 29 tests for the response in muscular strength to training.

Measurements of Rectus femoris diameter

Rectus femoris (RF) diameter was measured by M-mode ultrasonography, wall tracking ultrasound system (Siemens Vivid 07 GE) with a 12 MHz linear array transducer (12 L transducer GE). The transducer was placed perpendicular to the long axis of the thigh with excessive use of contact gel and minimal pressure to avoid compression of the muscle (3,8). The diameter of the RF was measured at the half point of the length between epicondylus lateralis and trochanter major of the femur. Measurements were taken on the patient's right leg with the patient lying in a supine position with both knees extended but relaxed and toes pointing the ceiling. A set of five pictures was taken and further analyzed offline. Both pre- and post-rehabilitation ultrasound measurements were analyzed blind and at random. At baseline 246 patients were measured and 173 after training. The main causes for missing values were technical in nature; a server crash of the ultrasound system or the inability to visualize the inner wall of the RF with ultrasound. All ultrasound measurements were performed by a single experienced investigator (T.T) and this method was validated against CT in a similar population (27).

Genotyping

Anonymously coded blood samples were drawn from each patient. Genotyping was performed in a blinded manner using iPLEX technology on a MassARRAY Compact Analyser (Sequenom Inc., San Diego, CA, USA). Selection of SNPs was based on recent review articles, GWAS and GWLS up to January 2011 in which potential candidate genes, SNPs and regions were identified for either aerobic capacity, muscular strength or muscular endurance as

phenotypes (5,6,10,11,25,31,36). Sixty-five SNPs in 30 genes were selected for genotyping based on earlier associations with related phenotypes (Table 1, Supplemental Digital Content 1, http://links.lww.com/MSS/A222, Selected candidate genes and SNPs). Nine SNPs had high linkage disequilibrium with other SNPs of the same gene and two SNPs had a genotyping success rate below 95%. Fifty-four SNPs were therefore withheld for further analysis.

Statistical analyses

Data were analyzed using SAS statistical software version 9.2 for Windows (SAS Institute Inc, Cary, NC, USA). Data were reported as means ± standard deviation (SD) for anthropometric measurements, RF diameter and muscle strength measurements or as number of patients with percentage for dichotomous variables. To test whether the observed genotype frequencies were in Hardy-Weinberg equilibrium a χ^2- test with one degree of freedom was used. Since multiple testing induces false positive or negative associations, and correction of p-values accordingly will lower the power to identify small genetic effects, 'increasing allele' genetic predispositions score (GPS) analysis was performed in which the number of increasing alleles was regressed against the phenotypes of interest. Based on our data, backward regression analysis was first applied to detect subsets of SNPs to be associated with the different muscular phenotypes and for which the GPS scores were calculated. Only these significant contributing SNPs were included in the GPS score. The number of significant contributing SNPs and its following GPS score were therefore different between the different phenotypes. An additive effect was hypothesized and equal weights were given for each increasing allele, because no well-defined effect sizes were known for the different SNPs and weighting of increasing alleles might only have limited effects (14). GPS was calculated for each individual by adding the As an

alternative approach we created a total GPS score (GPS_{54}) based on the total set of 54 SNPs for isokinetic KES at 180°/s, in order to compare the amount of explained variance of an overall approach (GPS_{54}) with the GPS based on a significant subgroup of SNPs after backward regression. Isokinetic KES at 180°/s was used as this is a measurement which has a wide spread use within muscle testing and since the training regimen in cardiac rehabilitation was predominantly dynamic in nature. ANCOVA analysis and multiple regression were used to test the association and percentage explained variance of GPS_{54} in baseline and response to training values. The power of the total GPS_{54} to discriminate between high and low responders was analysed using ROC and AUC and adjusted odds were calculated. All statistical tests were performed two-sided at a significance level of 5%.

RESULTS

Descriptive baseline patient characteristics, cardiac history, medication use and response to three months of cardiac rehabilitation are described in table 1. Baseline characteristics did not differ between the patients who dropped-out of the study and the patients who participated for three months. PeakVO_2 increased by 21.6 ± 15.9% ($p<0.001$) and peak heart rate by 8.2% ± 11.9% after 3 months of training, isometric KES increased by 11.5 ± 16.0% ($p<0.0001$), isokinetic KES (60°/s) by 17.0 ± 23.1% ($p<0.0001$), isokinetic KES (180°/s) by 16.5 ± 20.2% ($p<0.0001$) and isokinetic quadriceps muscle endurance by 18.8 ± 23.3% ($p<0.0001$) (Table 2). RF diameter was increased by 5.4 ± 11.2% ($p<0.0001$). Body weight remained similar whilst relative and absolute values of body fat decreased significantly by approximately 3% ($p<0.001$) (Table 2).

Backward regression analysis revealed subsets of SNPs to be significantly associated with the respective muscular phenotypes at baseline and the response to training (strength and RF diameter). In particular, we identified 2 SNPs for baseline isometric and isokinetic KES (60°/s) and up to 11 SNPs for the response of RF diameter and knee extensor endurance strength. Table 2 (Supplemental Digital Content 2, http://links.lww.com/MSS/A223) gives an overview of the SNPs included in the GPS score for each baseline and response phenotype. Only these significantly contributing SNPs were included in the GPS score. To avoid the possibility of false positive or negative results and the lack of statistical power by the smaller groups at the two tails -small number of patients with either a small or high number of increasing alleles- we combined the two lower and two upper GPS score groups respectively. Results of ANCOVA analyses of the GPS influence on baseline and response variables, with age, sex, height and FFM as covariates for baseline measurements and age, sex, height, change in FFM, TI and TF for response measurements, are shown in Figure 1. Proc GLM showed that each GPS-score was significantly associated with RF diameter ($p<0.01$) and RF diameter response ($p<0.0001$), isometric KES ($p<0.05$) and change in isometric KES ($p<0.01$), isokinetic KES at 60°/s ($p<0.05$) and 180°/s ($p<0.001$) and their respective response to training ($p<0.0001$) and isokinetic knee extensor muscle endurance ($p<0.001$) and its change after three months of training ($p<0.0001$).

Stepwise multiple regression analysis showed a total explained variance between 36% and 57% for the baseline muscle phenotypes (Table 3). GPS score was found as an independent determinant in all baseline muscle phenotypes except for baseline isometric knee extension strength with partial r between 0.16 and 0.30 (Table 3). The significant b-coefficients in table 3 indicate that each increasing allele in the GPS score results in an additional 6.65 Nm, 3.47 Nm, 124.62 J and 0.04 cm in baseline isokinetic KES (at 60°/s and 180°/s), baseline muscle

endurance and baseline RF diameter respectively. GPS_{54} was not significantly related to baseline isokinetic KES at 180°/s. Table 4 shows the multiple stepwise regression analysis for the training response parameters. Total explained variance ranged between 6% and 26% with GPS as a significant independent determinant in all response phenotypes (r between 0.25 and 0.48). Each additional increasing allele adds 6.92% to the gain in isometric KES, 1.38% in isokinetic KES at 60°/s, 6.60% in isokinetic KES at 180°/s, 7.60% in knee extension endurance and adds 3.38% to the gain in RF diameter. Analyses of the high vs. low-responder groups were performed for the GPS of all phenotypes after training. For all response phenotypes GPS was the only statistically significant contributing variable, except for the response in isokinetic KES at 180°/s for which change in fat free mass was an additional significant predictor. Results per phenotype are shown in table 5.

Additionally we performed an analysis on the GPS_{54} score, based on the 54 selected SNPs, for isokinetic KES at 180°/s and the response after training. Although there was a theoretical spread of GPS_{54} score between 0 and 108, the GPS_{54} ranged from 42 to 60 for baseline and between 40 and 65 for the sample with response data. The groups at the two tails were combined and the GPS_{54} was analyzed with a spread from 46 to 57 for baseline and 47 to 58 for the response. When GPS_{54} was analyzed without covariates for isokinetic KES (180°/s) at baseline a trend could be observed that a higher GPS_{54} results in a higher baseline KES (P=0.06). When age, sex, height and FFM were added as covariates the model was significant (P<0.001), however, GPS_{54} had no significant contribution (P=0.50). For the response in isokinetic KES (180°/s), GPS_{54} was a significant (P<0.001) independent variable when analyzed without covariates and the model with covariates was also significant (P<0.01). In the latter procedure, GPS was the only significant contributor to the model (partial P=0.0029). When GPS_{54} was

entered into a multiple stepwise regression analysis with all covariates to explain response in isokinetic KES at 180°/s, GPS_{54} had a partial R^2 of 9.5% and age had a partial R^2 of 2.6%. GPS_{54} contributed significantly to the distinction between high and low-responder groups (AUC=0.68; 95% CI: 0.56-0.80 and adjusted odds ratio=1.23; 95% CI: 1.06-1.42) (Figure 1, Supplemental Digital Content 3, http://links.lww.com/MSS/A224, Overlay of four different models to discriminate high vs. low responder in isokinetic KES (180°/s) after training). Change in FFM also contributed significantly to the high response status in isokinetic KES at 180°/s (AUC=0.64; 95% CI: 0.51-0.76) (P<0.05). When adding change in FFM to the model with GPS_{54}, the AUC increased to 0.72 (95% CI: 0.61-0.84) but the model did not differ significantly from the model with GPS_{54} alone (P=0.28).

DISCUSSION

Since most previous genetic association analysis studies mainly focus on one phenotype and one gene (variant), the first aim of this study was to identify combinations of gene variants that were associated with different muscular phenotypes and to quantify the degree of explained variances of these GPS-scores for the variability in strength and strength gains in CAD patients. In this study we found an increase in muscle strength between 11% and 17% and in RF diameter of 5% after three months of rehabilitation in CAD patients with a predominantly aerobic exercise-training program. However a large inter-individual variability could be observed.

Previous studies applying the candidate gene approach have found significant associations of SNPs with different muscular phenotypes but with only limited explained variance by a single SNP. Recent genome-wide studies have identified novel SNPs and regions of interest for associations with muscular phenotypes providing additional potential genetic

information. However, muscular strength phenotypes are multifactorial and polygenic traits. To the best of our knowledge, this was the first study to search for combinations of SNPs in different muscular (endophenotype) related genes. By means of backward regression analysis, we were able to identify 10 sets of SNPs to be associated with different muscular phenotypes. rs17602729 in the *AMPD1* gene and rs1016732 and rs2854248 in the *ATP1A2* gene showed a large overlap between the different strength phenotypes. Rs17602729 of the *AMPD1* gene has previously been shown to be associated with a diminished aerobic capacity and cardiorespiratory response to exercise (22,28) and a decrease in exercise duration over 20 years (25). Likewise markers from *ATP1A2* were associated with a decrease in exercise duration (25). The majority of SNPs included in the GPS-scores for these muscular phenotypes were located in genes that were functionally categorized into *muscle metabolism* or *muscle growth and differentiation*.

ANCOVA analyses showed that all created GPS-scores were significantly associated with their respective phenotype under study. At baseline, CAD patients with a higher GPS-score showed higher baseline isometric and isokinetic knee extensor strength, with every additional increasing allele accounting for a surplus in strength. Subjects with a higher GPS also showed a higher muscle strength or muscular endurance response to training. Moreover, we were able to show that GPS contributes significant to the discrimination between high and low responders status for the phenotypes under study. GPS had a significant AUC between 0.62 and 0.85 and adjusted odds ratio between 1.90 and 2.84 for the different muscular phenotypes. A patient with a high GPS has a higher probability to end up in the group with the 25% highest response after training. For all phenotypes under study, GPS was the best independent variable associated with a high-responder status and except for isokinetic KES at 180°/s, it was the only contributor to high-response values.

The GPS-score was an independent determinant for all, except isometric extensor strength, baseline phenotypes with a partial R^2 ranging between 3% and 9% (Table 3). For the response to training only the change in FFM and age were previously found as determining variables for some response phenotypes (Table 4). The partial explained variance by adding the GPS-score for the response to training had a R^2 between 6% and 23%. The higher explained variance by the GPS of the difference in response to training, compared to the baseline measurement, might be explained by the higher number of other determinants at baseline. At baseline the largest part of the variability in muscle strength and diameter could be explained by covariates such as age, sex, height and FFM, which are already under genetic influence. Furthermore, effect sizes of gene variants might be larger when the muscular system is challenged to be active in repair and metabolic optimization in response to regular training, compared to a stable baseline condition.

Related to the secondary aim of this study we compared two strategies to determine the genetic predisposition score. The option to determine each GPS-score phenotype-specific based on significantly contributing SNPs is less practical in further applications of this approach as the set of SNPs differs according to different phenotypes. This approach might also inflate the probability of finding significant GPS-phenotype associations. An overall GPS-score based on all 54 SNPs (GPS_{54}) was calculated and compared with the GPS-score based on the significant subsets of SNPs for isokinetic KES at 180°/s at baseline and the response after training. Indeed, the explained variance in response to training was higher when the GPS score was based on the significant subgroup of SNPs (13.7%) when compared to GPS_{54} (9.5%). Both approaches proved GPS to discriminate significantly ($p<0.01$) between high and low responders to training with a similar AUC of 0.70 (95% CI: 0.58-0.81) and 0.68 (95% CI: 0.56-0.80) (Supplemental Digital

Content, figure 1, Overlay of four different models to discriminate high vs. low responder in isokinetic KES (180°/s) after training) for subgroup and total group of SNPs respectively. However the adjusted odds ratio is clearly in favor of the smaller group of SNPs: 1.90 (95% CI: 1.24-2.93) vs. GPS_{54}: 1.23 (95% CI: 1.06-1.42). Constructing a GPS based on 54 SNPs induces background 'noise', meaning that subjects might carry a small or larger amount of 'increasing' alleles, while part of those alleles are only having very limited effect or no effect on the phenotype. For the GPS_{54} score groups, a non-linear GPS-phenotype curve is observed (plateau in response scores for GPS_{54} scores 51 up to 56, results not shown) which also induces a lower degree of explained variance (R^2). By first using a backward regression analysis on the total group of SNPs we, and others who applied this similar approach (5), were able to filter out the SNPs that had very small contributions or no influence on the phenotype under study. Inherent to the chosen construction of the GPS-scores (whether as a total set of SNPs or subset of SNPs), we assumed allelic effects to be co-dominant and each SNP to have an equal additional effect. Also by using GPS-scores, information on which combination of increasing alleles is responsible for the specific effect is lost, as individuals with 4 increasing alleles, might possess those from various combinations of increasing alleles of different gene variants. Finally, we are aware that the selection of genes in the initial gene list might have left out other SNPs that might be associated with muscular phenotypes.

There are some limitations to this study. This study was performed in a predominantly Caucasian, older, male CAD population. According to required sample sizes in genetic epidemiological studies, the sample size is small (4). This approach therefore needs further replication and results might be expected to be different in other populations or in response to other exercise regimens. The cardiac rehabilitation training was mostly focused on aerobic

training with a smaller part of dynamic resistance training (3 sets of 15 reps) of the lower extremities with own body weight as resistance. Unfortunately some measuring devices had technical errors so in some individuals not all tested parameters were available. Additionally there is a reasonable dropout rate in patients following cardiac rehabilitation due to work resume or long distance to the rehabilitation center, which led to a lower number of response measurements. However sub-analyses of dropout versus non-dropout groups did not reveal significant differences between both groups at the start of the rehabilitation.

CONCLUSIONS

Constructions of multiple SNPs into genetic predisposition scores (GPS) for different muscular phenotypes showed to be associated with baseline muscle strength, muscle diameter and the response of these parameters to training in cardiac rehabilitation patients. The GPS-score could explain up to 23% of the variance in these muscular phenotypes and was able to discriminate high versus low responder status on different muscular phenotypes. Phenotype-specific GPS-scores selected by backward regression show higher GPS-phenotype associations compared to the application of a GPS-score based on all listed gene variants.

Acknowledgements

This study was supported by grants from the Fund for Scientific Research –Flanders 'Fonds voor Wetenschappelijk Onderzoek - Vlaanderen', Belgium (F.W.O. grant G.0624.08 and G.0124.02). There are no conflicts of interest and the results of the present study do not constitute endorsement by ACSM.

REFERENCES

1. Abney M, McPeek MS, Ober C (2001) Broad and narrow heritabilities of quantitative traits in a founder population. *Am J Hum Genet*. 2001;68(5):1302–1307.
2. Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. *J Bone Miner Res*. 1997;12(12):2076–2081.
3. Arts IM, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ. Normal values for quantitative muscle ultrasonography in adults. Muscle Nerve. 2010;41:32-41.
4. Bouchard C. Overcoming barriers to progress in exercise genomics. *Exerc Sport Sci Rev.* 2011 Oct;39(4):212-7.
5. Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, Rao DC, Rankinen T. Genomic predictors of the maximal O□ uptake response to standardized exercise training programs. *J Appl Physiol*. 2011;110(5):1160-70.
6. Bray MS, Hagberg JM, PerusseL, Rankinen T, Roth SM, Wolfarth B, Bouchard C. The human gene map for performance and health-related fitness phenotypes: the 2006-2007 update. *Med Sci Sports Exerc*. 2009;41(1):35-73.
7. Carmelli D, Reed T. Stability and change in genetic and environmental influences on hand-grip strength in older male twins. *J Appl Physiol*. 2000;89(5):1879–1883
8. Delaney S, Worsley P, Warner M, Taylor M, Stokes M. Assessing contractile ability of the quadriceps muscle using ultrasound imaging. *Muscle Nerve*. 2010;42:530-538.
9. De Mars G, Thomis MA, Windelinckx A, Van Leemputte M, Maes HH, Blimkie CJ, Claessens AL, Vlietinck R, Beunen G. Covariance of isometric and dynamic arm contractions: multivariate genetic analysis. *Twin Res Hum Genet*. 2007;10(1):180–190

10. De Mars G, Windelinckx A, Huygens W, Peeters MW, Beunen GP, Aerssens J, Vlietinck R, Thomis MA. Genome-wide linkage scan for maximum and length-dependent knee muscle strength in young men: significant evidence for linkage at chromosome 14q24.3. *J Med Genet*. 2008;45(5):275–283.
11. De Mars G, Windelinckx A, Huygens W, Peeters MW, Beunen GP, Aerssens J, Vlietinck R, Thomis MA. Genome-wide linkage scan for contraction velocity characteristics of knee musculature in the Leuven Genes for Muscular Strength Study. *Physiol Genomics*. 2008;35(1):36-44.
12. Frederiksen H, Gaist D, Petersen HC, Hjelmborg J, McGue M, Vaupel JW, Christensen K. Hand grip strength: a phenotype suitable for identifying genetic variants affecting mid- and late-life physical functioning. *Genet Epidemiol*. 2002;23(2):110–122.
13. Ghroubi S, Chaari M, Elleuch H, Massmoudi K, Abdenadher M, Trabelssi I, Akrout M, Feki H, Frikha I, Dammak J, Kammoun S, Zouari N, Elleuch MH. The isokinetic assessment of peripheral muscle function in patients with coronary artery disease: correlations with cardiorespiratory capacity. *Ann Readapt Med Phys*. 2007;50:295-301.
14. Janssens AC, Moonesinghe R, Yang Q, Steyerberg EW, van Duijn CM, Khoury MJ. The impact of genotype frequencies on the clinical validity of genomic profiling for predicting common chronic diseases. *Genet Med*. 2007;9:528-35.
15. Kavanagh T, Mertens DJ, Hamm LF, Beyene J, Kennedy J, Corey P, Shephard RJ. Prediction of long-term prognosis in 12 169 men referred for cardiac rehabilitation. *Circ*. 2002;106:666-671.
16. Liu XG, Tan LJ, Lei SF, Liu YJ, Shen H, Wang L, Yan H, Guo YF, Xiong DH, Chen XD, Pan F, Yang TL, Zhang YP, Guo Y, Tang NL, Zhu XZ, Deng HY, Levy S, Recker RR,

Papasian CJ, Deng HW. Genome wide association and replication studies identified TRHR as an important gene for lean body mass. *Am J Hum Genet*. 2009;84,418-423

17. Loos R, Thomis M, Maes HH, Beunen G, Claessens AL, Derom C, Legius E, Derom R, Vlietinck R. Gender-specific regional changes in genetic structure of muscularity in early adolescence. *J Appl Physiol*. 1997;82 (6):1802–1810
18. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE: Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002, 346:793-801.
19. Rantanen T. Muscle strength, disability and mortality. *Scand J Med Sci Sports*. 2003;13:3-8.
20. Rantanen T., Harris T, Leveille SG, Visser M, Foley D, Masaki K, Guralnik JM. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. *J Gerontol A Biol Sci Med Sci*. 2000;55:M168-M173.
21. Reed T, Fabsitz RR, Selby JV, Carmelli D. Genetic influences and grip strength norms in the NHLBI twin study males aged 59–69. *Ann Hum Biol*. 1991;18(5):425–432
22. Rico-Sanz J, Rankinen T, Joanisse DR, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. Associations between cardiorespiratory responses to exercise and the C34T AMPD1 gene polymorphism in the HERITAGE Family study. *Physiol Genomics*. 2003;14:161–166.
23. Ruiz JR, Gómez-Gallego F, Santiago C, González-Freire M, Verde Z, Foster C, Lucia A. Is there an optimum endurance polygenic profile? *J Physiol*. 2009;587:1527-1534.
24. Santiago C, Ruiz JR, Muniesa CA, González-Freire M, Gómez-Gallego F, Lucia A. Does the polygenic profile determine the potential for becoming a world-class athlete? Insights from the sport of rowing. *Scand J Med Sci Sports*. 2009;20:e188-e194.

25. Sarzynski MA, Rankinen T, Sternfeld B, Grove ML, Fornage M, Jacobs DR Jr, Sidney S, Bouchard C. Association of single-nucleotide polymorphisms from 17 candidate genes with baseline symptom-limited exercise test duration and decrease in duration over 20 years: the Coronary Artery Risk Development in Young Adults (CARDIA) fitness study. *Circ Cardiovasc Genet*. 2010;3(6):531-8

26. Seeman E, Hopper JL, Young NR, Formica C, Goss P, Tsalamandris C. Do genetic factors explain associations between muscle strength, lean mass, and bone density? A twin study. *Am J Physiol*. 1996;270(2 Pt 1):E320–E327

27. Thomaes T, Thomis MA, Onkelinx S, Coudyzer W, Cornelissen V, Vanhees L. Reliability and validity of the ultrasound technique to measure the rectus femoris muscle diameter in older CAD-patients. *BMC Med Imaging*. 2012;12(1):7

28. Thomaes T, Thomis MA, Onkelinx S, Fagard R, Matthijs G, Buys R, Schepers D, Cornelissen V, Vanhees L. A genetic predisposition score for muscular endophenotypes predicts the increase in aerobic power after training: the CAREGENE study. *BMC Genet*. 2011;3;12:84.

29. Thomis MA, Van Leemputte M, Maes HH, Blimkie CJ, Claessens AL, Marchal G, Willems E, Vlietinck RF, Beunen GP. Multivariate genetic analysis of maximal isometric muscle force at different elbow angles. *J Appl Physiol*. 1997;82(3):959–967

30. Thomis MA, Beunen GP, Maes HH, Blimkie CJ, Van Leemputte M, Claessens AL, Marchal G, Willems E, Vlietinck RF. Strength training: importance of genetic factors. *Med Sci Sports Exerc*. 1998;30(5):724–731

31. Thomis MA, De Mars G, Windelinckx A, Peeters MW, Huygens W, Aerssens J, Beunen GP. Genome-wide linkage scan for resistance to muscle fatigue. *Scand J Med Sci Sports*. 2011;21(4):580-8.

32. Tiainen K, Sipila S, Alen M, Heikkinen E, Kaprio J, Koskenvuo M, Tolvanen A, Pajala S, Rantanen T. Heritability of maximal isometric muscle strength in older female twins. *J Appl Physiol*. 2004;96(1):173–180

33. Tiainen K, Sipila S, Alen M, Heikkinen E, Kaprio J, Koskenvuo M, Tolvanen A, Pajala S, Rantanen T. Shared genetic and environmental effects on strength and power in older female twins. *Med Sci Sports Exerc.* 2005;37 (1):72–78

34. Tiainen KM, Perola M, Kovanen VM, Sipila S, Tuononen KA, Rikalainen K, Kauppinen MA, Widen EI, Kaprio J, Rantanen T, Kujala UM. Genetics of maximal walking speed and skeletal muscle characteristics in older women. *Twin Res Hum Genet*. 2008;11(3):321–334.

35. Tiainen K, Sipila S, Kauppinen M, Kaprio J, Rantanen T. Genetic and environmental effects on isometric muscle strength and leg extensor power followed up for three years among older female twins. *J Appl Physiol*. 2009;106 (5):1604–1610.

36. Timmons JA, Knudsen S, Rankinen T, Koch LG, Sarzynski M, Jensen T, Keller P, Scheele C, Vollaard NB, Nielsen S, Akerström T, MacDougald OA, Jansson E, Greenhaff PL, Tarnopolsky MA, van Loon LJ, Pedersen BK, Sundberg CJ, Wahlestedt C, Britton SL, Bouchard C. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. *J Appl Physiol*. 2010;108(6):1487-96.

37. Vanhees L, Fagard R, Thijs L, Staessen J, Amery A. Prognostic significance of peak exercise capacity in patients with coronary artery disease. *J Am Coll Cardio*. 1994;23:358-363.

38. Vanhees L, Stevens A, Schepers D, Defoor J, Rademakers F, Fagard R. Determinants of the effects of physical training and of the complications requiring resuscitation during exercise in patients with cardiovascular disease. *Eur J Cardiovasc Prev Rehabil.* 2004 Aug;11(4):304-12.

39. Williams AG, Folland JP. Similarity of polygenic profiles limits the potential for elite human physical performance. *J Physiol*. 2008;586:113-121.

Figure 1. GPS scores for the different muscular phenotypes

A: baseline RF diameter (overall $P<0.01$), **B: response in RF diameter** (overall $P<0.0001$), **C: baseline isometric extensor strength (60°)** (overall $P<0.05$)**, D: response in isometric extensor strength (60°)** (overall $P<0.01$)**, E: baseline isokinetic extensor strength (60°/s)** (overall $P<0.05$), **F: response in isokinetic extensor strength (60°/s)** (overall $P<0.0001$), **G: baseline isokinetic extensor strength (180°/s)** (overall $P<0.001$), **H: response in isokinetic extensor strength (180°/s)** (overall $P<0.0001$)**, I: baseline knee extensor muscle endurance (25 repetitions 180°/s) (o**verall $P<0.001$)**, J: response in knee extensor muscle endurance (25 repetitions 180°/s** (overall $P<0.0001$)

Left Y-axis: Phenotype under study (square dots ± SE) corrected for age, sex, height and fat free mass

Right Y-axis: Number of patients in each increasing alleles group (bar graph)

X-axis: GPS - Number of increasing alleles

Figure 1

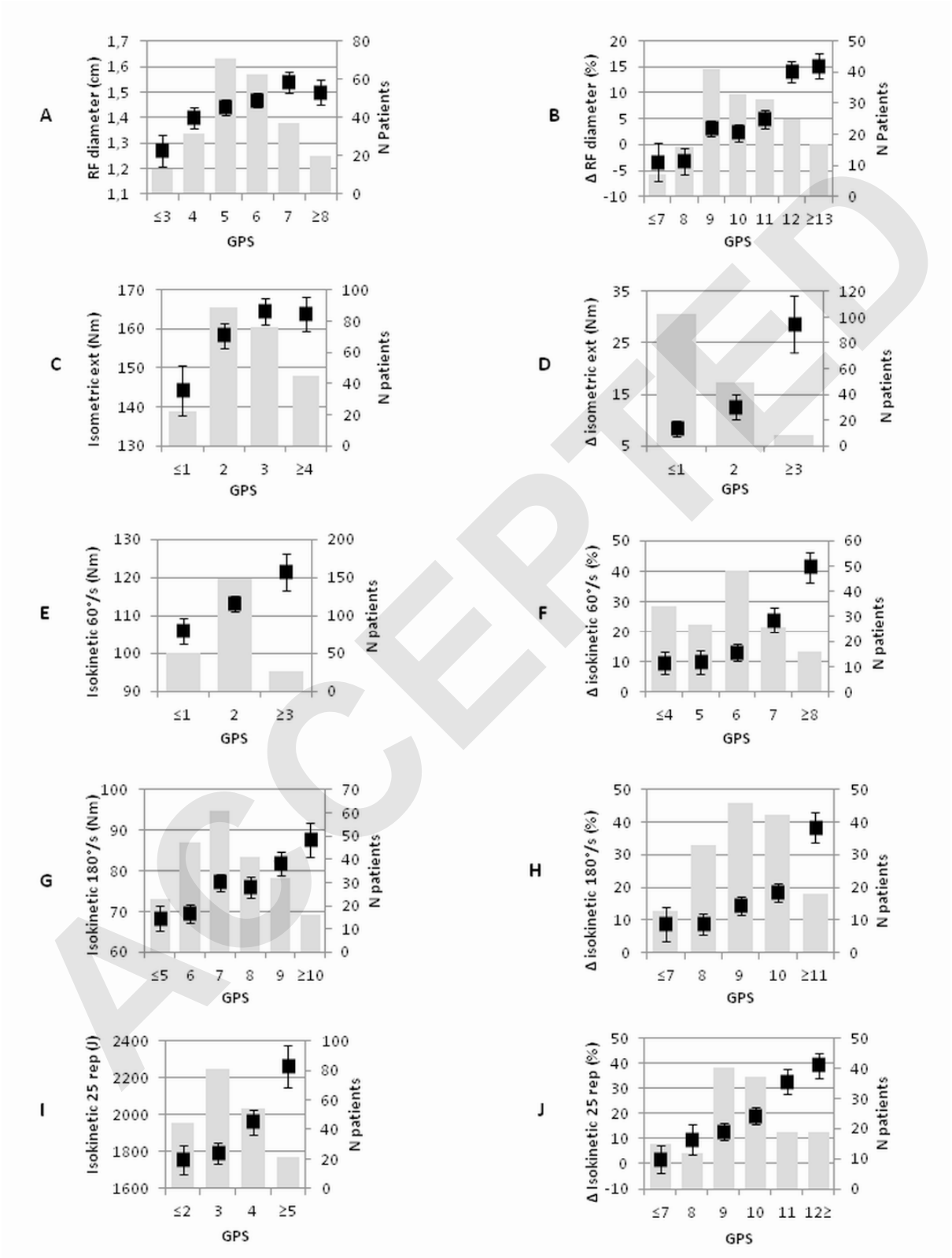


Table 1. Patient characteristics at baseline

Variable	Mean ± SD or number (%)
Sex (M/F)	223/37
Age (years)	60.5 ± 9.6
Height (cm)	171.5 ± 8.0
BMI (kg/m²)	27.5 ± 4.2
Reason for referral to CR*	
AMI	164 (63)
PCI	169 (65)
CABG	100 (38)
Risk factors	
History of diabetes	28 (11)
History of hypertension	128 (49)
Past smoking	130 (50)
Drug treatment	
B-Blocker	204 (78)
Anticoagulantion	22 (8)
ACE-inhibitor	143 (55)
Angiotensin II inhibitor	23 (9)
Anti-aggregantia	224 (86)
Ca-antagonist	30 (12)
Molsidomine	10 (4)
Diuretic	41(16)
Statin	214 (82)

CR: Cardiac rehabilitation; AMI: Acute myocardial infarction; CABG: Coronary artery bypass grafting, PCI: Percutaneous coronary intervention. * Some patients had comorbidity for PCI and CABG.

Table 2. Baseline measurements and changes after three months of cardiac rehabilitation training in CAREGENE II

Variable	Baseline 1 (N=260)	Baseline: 2 (N=204)	N	Post-training	Δ (%) ± SD
Weight (kg)	80.7 ± 13.3	79.4 ± 13	204	80.3 ± 0.9	-0.04 ± 5.7
FFM (kg)	57.1 ± 9.8	57.0 ± 9.7	200	57.5 ± 0.7**	1.2 ± 3.6**
Impedance body fat (%)	29.3 ± 6.3	29.0 ± 6.4	201	28.1 ± 0.5**	-3.2 ± 8.0**
Impedance body fat (kg)	23.5 ± 6.7	23.2 ± 6.8	201	22.4 ± 0.5**	-3.0 ± 10.4**
Data at peak exercise					
peakVO_2 (ml/min)	1558 ± 470	1564 ± 478	204	1883 ± 39**	21.6 ± 15.9**
HR (b/min)	125 ± 20	124 ± 20	204	134 ± 1 **	8.2 ± 11.9**
RER	1.20 ± 0.11	1.21 ± 0.11	204	1.22 ± 0.01*	1.60 ± 9.4*
EqO_2	42.4 ± 9.3	42.3 ± 8.5	204	42.2 ± 0.56	0.50 ± 14.5
Rectus Femoris diameter (cm)	1.45 ± 0.27	1.45 ± 0.27	173	1.53 ± 0.02**	5.4 ± 11.2**
Extension strength (quadriceps)					
Isometric 60° (Nm)	159 ± 42	159 ± 42	175	176 ± 3**	11.5 ± 16.0**
Isokinetic 60°/s (Nm)	112 ± 33	112 ± 32	163	128 ± 3**	17.0 ± 23.1**
Isokinetic 180°/s (Nm)	75.4 ± 24.2	75.5 ± 23.5	164	86.6 ± 2.0**	16.5 ± 20.2**
Muscular endurance (extension) (180°/s) (J)	1874 ± 683	1882 ± 661	155	2093 ± 51**	18.8 ± 23.3**

Baseline 1: Baseline measurements of the total group; Baseline 2: Baseline measurements of the group without drop out; N, number of patients; SD: Standard Deviation; HR: peak heart rate; RER: VCO_2/VO_2; $EqO_2=V_E/VO_2$ *p<0.05; **p<0.0001

Table 3. Significant partial correlation coefficients and parameter estimates of baseline knee extension strength parameters and rectus femoris diameter.

	Isometric quadriceps muscle strength		Isokinetic quadriceps strength (60°/s)		Isokinetic quadriceps strength (180°/s)		Muscular endurance (extension)		RF diameter	
	r	b-coefficient	r	b-coefficient	r	b-coefficient	r	b-coefficient	r	b-coefficient
Age	-0.28	-0.99****	-0.25	-0.68***	-0.24	-0.45***	-0.34	-19.54****	-0.31	-0.008****
Sex	-	-	-	-	-	-	-	-	-0.47	-0.35****
Height	0.24	1.35***	-	-	-	-	-	-	-	-
FFM	0.25	1.26***	0.48	1.55****	0.58	1.41****	0.49	33.46****	-	-
RF diameter	0.20	25.9**	0.23	24.36***	0.21	13.17**	0.15	304.98*	-	-
Diabetes	-	-	-	-	-0.15	-7.75*	-0.24	-369.41**	-	-
CABG	-	-	-	-	-	-	-	-	-0.15	-0.07*
β-Blocker	-0.18	-13.13**	-	-	-	-	-	-	-	-
Anticoagulantia	-0.17	-18.05*	-0.15	-13.25*	-0.17	-10.22*	-0.20	-338.77*	-	-
Claudication	-0.23	-30.62***	-	-	-	-	-	-	-	-
ACE	-	-	-	-	-	-	0.17	166.89*	-	-
GPS	-	-	0.16	6.65*	0.30[$]	3.47[$]****	0.23	124.62**	0.24	0.04***
Total variance	R^2=0.53 (N=219)		R^2=0.49 (N=214)		R^2=0.57 (N=213)		R^2=0.54 (N=219)		R^2=0.36 (N=236)	

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; FFM: fat free mass; RF: Rectus femoris; CABG: Coronary artery bypass grafting. [$]r and b-coefficient were non-significant for GPS_{54}

Table 4. Significant partial correlation coefficients and parameter estimates of response in knee extension strength parameters and rectus femoris diameter.

	ΔIsometric quadriceps muscle strength		ΔIsokinetic quadriceps strength (60°/s)		ΔIsokinetic quadriceps strength (180°/s)		ΔMuscular endurance (extension)		ΔRF diameter	
	r	b-coefficient	r	b-coefficient	r	b-coefficient	r	b-coefficient	r	b-coefficient
Age	-	-	-	-	0.18 / $^{\$}$0.16	-0.39* / $^{\$}$-0.35*	-	-	-	-
Sex	-	-	-	-	-	-	-	-	-	-
Height	-	-	-	-	-	-	-	-	-	-
ΔFFM	-	-	0.40	6.92***	-	-	0.18	0.57*	0.16	0.25*
Tr frequency	-	-	-	-	-	-	-	-	-	-
Tr Intensity	-	-	-	-	-	-	-	-	-	-
GPS/GPS$_{54}$	0.25	6.92**	0.41	1.38***	0.37/$^{\$}$0.31	6.60***/$^{\$}$2.01***	0.47	7.60***	0.48	3.38***
Total variance	R^2=0.06 (N=160)		R^2=0.26 (N=151)		R^2=0.16 / $^{\$}R^2$=0.13 (N=152)		R^2=0.23 (N=142)		R^2=0.25 (N=142)	

*p<0.05, **p<0.01, ***p<0.0001; FFM: fat free mass; RF: Rectus femoris; CABG: Coronary artery bypass grafting. GPS: genetic predisposition score based on phenotype-specific subset of SNPs, GPS$_{54}$: genetic predisposition score based on total number of SNPs for baseline isokinetic quadriceps strength at 180°/s. $^{\$}$: estimates for GPS$_{54}$

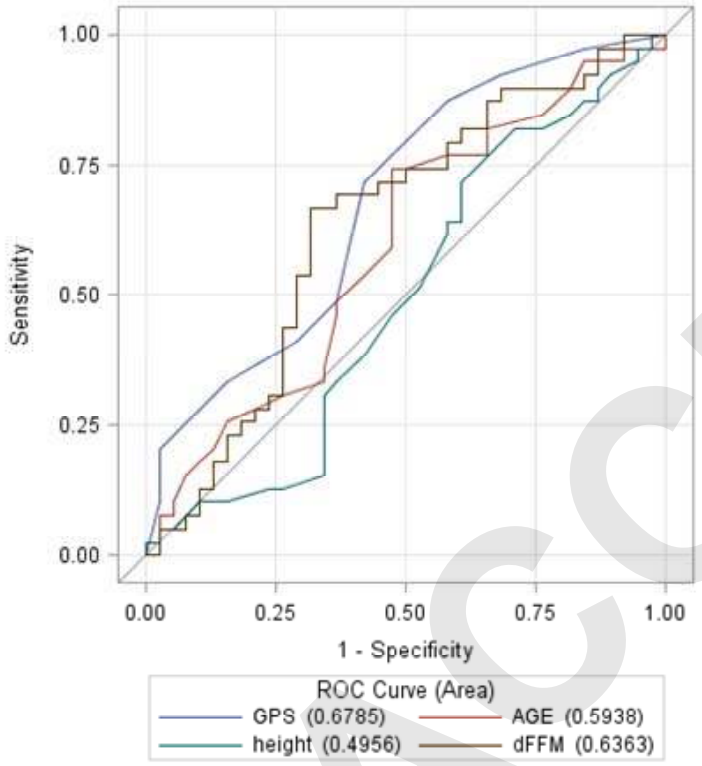
Table 5. Area under the receiver operating characteristic curve (AUC) and adjusted odds ratios for GPS score as determinant of high or low responder status for the different response phenotypes.

Reponse phenotype		AUC (95% CI)	Odds ratio (95% CI)
ΔIsometric quadriceps muscle strength	GPS	0.62 (0.51-0.72)*	2.57 (1.17-5.64)
ΔIsokinetic quadriceps strength (60°/s)	GPS	0.72 (0.61-0.84)**	2.01 (1.32-3.06)
ΔIsokinetic quadriceps strength (180°/s)	GPS *GPS_{54}*	0.70 (0.58-0.81)** *0.68 (0.56-0.80)***	1.90 (1.24-2.93) *1.23 (1.06-1.42)*
ΔMuscular endurance (extension)	GPS	0.83 (0.74-0.92)**	2.84 (1.73-4.66)
ΔRF diameter	GPS	0.85 (0.77-0.93)***	2.65 (1.76-3.97)

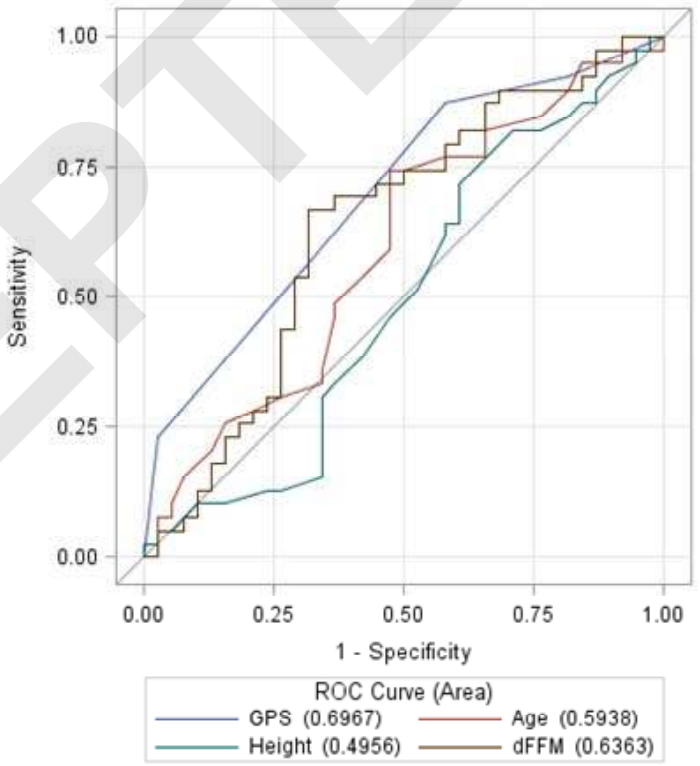
GPS: genetic predisposition score based on phenotype-specific subset of SNPs, *GPS54*: genetic predisposition score based on total number of SNPs for response to training in isokinetic quadriceps strength at 180°/s. CI: Confidence Interval; *p<0.05; **p<0.01; ***p<0.0001

Supplemental material

Figure 1: Overlay of four different models to discriminate high vs. low responder in isokinetic KES (180°/s) after training.
Model age (AUC: 0.59; 95% CI: 0.47 - 0.72)
Model height (AUC: 0.50; 95% CI: 0.36 - 0.63)
Model change in FFM (dFFM) (AUC: 0.64; 95% CI: 0.51 - 0.76)
Figure A: GPS_{54} model with 54 SNPs: AUC: 0.68; 95% CI: 0.56 - 0.80
Figure B: GPS model with 9 significant SNPs: AUC: 0.70; 95% CI: 0.58 - 0.81



A. GPS model with 54 SNPs to predict high vs. low responder on isokinetic KES at 180°/s



B. GPS model with 9 SNPs to predict high vs. low responder on isokinetic KES at 180°/s

Supplemental table 1. Selected candidate genes and SNPs

Gene abbreviation	Gene Name	Selected SNPs
Metabolism		
ACSL1	acyl-CoA synthetase long-chain family member 1	rs6552828
AMPD1	adenosine monophosphate deaminase 1	rs17602729
APOA1	apolipoprotein A-I	rs12721026
ATP1A2	ATPase, Na+/K+ transporting, alpha 2 polypeptide	rs2070704, rs1016732, rs2854248, rs2295623
CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	rs7415038, rs2296383
HIF1A	hypoxia inducible factor 1, alpha subunit	rs1957757, rs11549465, rs1709920, rs1087314, rs41508050*
RYR1	ryanodine receptor 1 (skeletal)	rs11083462^{+}, rs2229146^{+}, rs2229139, rs2228069, rs2071089
TPM1	tropomyosin 1	rs707602
Growth/differentiation factor		
ACVR1B	activin A receptor, type IB	rs2854464, rs10783486^{+}, rs11612312^{+}, rs746434, rs10783485
AKT1	v-akt murine thymoma viral oncogene homolog 1	rs10141867^{+}, rs1130214, rs33925946^{+}
DAAM1	dishevelled associated activator of morphogenesis 1	rs1956197
H19	imprinted maternally expressed transcript (RNA-gene)	rs2251375, rs4929984
IGF1	insulin-like growth factor 1	rs35767, rs2033178, rs17727841
IGF2	insulin-like growth factor 2	rs680, rs3213221
IGF2AS	insulin-like growth factor 2 antisense (RNA-gene)	rs7924316
IGFBP1	insulin-like growth factor binding protein 1	rs1065780
IGFBP3	insulin-like growth factor binding protein 3	rs2132570, rs6670, rs3110697, rs2834747, rs2854744
INHBC	inhibin, beta C	rs533975*, rs2943693
MYOD1	myogenic differentiation 1	rs3911833, rs2526547

MYOG	myogenin	rs4950877, rs2071452
MSTN/GDF-8	myostatin	rs1805086, rs7570532[+], rs2293284[+], rs3762546, rs3791783[+], rs11681628[+]
Muscle structure		
ACTN3	actinin, alpha 3	rs1815739
SVIL	supervillin	rs6481619
TTN	titin	rs10497520
Other		
GR/NR3C1	nuclear receptor subfamily 3, group C, member 1	rs6190
INS	insulin	rs689
VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	rs731236, rs4516035, rs1544410[+], rs7975232
CNTF	ciliary neurotrophic factor	rs1800169
CAMTA1	calmodulin binding transcription activator 1	rs884736
ID3	inhibitor of DNA binding 3	rs11574

* Genotyping success rate <95%, [+] high LD with other SNPs in the same gene

Supplemental material

Table 2. Overview of the significant SNPs contributing to the GPS-score for baseline and response to training phenotypes by backward regression analysis

	RF diameter	ΔRF diameter	Isom 60°	ΔIsom 60°	Isok 60°/s	ΔIsok 60°/s	Isok 180°/s	ΔIsok 180°/s	Isok 25rep	ΔIsok 25rep
4*CVR1B*_rs10783485		X								
4*CVR1B*_rs2854464		X				X		X		
4*CVR1B*_rs746434				X						
4*KT1*_rs1130214							X			
4*MPD1*_rs17602729	X		X	X	X	X	X			X
4*POA1*_rs12721026			X		X		X			
4*TP1A2*_rs1016732						X	X	X	X	X
4*TP1A2*_rs2070704									X	X
4*TP1A2*_rs2295623		X								
4*TP1A2*_rs2854248		X				X		X	X	X
*CACNA1S*_rs2296383										X
*CACNA1S*_rs7415038										X
*CNTF*_rs1800169										X
*DAAM1*_rs1956197		X	X							
*GR*_rs6190		X								X
*H19*_rs2251375						X				
*H19*_rs4929984		X								
*HIF1A*_rs11549465									X	
*ID3*_rs11574		X	X					X		
*IGF1*_rs17727841	X									
*IGF1*_rs35767								X		
*IGF2*_rs3213221	X									
*IGF2*_rs680								X		
*IGF2AS*_rs7924316								X		
*IGFBP1*_rs1065780						X				
*IGFBP3*_rs2132570							X			
*IGFBP3*_rs2854744							X			
*IGFBP3*_rs2834747								X		
*IGFBP3*_rs3110697		X								X
*IGFBP3*_rs6670	X									
*INS*_rs689								X		
*MSTN*_rs3762546		X								
*MYOD1*_rs3911833		X								
*MYOD1*_rs2526547							X			
*MYOG*_rs4950877										X
*RYR1*_rs2228069										X
*RYR1*_rs2229139							X			
*TPM1*_rs707602						X				
*TTN*_rs10497520							X			
*VDR*_rs4516035									X	
*VDR*_rs7975232	X									
N SNPs	**5**	**11**	**4**	**2**	**2**	**7**	**9**	**9**	**5**	**11**