Received Date : 10-Sep-2015

Revised Date : 20-Jan-2016

Accepted Date : 22-Feb-2016

Article type : Original Paper

GWAS Analysis of Hand Grip and Lower Body Strength in Older Adults in the CHARGE Consortium

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Running Title: Genome-wide association of strength in older adults

Keywords: genome-wide association, muscle strength, aging, older adults, meta-analysis, SNP

AGING CELL AUTHOR CHECKLIST.

Title	**GWAS Analysis of Hand Grip and Lower Body Strength in Older Adults in the CHARGE Consortium**
Authors	There are 66 co-authors to this manuscript, as part of the Cohorts for Heart and Aging Research Genomic Epidemiology (CHARGE) Consortium. Author names, affiliations and email addresses are attached.
Manuscript Type	Primary research article
Total Character Count (including spaces)[1]	36,874

Word count of Summary[2]	250						
Number of papers cited in the References[3]	32						
Listing of all Tables (Table1, Table 2 etc)[4]							
Table 1	Top SNP in each region with suggestive association with hand grip in discovery and replication sets						
Table 2	Functional Annotations of the GWAS SNPs by histone marks, ChIP-seq and DNase-seq from ENCODE Project and Epigenetic Roadmap Project						
Figure specifications (please complete one row per figure)[5] ***Figure no.***	Colour ***(yes/no)***	Greyscale ***(yes/no)***	Black and white ***(yes/no)***	Single column (80mm) ***(yes/no)***	Double column (167mm) ***(yes/no)***	Size of figure at full scale (mm x mm) ***(insert details)***	Smallest font size used in the figure at full scale (minimum 6pt) ***(insert details)***
Figure 1a	yes	no	no	no	yes	84.3mmx 131.8mm	7 pt
Figure 1b	yes	no	no	no	yes	84.3mmx 131.8mm	7 pt
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Abstract

Background. Decline in muscle strength with aging is an important predictor of health trajectory in the elderly. Several factors, including genetics, are proposed contributors to variability in muscle strength.

Methods. To identify genetic contributors to muscle strength, a meta-analysis of genome-wide association studies of hand grip was conducted. Grip strength was measured using a handheld dynamometer in 27,581 individuals of European descent over 65 years of age from 14 cohort studies. Genome-wide association analysis was conducted on ~2.5 million imputed and genotyped variants (SNPs). Replication of the most significant findings was conducted using data from 6,393 individuals from three cohorts. GWAS of lower body strength was also characterized in a subset of cohorts.

Results. Two genome-wide significant (p-value< $5x10^{-8}$) and 39 suggestive (p-value< $5x10^{-5}$) associations were observed from meta-analysis of the discovery cohorts. After meta-analysis with replication cohorts, genome-wide significant association was observed for rs752045 on chromosome 8 (β=0.47, SE=0.08, p-value= $5.20x10^{-10}$). This SNP is mapped to an intergenic region and is located within an accessible chromatin region (DNase hypersensitivity site) in skeletal muscle myotubes differentiated from the human skeletal muscle myoblasts cell line. This locus alters a binding motif of the CCAAT/enhancer-binding protein β (CEBPB) that is implicated in muscle repair mechanisms. GWAS of lower body strength did not yield significant results.

Conclusion. A common genetic variant in a chromosomal region that regulates myotube differentiation and muscle repair may contribute to variability in grip strength in the elderly. Further studies are needed to uncover the mechanisms that link this genetic variant with muscle strength.

Introduction

Loss of muscle strength, "dynapenia," is a common characteristic of aging and is associated with increased risk of frailty, falls, hospitalizations and mortality(Marsh et al. 2011; Xue et al. 2010; Moreland et al. 2004). In particular, hand grip strength is found to be predictive of overall and exceptional survival (Willcox et al. 2006) and other key age-related outcomes (McLean et al. 2014; Marsh et al. 2011). For

example, poor hand grip strength among healthy middle-aged subjects was found to significantly predict functional limitations and disability 25 years later(Rantanen et al. 1999). The biology that drives muscle strength decline is complex, with hormonal changes, inflammatory pathway activation, mitochondrial physiology, malnutrition, and exercise all likely playing a role (Gonzalez-Freire et al. 2014; Walston 2012). Further identification of biologically relevant pathways that influence muscle strength maintenance and decline could be important in the development of future treatment or prevention strategies. Hence, genetic approaches to the identification of novel biology may be helpful.

The heritability of muscle strength in older adults has been estimated to be between 40 and 65% (Matteini et al. 2010; Tiainen et al. 2004). Previously published reports have been limited to candidate gene analyses in small cohorts of older adults (Arking et al. 2006; Serena Dato et al. 2012; S Dato et al. 2014). These studies have highlighted potentially important biologic pathways associated with hand grip strength but have been unable to identify a significant replicated locus. In spite of the importance of this phenotype for health and function, to date, no genome-wide association study (GWAS) has been published on hand grip strength.

Because of the large, well characterized cohorts represented in the CHARGE consortium, grip strength and genome-wide genotype data from 17 cohort studies (14 discovery and 3 replication cohorts) of older adults were included in this meta-analysis. We sought to identify potential genetic influences that underlie measures of strength in adults age 65 and older.

Results

Discovery Set

A genome-wide meta-analysis included 27,581 community-dwelling men and women of European ancestry from a discovery set of 14 participating cohorts. On average across the cohorts, there were 2,725,778 SNPs analyzed, with SNPs analyzed per cohort ranging from 2,332,998 to 4,930,728. Sample size and cohort characteristics are found in **Supplemental Table S1**. There were no significant differences in age, strength or gender distributions between the discovery and replication cohorts. Q-Q and Manhattan plots are shown in **Supplemental Figures S1-S2**. In the discovery set meta-analysis, 2 SNPs reached genome-wide significance (rs3121278 chr10: p-value = 2.68×10^{-8} and rs752045 chr8: p-value = 3.09×10^{-8}). An additional 39 SNPs reached suggestive significance in 8 regions on chromosomes 1 (1 SNP), 5 (2 highly correlated SNPs), 7 (7 SNPs), 8p23 (2 SNPs), 8q12 (14 SNPs), 10 (11 SNPs), 11 (3

SNPs), and 12 (1 SNP) (**Supplemental Table S4**). Chromosomes 1, 5 and 12 loci were not pursued in subsequent analysis due to the fact that there was only a single SNP in the locus with suggestive significance. The five regions that remained suggestive are intergenic. **Table 1** shows the lead SNP per region with meta-analyzed results from discovery, replication as well as combined discovery and replication cohorts. Regional plots (created using Locus zoom http://csg.sph.umich.edu/locuszoom/) are displayed in **Figure 1**.

Replication Cohorts

Significant and suggestive SNPs on chromosomes 7, 8p23, 8q12, 10 and 11 were tested in the replication cohorts and in the combined discovery/replication set. First, the most significant discovery SNP, rs3121278, was significant in the replication ($p\text{-value}_{rep}$=0.01), yet the effect was in the opposite direction from the discovery set resulting in a decrease in significance in the combined analysis ($p\text{-value}_{disc+rep}$= $6.18\text{x}10^{-5}$). Next, SNP rs752045 on chromosome 8p23 showed an association with grip strength upon replication and the direction was consistent with that of the discovery set ($p\text{-value}_{rep}$ = $4.80\text{x}10^{-3}$), leading to increased significance in the combined set ($p\text{-value}_{disc+rep}$= $5.20\text{x}10^{-10}$). Likewise, the second best SNP on chromosome 11 rs11235843 showed consistent direction and magnitude of effect in the replication cohorts ($p\text{-value}_{rep}$ = $4.70\text{x}10^{-2}$) and significance in the combined set increased ($p\text{-value}_{disc+rep}$= $1.19\text{x}10^{-6}$), although it still failed to reach the preset threshold for genome-wide significance. Lastly, SNPs in suggestive areas of chromosome 7 and 8q12 showed no effect upon replication. Combined results from these regions showed slightly decreased significance, although p-values were still in the range of suggestive association.

Lower body strength

A meta-analysis of genome-wide association analysis of lower body strength was conducted as an secondary muscle strength phenotype. There were no genome-wide significant associations identified **(Supplemental Figure S3)**. The most significant association was observed for rs16831 on chr11 (P=$6.07\text{x}10^{-7}$; **Supplemental Table S5)**. The closest gene was an uncharacterized gene LOC101929497 approximately 187Mb away. We also looked up the top signals from the grip strength analysis, however these loci were not significantly associated with lower body strength (P>0.05; **Supplemental Table S6**).

Functional Annotation

Results from the functional annotation analysis are shown in **Table 2**. SNPs in the chromosome 7, 10 and 11 regions showed direct links to the regulatory chromatin states in muscle tissue or accessible chromatin states according to ChIP-seq and DNase-seq data. First, top discovery SNPs rs3121278 and rs752045 were located within accessible chromatin regions in skeletal muscle myotubes differentiated from the skeletal muscle myoblast (HSMM) cell lines. The suggestive SNP rs2796549 also was located within an accessible chromatin region in skeletal muscle myoblasts. Next, the three suggestive chromosome 11 SNPs localized to motifs predicted to be regulatory elements, promoters and enhancers, in skeletal muscle myoblasts. The top suggestive chromosome 7 SNP rs1819054 was not shown to affect gene regulatory elements in muscle-related tissues; however, three SNPs within the region were predicted to localize in regulatory enhancers in skeletal muscle myoblasts. This chromosome 7 locus was significantly enriched for enhancer/promoter elements in muscle cells compared to other muscle types (p-value=9.9×10^{-5}). Suggestive SNPs on chromosomes 7, 8p12, and 10 were also predicted to alter binding motifs of the CCAAT/enhancer-binding protein beta, delta and gamma family (CEBPB, CEBPD, CEBPG), zinc finger protein 263 (ZNF263) and the Nuclear factor kappa beta (NFkB).

eQTL Analysis

The top five SNPs listed in **Table 1** were queried as index SNP in skeletal muscle and brain tissue eQTL. For the locus on chromosome 10 (rs3121278), a proxy SNP rs3121327 (r^2=0.87) was significantly associated with gene transcript zinc finger protein 33B (*ZNF33B*) in prefrontal cortex tissue. No other associations were observed for the other loci queried.

Discussion

The combined discovery and replication meta-analysis resulted in increased significance in the chr8p23 locus, exceeding genome-wide significance (rs752045, p-value=3.18×10^{-10} and rs890022, p-value=4.80×10^{-8}). We conducted a genome-wide association analysis of lower body strength in a smaller sample as a second trait for muscle strength. However, there were no significant genetic associations observed for lower body strength and the results did not confirm the top signals from the grip strength analysis.

The chromosome 8p23 locus - rs752045 - is over 500 kb away from the closest gene genome-wide significant association. However, according to the ENCODE's DNase-I hypersensitivity data, rs752045 is located in an accessible chromatin region, indicating possible regulatory activities in skeletal muscle

myotubes differentiated from the HSMM cell line. This SNP alters a binding motif of the CCAAT/enhancer-binding protein beta (CEBPB). The effect allele (G) decreases a score developed to define the effect of variants on regulatory motifs (the position weight matrix (PWM) score). In this case the PWM score for CEBPB decreased from 11.6 to -0.2, indicating a prediction of decreased binding affinity of CEBPB. The PWM scores were reported as part of the HaploReg database (http://www.broadinstitute.org/mammals/haploreg/detail_v4.1.php?query=&id=rs752045). CEBPB is a transcription factor that regulates genes for inflammatory responses, including the IL-1 response element in the *IL6* gene (Harries et al. 2012). IL-6 levels are strongly related to muscle strength, functional decline and sarcopenia in older adults (Kilgour et al. 2013; Cesari et al. 2004). CEBPB is also important in macrophage function, which plays a crucial role in normal skeletal muscle repair (Rahman et al. 2012). In addition, expression of CEBPB in blood leukocytes has been positively associated with muscle strength in humans, further supporting the possible link between gene variants and a decline in skeletal muscle function in older age groups (Ruffell et al. 2009).

SNPs in associated regions on chromosomes 7 and 11 are proximal to genes *PLEKHB1* (chr11), *FAM3C* (chr7) and *WNT16* (chr7), the latter has been associated with bone mineral density, osteoporosis and fracture risk. Both loci represent promoters or enhancers in regulatory chromatin states in skeletal muscle myoblasts in ENCODE and Epigenetic Roadmap data. PLEKHB1 protein interacts with ACVR1, which is involved in fibrodysplasia ossificans progressiva (FOP), a rare congenital disorder that causes bone formation in muscles, tendons, ligaments and connective tissues. Additionally, SNPs on the chromosome 7 locus were predicted to alter binding motifs of the CCAAT/enhancer-binding protein beta, delta and gamma family (CEBPB, CEBPD, CEBPG) and the Nuclear factor kappa β (NFkB). In addition to the *CEBPB* association to muscle discussed above, *CEBPD* has also been linked to differential expression of myostatin, a skeletal muscle inhibitory factor that can lead to muscle strength declines (Allen et al. 2010). CEBPG likely plays a role in cell growth arrest in the setting of inflammation activation (Huggins et al. 2013). NFkB is the nuclear transcription factor that acts as a gate-keeping molecule for activation of inflammatory signaling (Ershler 2007; Guttridge et al. 2000). Subtle alteration in expression of these factors may well alter muscle tissue maintenance with aging and would in turn lead to grip strength declines.

Last, the suggestive region of chromosome 10 is 20 kb away from the *BMS1L* gene, a ribosome assembly protein which has no known function in skeletal muscle. This group of three SNPs also had relevant data

from ENCODE indicating that DNase hypersensitive sites were found in skeletal muscle myotubes, in particular those differentiated from HSMM cell lines and osteoblasts.

There are several strengths to this study. First, we have identified 14 cohorts including 27,581 older adults that have appropriate hand grip strength measurements and genotypes necessary to perform a study of this kind. Next, the ability to explore potential findings with the ENCODE data provides an important biological window into the potential relevance of the genetic findings. There are potential limitations to this study as well. First, a cross-sectional, one time hand grip or lower body strength measure may not be the best phenotypic measurement to capture age-related strength decline as a phenotype. Although the lower body strength analysis was consistent with grip strength, due to sample size restrictions, the age cutoff for lower body strength was set at 50 years of age. The correlation between grip and lower body strength has been reported to be in the range of 0.4-0.6, suggesting that both measure the same construct of muscle strength (Bohannon et al. 2012).

This cross-sectional study was designed to determine genetic variants associated with grip strength in persons over the age of 65 years. Strength in old age is thought to be a reflection of both the peak strength as well as rate of decline. Similarly, cross-sectional analysis with phenotypes such as bone density or cognitive performance still have been useful for understanding rate of decline with age. Here we studied individuals over 65 years of age, thus the majority are predicted to have already entered the decline phase. Future genetic studies should consider examining changes in muscle strength to focus on the potential determinants of age related decreases that are commonly observed with aging, as trajectories of strength decline were not widely available among these cohorts

Despite limitations, these results suggest biologically plausibility. Chromosome 7 locus was significantly enriched for enhancer/promoter elements in muscle cells compared to other muscle types. C/EBP transcription factors have been linked to a number of metabolic and inflammatory processes that would be expected to influence skeletal muscle, and have been previously implicated in other cohorts. These findings provide additional rationale for the further study of C/EBP related pathways and their overall influence in the development of dynapenia in older adults. Future studies should follow up these findings to determine if there are potential epigenetic changes, or even whether there are significant CEBPB expression differences in skeletal muscle samples between young and old humans.

Experimental Procedures

Subjects

The discovery phase of this GWAS was conducted on 27,581 subjects from the following 14 participating studies of the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE). the Age, Gene/Environment Susceptibility Study (AGES); the Cardiovascular Health Study (CHS): the Framingham Heart Study (FHS); the Health, Aging, and Body Composition (Health ABC) Study; the Health and Retirement Study (HRS); the InCHIANTI Study; the Lothian Birth Cohort Studies (1921 and 1936); the Osteoporotic Fractures in Men Study (MrOS); Religious Order Study, Memory and Aging Project (MAP/ROS); the Study of Health in Pomerania (SHIP); the Study of Osteoporotic Fractures (SOF); the Tasmanian Study of Cognition and Gait (TasCog); the Twins UK Study. Replication cohorts contributed 6,393 subjects from three cohorts, the Atherosclerosis Risk in Communities Study (ARIC) and the Rotterdam Studies I and II. Detailed description of each cohort and references are included in the Supplemental Materials. Each cohort's study protocol was reviewed and approved by their respective institutional review board.

In parallel to grip strength analysis, a GWAS analysis of lower body strength was conducted as an additional measure of muscle strength in 9,822 individuals over the age of 50 years from 7 studies: AGES, Baltimore Longitudinal Study on Aging (BLSA), InCHIANTI, CHS, FHS, Health ABC, and MAP/ROS.

Phenotyping

All participants with at least one recorded grip strength measurement (kg) **(Supplemental Table S1)** were included in the analysis. The primary outcome was defined as the maximal value across available trials. Exclusion criteria for grip strength analysis included age less than 65 years, non-Caucasian origin via self-report or identical-by-state (IBS) clustering of the GWAS data, and missing grip strength data. Additional exclusion based on self-reported pain, surgery or osteoarthritis in the dominant hand was considered. However, since adequate data across all cohorts were not available, these exclusions were not implemented in this analysis. Hand grip was employed as a non-transformed, continuous trait.

For lower body strength, all studies used performance based assessment methods reporting measures in kg or in Newton-meter **(Supplemental Table S2)**. If multiple examinations were performed, the maximum measurement was used. Exclusion for lower body strength analysis was consistent with grip strength; however, due to sample size restrictions, the age cutoff was set at 50 years of age. Lower leg strength was analyzed as a non-transformed, continuous trait.

Additional variables used in this study included gender, age, standing height and weight for both grip and lower body strength. Each of these characteristics was collected with hand grip and/or lower body strength according to study-specific protocols.

Genotyping

Each cohort performed its own genome-wide genotyping and genotype imputation based on NCBI Build 36 (http://www.ncbi.nlm.nih.gov/SNP/). Supplemental Table S3 summarizes genotyping platform, imputation methods, quality control methods and final SNP count per cohort. Results are reported for each SNP for as many cohorts as were available via genotyping and imputation.

Statistical Analysis

Multiple linear regression models were built for genotyped and imputed SNPs on maximal grip strength (kg), adjusted for age, gender, height, weight, study site (when necessary), and principal components to control for population stratification (Price et al. 2006). An additive model with the count of the number of variant alleles was used for all analyses. Hand grip strength was used as a continuous trait and the regression results reflect an increase or decrease in strength (kg) per additive allele. Test statistics for genome-wide association analysis were combined using METAL (Willer, Li, and Abecasis 2010). Inverse variance weighted meta-analysis was performed using a fixed effects model of β estimates and standard errors from each cohort. In the meta-analysis of discovery GWAS, between-study heterogeneity was tested using Cochran's Q test as implemented in METAL. A threshold of p-value less than $5x10^{-8}$ was utilized to determine genome-wide statistical significance, while p-values less than $1x10^{-5}$ were considered suggestive. SNPs that met these significance thresholds were then evaluated in a set of 3 replications cohorts, as well as analyzed jointly in discovery and replication cohorts (n=33,974).

For the leg strength analysis, since the unit of measure differed by cohort (kg or Nm), a sample-size weighted meta-analysis was conducted where an arbitrary reference allele is selected and a z-statistic summarizing the magnitude and the direction of effect relative to the reference allele was calculated and weighted by the square root of the sample size of each study. Thresholds for statistical significance set for the hand grip analysis were utilized for the leg strength results as well.

Using the HaploReg tool (http://compbio.mit.edu/HaploReg.), we annotated potential regulatory functions of our GWAS SNPs and loci based on experimental epigenetic data, including open chromatin and histone modifications, and transcription factor binding sites in human cell lines and tissues (Ward

and Kellis 2012). First, we constructed haplotype blocks for GWAS most significant, or lead, SNPs and SNPs in high linkage disequilibrium (LD, $r^2 > 0.8$) with GWAS lead SNPs. Then, we identified regulatory elements including enhancers and promoters estimated by chromatin states in the haplotype blocks across 98 healthy human tissues/normal cell lines available in the ENCODE Project and the Epigenomics Roadmap Project (Encode and Consortium 2011; Chadwick 2012). The regulatory elements were annotated by an algorithm named ChromHMM and data were downloaded from HaploReg3 (Ernst and Kellis 2012; Ward and Kellis 2012). To evaluate whether GWAS loci were enriched with regulatory elements and corresponded to the DNase I hypersensitive sites (DHSs) in muscle tissues, we performed a promoter/enhancer enrichment analysis using a hypergeometric test to compare the abundance of regulatory elements in muscle tissues (9 relevant muscle tissues/cell lines) to non-muscle tissues (89 tissues/cell lines) in the haplotype blocks of a GWAS locus. A permutation was performed to correct for multiple testing. Permutation p-values less than 0.05 were considered statistically significant.

Expression quantitative trait loci (eQTL) analysis

Proxy SNPs in linkage disequilibrium ($r^2>0.8$) in European ancestry populations were identified for hand grip for the top five most significant SNPs as the lead SNPs using SNAP (Johnson et al. 2008). Index SNPs and proxies were identified in a collected database of expression SNP (eSNP) results. The collected eSNP results met criteria for statistical thresholds for association with gene transcript levels as described in the original papers. A general overview of a subset of >50 eQTL studies has been published (Zhang et al. 2014), with specific citations for >100 studies. For the current query, we focused our search to skeletal muscle and brain tissue (Zhang et al. 2014; Keildson et al. 2014). Details on tissue samples can be found in the Supplemental Text.

Acknowledgements

The Longevity Consortium, funded by the National Institute of Aging, grant number U19 AG023122, provided administrative resources for this work. Dr. Bruce Psaty serves on the DSMB for a clinical trial of a device funded by the manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. Dr. Kiel has received grant funding from Eli Lilly, and has served on a scientific advisory board from Novartis. There are no additional conflicts of interest.

Author Contributions

All authors were involved in data collection, study design, methods development, and review and final approval of the manuscript. In addition, AMM, TT, WCC, JDE, ADJ, AMA, MLC, GD, DSE, BH, KL, KLL, MM, AVS, JAS, AT, and LY, DEA, ASB, AH, YH, FR, AU were involved in data analysis. AMM, TT, DK, GA, WCC, ADE, ADJ, ABN, JDW, DPK and JMM were responsible for writing the manuscript.

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565 **Supporting Information**

566 1) Supplemental Text (**matteini_supp_text.docx**): Contains detailed description of the discovery
567 cohorts, tissue sample description, funding/support information per cohort and supplemental
568 references
569 2) Supplemental Tables (**matteini_supp_tables_resub.docx**): Contains Tables S1-S6
570 a. Supplemental Table S1. Details of Hand Grip Measure Collection per Cohort
571 b. Supplemental Table S2. Assessment methods and cohort descriptive for lower leg
572 strength analysis.
573 c. Supplemental Table S3. Genotyping and Data Cleaning Details per Discovery Cohort

d. Supplemental Table S4. Top SNPs from the meta-analysis of grip strength genome-wide associations in 14 discovery cohorts.

e. Supplemental Table S5. Most significant non-redundant association from meta-analysis of lower body strength in 9,822 individuals

f. Supplemental Table S6. Associations from meta-analysis of lower body strength for the top signals from the grip strength meta-analysis.

3) Supplemental Figures (**matteini_supp_figures.docx)**: Contains Figures S1-S3

a. Supplemental Figure S1. Quantile-Quantile plot of expected versus observed –log10 p-values for meta-analysis of genome-wide association of grip strength.

b. Supplemental Figure S2. Genome-wide scans of grip strength of CHARGE cohorts. Genome-wide associations of grip strength for ~2.5 million imputed and genotyped HapMap SNPs.

Supplemental Figure S3. Quantile-Quantile plot of expected versus observed –log10 p-values for meta-analysis of genome-wide association of leg strength.

SNP	Chr	Position (hg18)	Effect /Non-effect Allele	Frequency of Effect Allele	Closest Gene Structure	Regulatory Motifs altered	Proximal Gene(s)	Distance to DNase-seq (kb)	Discovery Set (n=28,547) Muscle-related tissue Beta (SE)	Discovery Set (n=28,547) Muscle-related tissue P-value$_{disc}$	Replication Set (n=6,363) Muscle-related regulatory chromatin states Beta (SE)	Replication Set (n=6,363) Muscle-related regulatory chromatin states P-value$_{rep}$	Enhancer/promoter enrichment in muscle cells* Discovery + Replication Set (n=34,910) # SNPs in LD‡	Enhancer/promoter enrichment in muscle cells* Discovery + Replication Set (n=34,910) Beta (SE)	Enhancer/promoter enrichment in muscle cells* Discovery + Replication Set (n=34,910) Permutation P-Value P-values§ $_{disc+rep}$
rs1819054	7	120926996	G/A	0.40	Intergenic	*FAM3C*		103	0.27 (0.06)	$8.23x10^{-7}$	0.15 (0.13)	0.24	0.25	0.25 (0.05)	$6.13 x10^{-7}$
						PTPRZ1		37							
rs752045	8	5937538	G/A	0.18	Intergenic	*CSMD1*		1,098	0.47 (0.09)	$3.09x10^{-8}$	0.45 (0.16)	4.80E-03	0.47	0.47 (0.08)	$5.20 x10^{-10}$
						LOC100287015		311							
rs1508086	8	57980052	T/C	0.44	Intergenic	*LINC00968*		345	0.25 (0.05)	$2.71 x10^{-6}$	0.09 (0.12)	0.45	0.22	0.22 (0.05)	$4.21 x10^{-6}$
						IMPAD1		53							
rs3121278	10	42695652	T/G	0.18	Intergenic	*BMS1L*		45	-0.39 (0.07)	$2.68 x10^{-8}$	0.38 (0.15)	1.00E-02	-0.26	-0.26 (0.06)	$6.18 x10^{-5}$
						LINC01264		98							
rs11235843	11	73051644	A/G	0.10	Downstream	*PLEKHB1*			-0.38 (0.08)	$9.23 x10^{-6}$	-0.40 (0.20)	4.70E-02	-0.38	-0.38 (0.08)	$1.19 x10^{-6}$

Table 1: Top SNP in each region with suggestive association with hand grip in discovery and replication sets

Table 2. . Functional Annotations of the GWAS SNPs by histone marks, ChIP-seq and DNase-seq from ENCODE Project and Epigenetic Roadmap Project

rs3857836	7	120931488	Intergeninc	FAM3C (108) PTPRZ1 (369)			weak enhancer in skeletal muscle myoblasts[1]	33	**9.9×10^{-5}**
rs11761290	7	120932659	Intergenic	FAM3C (109) PTPRZ1 (368)			strong enhancer in skeletal muscle myoblasts[1] and skeletal muscle[2]	33	**9.9×10^{-5}**
rs10228676	7	120932913	Intergenic	FAM3C (109) PTPRZ1 (368)	CEBPG; Hoxa5		weak enhancer in skeletal muscle myoblasts[1]	33	**9.9×10^{-5}**
rs1013711	7	120943334	Intergenic	FAM3C (120) PTPRZ1 (357)	CEBPB; CEBPD		weak enhancer in colon smooth muscle[2]	8	**9.9×10^{-5}**
rs1528351	7	120955111	Intergenic	FAM3C (131) PTPRZ1 (345)	Nkx2				
rs752045	8	5937538	Intergenic	CSMD1 (1,098) LOC100287015 (311)	CEBPB; GR	skeletal muscle myotubes differentiated from HSMM cell line		12	1
rs2142991	10	42661111	Intergenic	BMS1 (11) LINC01264 (133)	CEBPB; CTCF; Smad4			40	1
rs2796549	10	42686043	Intergenic	BMS1 (36) LINC01264 (108)		skeletal muscle myoblasts; aortic smooth muscle		1	1
rs3121278	10	42695652	Intergenic	BMS1 (45) LINC01264 (99)	GR	skeletal muscle myotubes differentiated from HSMM cell line; osteoblasts		35	1
rs7128512	11	73049947	Intronic	PLEKHB1	Roaz		weak promoter in skeletal muscle myoblasts[1]	3	0.266
rs6590	11	73051200	UTR3	PLEKHB1	NRSF		enhancer in skeletal muscle[2]; weak	15	0.057

rs11235843	11	73051644	Downstream	PLEKHB1	Nrf-2	enhancer in stomach smooth muscle[2] enhancer in skeletal muscle[2]; weak enhancer in stomach smooth muscle[2]	15	0.057

Table 2 (continued)

*** Enhancer/promoter enrichment in muscle cells including SNPs in linkage disequilibrium with GWAS lead SNPs**

[a] the change in log-odds (LOD) scores of Regulatory motifs larger than 10 were reported ; [1]Annotation from ENCODE Database; [2] Annotation from Epigenetic Roadmap

[‡]SNPs in LD: Number of SNPs in LD ($r^2 \geq 0.8$ and MAF ≥ 1%, based on 1000 Genome Project) with the lead GWAS SNP in each locus

[§]Permutation p-values corrected for multiple testing: This analysis included all SNPs in LD with the GWS lead SNPs. Multiple testing corrected permutation p-values < 0.05 are considered statistically significant.

Figure 1 Legend. Regional association plots for the most significant associations from the meta-analysis of hand grip strength in the discovery set. The figures display –log10 p-values for SNPs that passed quality control for the analysis of hand grip strength for locus on (A) chromosome 7, (B) chromosome 8p23, (C) chromosome 8q12, (D) chromosome 10, and (E) chromosome 11. The degree of linkage disequilibrium (r^2) is displayed as shades of gray in the following categories: r2 $\geq$ 0.8, $\geq$ 0.6, $\geq$0.4, $\geq$0.2, and $\geq$0.

Figure 1A) Chromosome 7

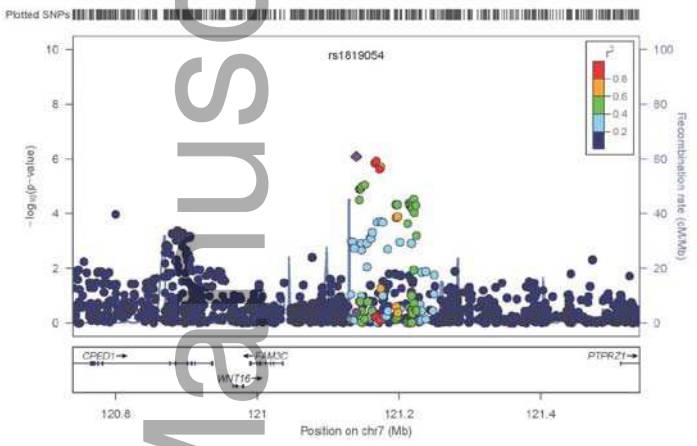

Figure 1B) Chromosome 8p23

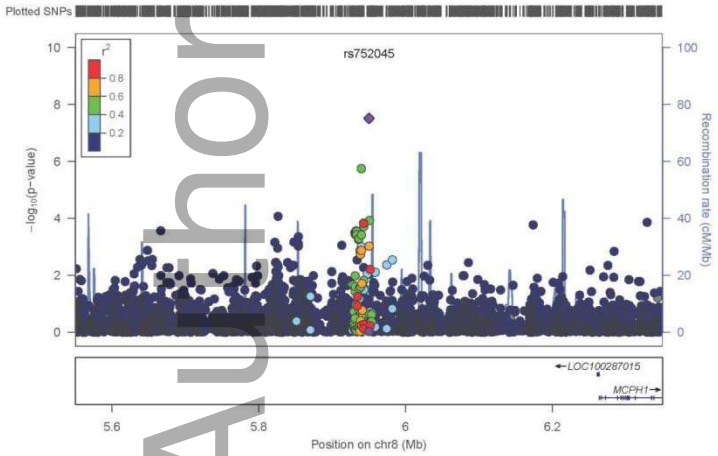

Figure 1C) Chromsome 8q12

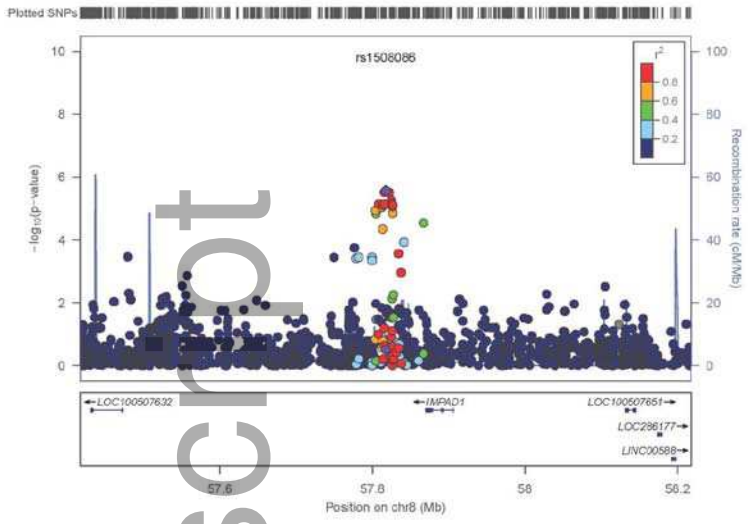

Figure 1D) Chromosome 10

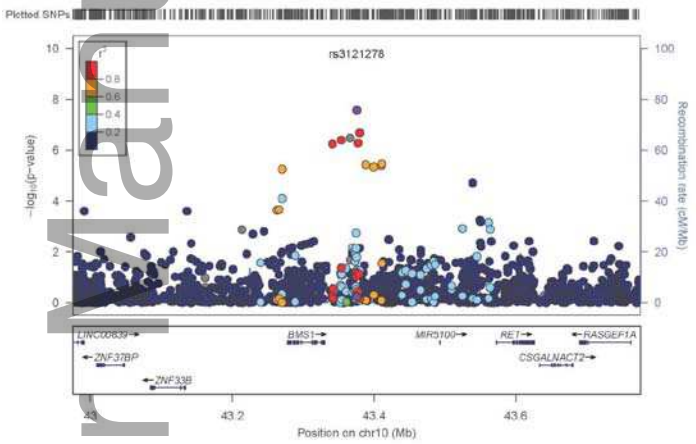

Figure 1E) Chromosome 11