Genetics of Maximal Walking Speed and Skeletal Muscle Characteristics in Older Women

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The aim of this study was to examine whether maximal walking speed, maximal isometric muscle strength, leg extensor power and lower leg muscle cross-sectional area (CSA) shared a genetic effect in common. In addition, we wanted to identify the chromosomal areas linked to maximal walking speed and these muscle characteristics and also investigate whether maximal walking speed and these three skeletal muscle characteristics are regulated by the same chromosomal areas. We studied 217 monozygotic (MZ) and dizygotic (DZ) female twin pairs aged 66 to 75 years in the Finnish Twin Study on Aging study. The DZ pairs (94) were genotyped for 397 microsatellite markers in 22 autosomes and X-chromosome. Genetic modeling showed that, muscle CSA, strength, power and walking speed shared a genetic effect in common which accounted for 7% of the variation in CSA, 51% in strength, 37% in power and 35% in walking speed. The results of an explorative multipoint linkage analysis suggested that the highest LOD score found for each phenotype was 2.41 for walking speed on chromosome 13q22.1, 2.14 for strength on chromosome 15q14, 2.84 for power on chromosome 8q24.23, and 2.93 for muscle CSA on chromosome 20q13.31. Also a suggestive LOD score, 2.68, for muscle CSA was found on chromosome 9q34.3. The chromosomal areas of a suggestive linkage for strength and power partly overlapped LOD scores higher than 1.0 being seen for these phenotypes on chromosome 15. The present study was the first genome-wide linkage analysis to be conducted for these multifactorial and clinically important phenotypes underlying functional independence in older women.

Walking ability is one of the main determinants of functional independence among older persons. Maximal walking speed decreases with aging (Bohannon, 1997). Mean maximal walking speed among 30-year-old women is 2.3 m/s, while the maximal walking speed among 60- to 70-year-old women decreases to 1.7 m/s (Bohannon, 1997; Bohannon et al., 1996). Reduced walking speed increases risk of mobility limitations and disability, particularly among older people (Guralnik et al., 1995, 2000; Onder et al., 2005).

The muscle strength and power of the lower extremities are strongly related to walking speed (Bean et al., 2003; Bohannon et al., 1996; Foldvari et al., 2000; Lauretani et al., 2003; Rantanen & Avela, 1997; Rantanen, Guralnik, et al., 1998). Maximal isometric muscle strength has been defined as the maximum voluntary contraction performed at a specific joint angle against an unyielding resistance (Enoka, 1994). Muscle power is the product of force generation and speed of muscle contraction and refers to the ability of the neuromuscular system to produce the greatest possible force as fast as possible (Enoka, 1994). Muscle strength (Frontera et al., 1991; Rantanen, Masaki, et al., 1998) and power (Frontera et al., 2000) decrease after 30 years of age and the decrease accelerates around the age of 60 due to diminished use as well as structural and functional

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changes in the neuromuscular system with aging (Wang et al., 1999). Also muscle cross-sectional area reaches its peak at around 30 years of age (Frontera et al., 1991). Muscle cross-sectional area and muscle strength correlate strongly. The loss of muscle mass with age, termed sarcopenia, is a multifactorial age-related phenomenon that impairs physical functions, including walking speed (Rosenberg, 1989; Roubenoff, 2000).

Our earlier studies have shown that among older female twins, genetic effects accounted for 17 to 34 % of the total variance in maximal walking speed (Pajala et al., 2005; Tiainen et al., 2007) and one third of the variance in muscle strength and power (Tiainen et al., 2004, 2005). The results of genetic modeling have also indicated that in part the same genes are related to muscle strength, muscle power and maximal walking speed (Tiainen et al., 2007). Genetic effects in common accounted for 52% of the variation in isometric knee extensor strength, 36% in leg extensor power and 34% in maximal walking speed (Tiainen et al., 2007). Results of different studies indicate that muscle cross-sectional area (CSA) seems to be under rather strong genetic regulation. Genetic effects accounted for 66% to 92% of the variation in CSA depending on the muscle measured and subjects' age (Loos et al., 1997; Thomis et al., 1997). Overlap between muscle CSA, muscle strength and power remains to be elucidated.

As far as we know, whole genome linkage analysis has not been performed either for walking speed or for muscle strength, muscle power and muscle CSA. In the study by Huygens et al. (2004) nine candidate genes from myostatin pathway were analyzed. Results of the linkage analyses showed that in chromosome 12 and 13 might be genes related to muscle strength among young healthy men. However, thus far research has been focused on the association studies between single candidate genes and muscle phenotypes. Obviously, walking speed and the three muscle characteristics studied here are complex multifactorial phenotypes that are affected by many different genes. According to the review by Rankinen et al. (2006), candidate genes which may have associations with muscle strength, power and muscle mass include myostatin (GDF8), ciliary neurotrophic factor (CNTF), the ciliary neurotrophic factor receptor (CNTFR), insulin-like growth factor 2 (IGF2), vitamin D receptor (VDR), type 1 collagen alpha 1 (COL1A1), angiotensin converting enzyme (ACE), α-actinin 3 (ACTN3), cyctic fibrosis transmembrane conductance regulator (CFTR), deiodinase, iodothyronine type 1 (DIO1), myosin light polypeptide kinase (MYLK), nuclear receptor subfamily 3 (NR3C1), and tumor necrosis factor gene (TNF). Candidate genes for walking speed have not been proposed. Genetic variability and the importance of specific genes and their expressions vary over time/gender and in the interaction with individual and environmental factors. Therefore, it is important to identify the chromosomal areas and candidate genes related to skeletal muscle characteristics and walking speed among people at older ages.

The aim of the present study was to examine whether maximal walking speed, maximal isometric knee extensor strength, leg extensor power and lower leg muscle CSA shared genetic effects in common in older women. In addition, we wanted to identify the chromosomal areas linked to maximal walking speed and these muscle characteristics and also to investigate whether maximal walking speed and these three skeletal muscle characteristics are regulated by the same chromosomal areas.

Materials and Methods

Subjects

This study is part of the Finnish Twin Study on Aging (FITSA), a study of genetic and environmental effects on the disablement process in older female twins. The recruitment procedures, methods used to determine zygosity and characteristics of the participants have been described in detail elsewhere (Tiainen et al., 2004). Briefly, the participants were recruited from the Finnish Twin Cohort, which consisted of 13,888 adult twin pairs, first studied in 1975 (Kaprio & Koskenvuo, 2002; Kaprio et al., 1978). Among them were originally 1260 respondent female twin pairs born between 1924 and 1937. Of this group, an invitation to take part in the FITSA study was sent in the year 2000 to 178 monozygotic (MZ) and 212 dizygotic (DZ) twin pairs, and to 24 twin pairs of uncertain zygosity (XZ). To be recruited for the study, both individuals in the pair had to agree to participate. The reasons for nonparticipation were that one or both sisters were unwilling to take part in the study (50 MZ, 51 DZ, 5 XZ twin pairs) or had poor health status (28 MZ, 52 DZ, 5 XZ twin pairs) or had died (2 MZ, 3 DZ, 1 XZ) since the cohort's last vital status update. The zygosity of the twins was determined at the baseline in 1975 by a validated questionnaire (Sarna & Kaprio, 1980; Sarna et al., 1978). In the present study zygosity was assessed using DNA extracted from a venous blood sample by a battery of 10 highly polymorphic gene markers.

The final sample of the FITSA study after the zygosity determination was 103 MZ and 114 DZ twin pairs. In the genome-wide linkage analysis for muscle strength, muscle power, lower leg muscle CSA and maximal walking speed the sample consisted of 188 66- to 75-year-old DZ twin individuals from 94 pairs. As a consequence of technical or medical problems some individuals have missing results. Before the laboratory examinations, the subjects provided a written informed consent. The study protocol was approved by the ethics committee of the Central Hospital District of Central Finland.

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Phenotypes

Maximal walking speed over 10 meters. This was measured in the laboratory corridor using photocells for timing as described in detail earlier (Pajala et al., 2005). The test was done twice and the faster performance was documented as the result. For the analyses, maximal walking speed (m/s) was calculated. The coefficient of variation in the maximal walking speed measurement in the present study was 5 % between two consecutive measurement occasions with a one to two week interval.

Maximal isometric knee extensor strength. This was measured from the dominant hand side in a sitting position using an adjustable dynamometer chair (Good Strength, Metitur LTD, Jyväskylä, Finland). The dominant leg was defined as the leg on the side of the dominant hand. The measurement was done at the knee angle of 60° from full extension. A detailed description of the measurement has been given earlier (Tiainen et al., 2004). The data were digitized into Newtons (N), recorded and stored on a computer using Good Strength software package (Metitur LTD, Jyväskylä, Finland). For each subject the best performance with the highest value was accepted as the result. The test-retest Pearson correlation coefficient for maximal isometric knee extensor strength was .97 and a coefficient of variation was 6% between two consecutive measurement occasions with a one to two week interval among 80-year-old men and women (Rantanen et al., 1997).

Leg extensor power. This was measured using the Nottingham Leg Extensor Power Rig according to published guidelines in an upright sitting position with the active leg towards the push-pedal in front of the seat (Bassey & Short, 1990). First, the leg of the dominant hand side was measured, followed by the leg of the nondominant side. The average power of each push was calculated according to published guidelines using The Leg Rig software package (University of Nottingham, Nottingham, UK) and expressed in watts (W). For each subject, the best performance with the highest value was accepted as the result. The test–retest intraclass correlation coefficient (ICC) for the leg extensor power was .92 and the coefficient of variation between test–retest was 8% (Tiainen et al., 2005). The Nottingham Leg Extensor Power Rig (Bassey & Short, 1990) and the dynamometer chair used here (Rantanen et al., 1997) have previously been shown to be reliable and valid as well as acceptable and safe also for older people.

Lower leg muscle cross-sectional area (CSA). This was measured with peripheral quantitative computed tomography (pQCT, XCT-2000, Stratec Medizintechnik, Pforzheim, Germany) on the dominant hand side. Two millimeters thick tomography slices were taken at 55% of the tibia length upwards from the distal joint surface of the tibia obtained from a scout view. The whole CSA without subcutaneus fat tissue and bones was included in the muscle CSA. Image processing and calculation of parameters were done using Bonalyse 1.3 (Bonalyse Ltd, Espoo, Finland). The coefficient of variation for CSA was 1%.

Genotyping

DNA was extracted from EDTA-anticoagulated whole blood according to standard procedures. In the linkage analysis 397 microsatellite markers, spaced on average 10.0 cM apart, were genotyped on all 22 autosomes and X chromosome. The marker set used was the ABI PRISM Linkage mapping Set MD10 (Applied Biosystems). Standard polymerase chain reaction (PCR) protocols were used for the amplification of fragments using 10ng of genomic DNA as a template. The fluorescently labeled PCR products were separated by capillary array electrophoresis. Because the genotyping was part of a larger project, the GenomEUtwin project, three different genotyping platforms, the MegaBACE1000 (Amersham Biosciences) electrophoresis system, the ABI3700, and the ABI3730 (Applied Biosystems) automated electrophoresis systems were used. The genotype calls were made with the GeneticProfiler1.5 (MegaBACE1000) and GeneMapper3.7 (ABI3700 and ABI3730) software. All allele calls were verified by two independent reviewers and any discrepancies were resolved. The GRR program (Graphical Representation of Relationships), which calculates the average IBS allele-sharing between all pairs in a dataset, was used to identify sample mix-ups, pairs with identical genotypes, and to check the accuracy of the pedigree structures. In-house scripts were used to identify any females homozygous for all X-chromosomal markers. The female-specific genetic map in Kosambi cM was constructed using an in-house developed web-based program, Cartographer (Sammalisto et al., 2005), which uses marker's physical location for the ordering of the markers and then obtains the genetic distances from deCODE genetic map locations (Kong et al., 2002). LOD score results are presented per 1 cM. Relevant candidate genes for future studies were searched for the areas where LOD score was 2.0 or greater (the nearest markers with ± 5 Mb).

Statistical Analysis

Preliminary statistical analyses for the phenotypes were performed by the SPSS program (SPSS Inc, 2001). The independent pathway model was used as a preliminary analysis and as a framework to evaluate whether lower leg muscle CSA, maximal isometric knee extensor strength, lower leg muscle power and maximal walking speed share a genetic component. The observed variance in a phenotype is decomposed into four sources of variance: additive genetic (A), nonadditive genetic (D), shared environment (C), and nonshared environment (E; Posthuma et al., 2003).

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The expected correlations for A, D and C between the twins of the MZ pair are 1.0, while for DZ pairs the expected correlation for A is .5, for D .25, and for C 1.0. E effects are uncorrelated in both the MZ and DZ twins. E also includes random effect. On the basis of these expectations difference models (ACE, ADE, AE, CE and E) are fitted to the data, the aim being to obtain the most parsimonious and best fitting model to explain the observed pattern of twin similarity in MZ and DZ pairs. D was not included in the models, as the phenotypic correlations between the twin sisters suggested no contribution of dominance genetic effects (Neale & Cardon, 1992).

The full independent pathway model consists of genetic and environmental effects which are common to all four traits (Ac, Cc, Ec) as well as genetic (As_1, As_2, As_3, As_4) shared environmental (Cs_1, Cs_2, Cs_3, Cs_4), and nonshared environmental (Es_1, Es_2, Es_3, Es_4) effects, which are specific for each trait. A full ACE model includes all plausible parameters. To obtain a more parsimonious model, the full model was modified by dropping the least significant (i.e., parameter estimate zero or very small) parameters one at a time, until the model with the best fit with the data was achieved. In the independent pathway model the saturated model was used to evaluate the fit of the ACE model. In the saturated model means are modeled in a similar way as in the ACE-model, while covariance matrices are unconstrained, and all variances and covariances in MZ and DZ twins are estimated. The fit of the reduced nested models was assessed by subtracting the –2 log likelihood (LL) and degrees of freedom (*df*) of the obtained model from the –2LL and *df* of the full ACE model. In all models, age was used as a covariate. The genetic analyses were carried out using raw data input with full information maximum likelihood in Mx (Neale et al., 2003), which permits twin pairs with incomplete data to be included.

Next to identify the chromosomal areas involved in lower leg muscle CSA, maximal isometric knee extensor strength, leg extensor power and maximal walking speed, genome-wide linkage analysis was used. Multipoint linkage analysis for skeletal muscle characteristics and maximal walking speed were done in four univariate runs using the variance-components models implemented in MERLIN (Abecasis et al., 2002), using age as a covariate. To estimate empirical *p* values for obtained results, a total of 100 replicates were created and analyzed using MERLIN's simulate option. The power analyses were done using Solar program (Almasy & Blangero, 1998).

Results

Table 1 summarizes the physical characteristics and the results for maximal walking speed, maximal isometric knee extensor strength, leg extensor power and lower leg muscle CSA. The phenotypic correlations for whole sample between isometric knee extensor strength and leg extensor power was .56 and between lower leg muscle CSA and maximal walking speed .08. Otherwise phenotypic correlations ranged from .31 to .42.

We have previously published heritability estimates for walking speed, muscle strength and power (Tiainen et al., 2007): the age-adjusted heritability estimate for maximal walking speed was .34 (95% confidence interval [CI] .23–.47), for isometric knee extensor strength .52 (95% CI .39–.62) and for leg extensor power .36 (95% CI .24-.48). The MZ age-adjusted intraclass correlation coefficient for lower leg muscle CSA was .72 (95% CI .63-.82), the DZ correlation was .32 (95% CI .15-.50) and in the age-adjusted genetic model with additive genetic effects and nonshared environmental effects, the heritability estimate was .75.

In the genetic modeling, lower leg muscle CSA, maximal isometric knee extensor strength, leg extensor power and maximal walking speed had an additive genetic effect in common (Ac) which accounted for 7% (95% CI 1–15%) of the variation in muscle CSA, 51% (95% CI 39–62%) in strength, 37% (95% CI 25–49%) in power and 35% (95% CI 23–47%) in walking speed (Figure 1). Lower leg muscle CSA and leg extensor power also had a shared environmental effect in common (Cc) explaining 5% (95% CI 0–14%) of the variation in muscle CSA and 26% (95% CI 16–36%) in power. Nonshared environmental effect in common (Ec) accounted for 3% of the variation in muscle CSA (95% CI 1–7%), 49 % (95% CI 38–61%) in strength and 3% (95% CI 1–8%) in power. The remaining variance was accounted for by trait-specific genetic and environmental effects. The final reduced ACE model fitted the data well (–2LL = 13,090.3, $df = 1645$, $p = .97$).

Figure 2 shows LOD score curves for all the studied phenotypes in the whole genome. LOD scores of 2.0 and higher for the investigated phenotypes are shown in Table 2. The highest multipoint LOD score for maximal walking speed, 2.41, was found on chromosome 13q22.1 (Figure 3a). The highest multipoint LOD score for maximal isometric knee extensor strength, 2.14, was found on chromosome 15q14 (Figure 3b). The highest LOD score, 2.84, for leg extensor power was found on chromosome 8q24.23 (Figure 3c). The highest LOD score for lower leg muscle CSA, 2.93, was found on chromosome 20q13.31 (Figure 3d). In the permutation analyses, a larger LOD score than 2.92 was found in 18 replicates out of 100 giving an empirical *p* value of .18

The chromosomal areas of linkage to isometric muscle strength and leg extensor power were close to each other on the chromosome 15. LOD scores higher than 1.0 were seen for these phenotypes on chromosome 15 on the area containing 1.00–26.00 cM from pter (Figure 4).

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Table 1

Means and Standard Deviations (*SD*) for Physical Characteristics, Skeletal Muscle Characteristics and Maximal Walking Speed in Older Female Monozygotic (MZ) and Dizygotic (DZ) Twin Individuals

	MZ individuals $n = 206$		DZ individuals $n = 228$	
Variables	Mean	*SD*	Mean	*SD*
Age (years)	68.3	3.7	68.9	3.1
Weight (kg)	69.6	11.8	70.6	12.2
Height (cm)	158.0	6.4	159.1	5.8
Maximal walking speed (m/s)	1.7	0.4	1.7	0.3
Isometric knee extensor strength (N)	296.3	81.0	287.0	85.6
Leg extensor power (W)	102.9	36.0	97.7	32.6
Lower leg muscle CSA (cm^2)	60.1	8.9	60.0	9.5

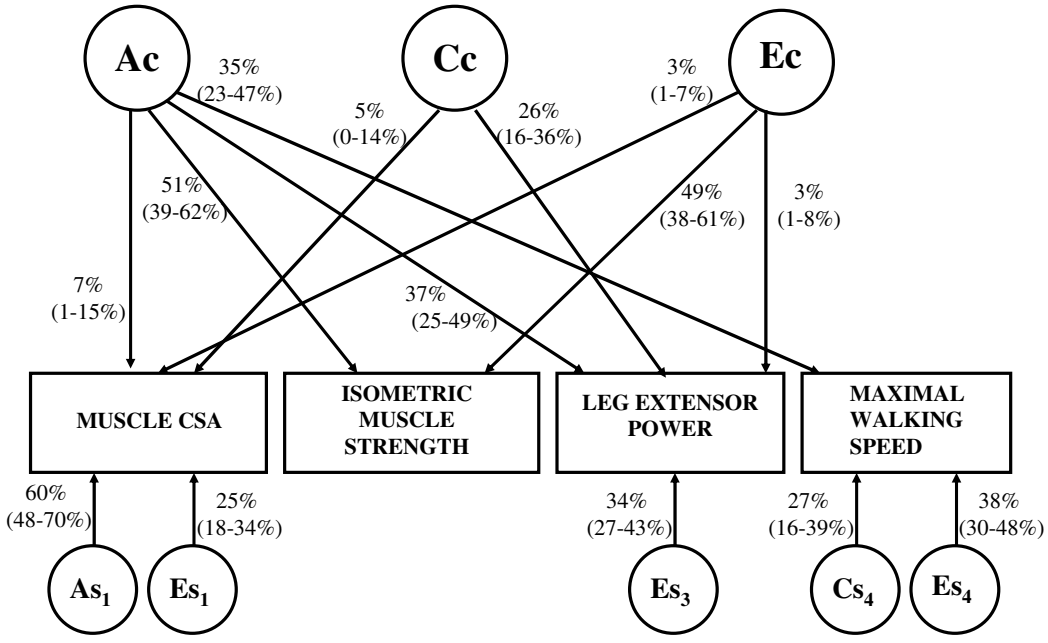

Figure 1

Reduced ACE model for lower leg muscle cross-sectional area (CSA), maximal isometric knee extensor strength, leg extensor power and maximal walking speed. Lower leg muscle CSA, maximal isometric knee extensor strength, leg extensor power and maximal walking speed shared genetic effect in common (Ac). Model also consists of shared environmental effect in common (Cc) for muscle CSA and power and nonshared environmental effect in common (Ec) for muscle CSA, strength and power. Remaining variance was accounted for by trait specific effects.

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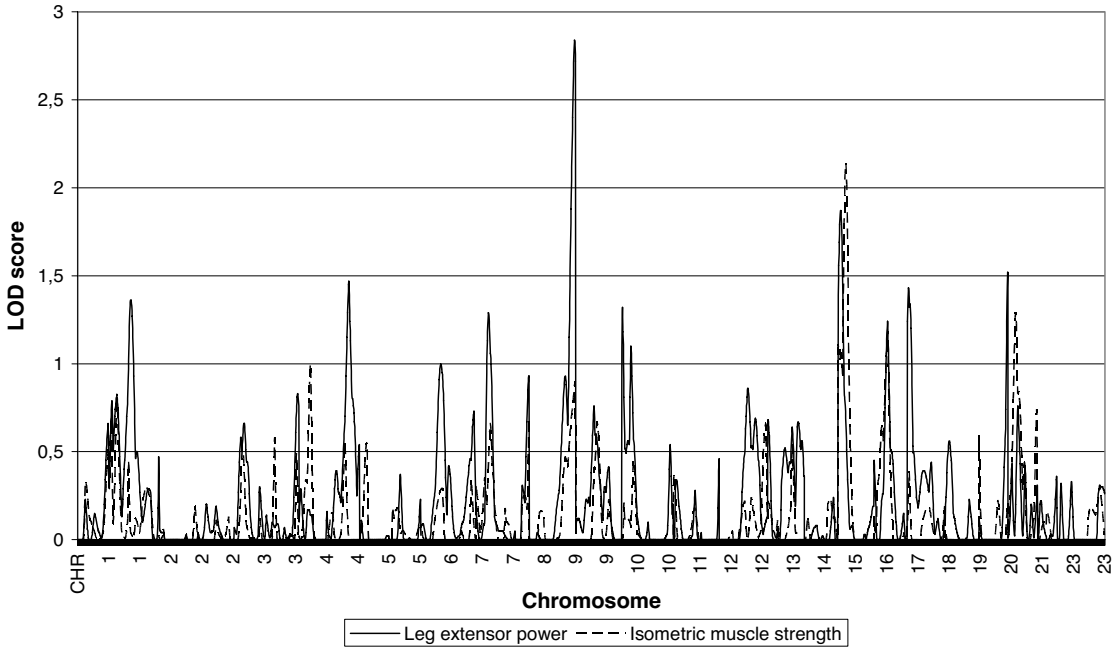

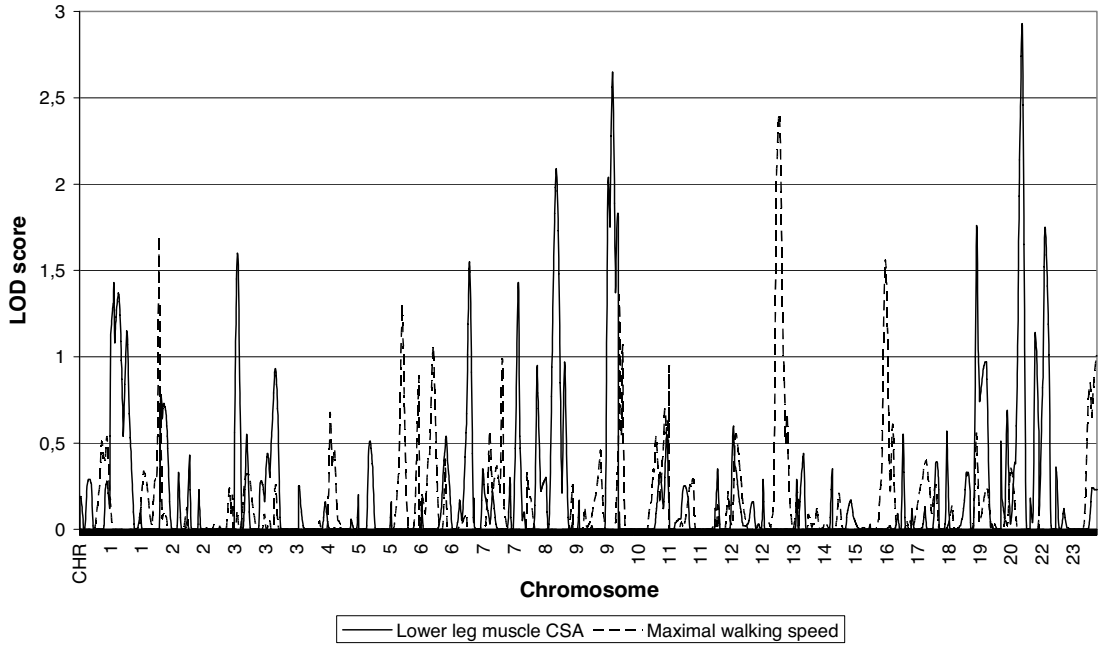

Figure 2

LOD scores for genome-wide linkage analysis for lower leg muscle CSA, isometric knee extensor strength, leg extensor power and maximal walking speed in the whole genome.

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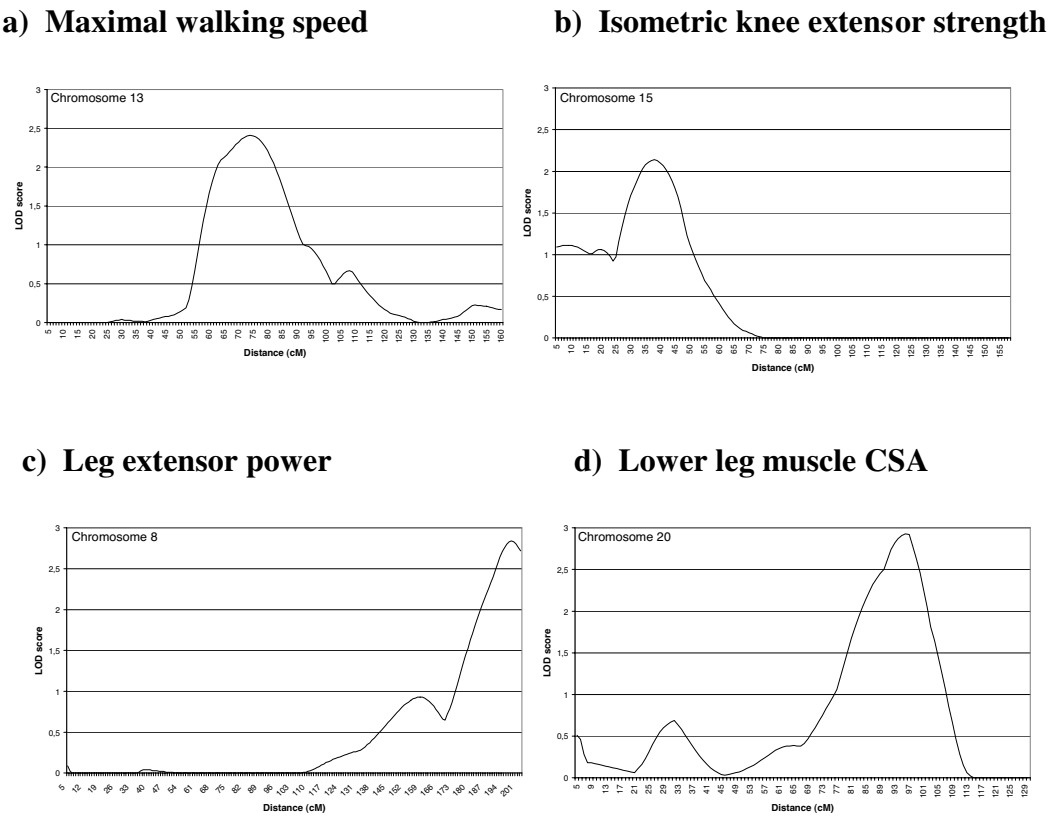

Figure 3

LOD scores for genome-wide linkage analysis for (a) maximal walking speed on chromosome 13, (b) isometric muscle strength on chromosome 15, (c) leg extensor power on chromosome 8, and (d) lower leg muscle CSA on chromosome 20. X-axis indicates distance in cM from pter and y-axis indicates LOD score values.

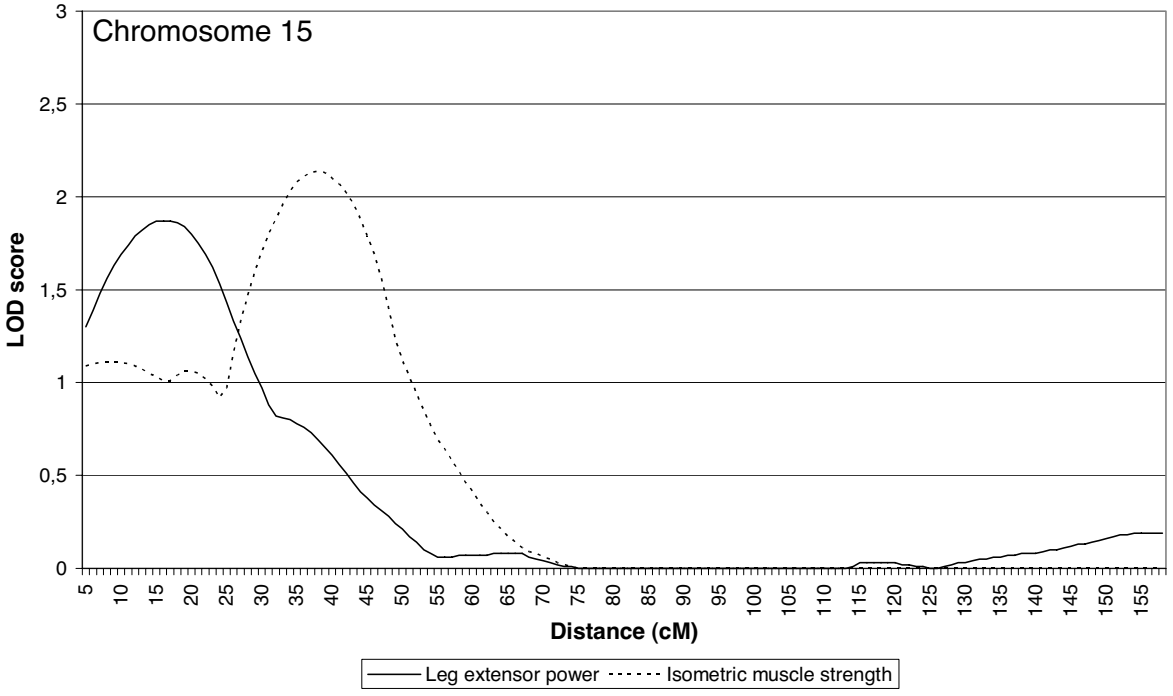

Figure 4

The chromosomal area in common for isometric muscle strength and leg extensor power on chromosome 15.

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Table 2

Chromosomes and Chromosome Locations (cM) with LOD Score ≥ 2.0 in Multipoint Linkage Analyses for Isometric Knee Extensor Strength, Leg Extensor Power, Lower Leg Muscle CSA and Maximal Walking Speed

Chromosome	Chromosome location (cM)	LOD scores for			
		Isometric muscle strength	Leg extensor power	Lower leg muscle CSA	Maximal walking speed
8	126	0.01	0.26	**2.02**	0
	127	0.01	0.26	**2.06**	0
	128	0.01	0.27	**2.09**	0
	129	0.01	0.28	**2.09**	0
	130	0.02	0.30	**2.06**	0
	131	0.02	0.32	**2.04**	0
	132	0.03	0.35	**2.00**	0
	:				
	182	0.71	**2.04**	0.02	0
	183	0.72	**2.12**	0.01	0.01
	184	0.73	**2.19**	0	0.01
	185	0.74	**2.26**	0	0.02
	186	0.75	**2.33**	0	0.03
	187	0.76	**2.40**	0	0.04
	188	0.77	**2.48**	0	0.05
	189	0.79	**2.57**	0	0.06
	190	0.81	**2.64**	0	0.08
	191	0.82	**2.71**	0	0.1
	192	0.84	**2.76**	0	0.12
	193	0.85	**2.80**	0.01	0.14
	194	0.87	**2.83**	0.01	0.17
	195	0.88	**2.84**	0.02	0.19
	196	0.89	**2.83**	0.02	0.21
	197	0.89	**2.80**	0.03	0.23
	198	0.90	**2.76**	0.04	0.25
	199	0.90	**2.72**	0.04	0.26
9	154	0.92	0.09	**2.02**	0
	155	0.86	0.07	**2.04**	0
	156	0.86	0.07	**2.01**	0
	:			:	
	165	0	0.01	**2.07**	0
	166	0	0	**2.18**	0
	167	0	0	**2.28**	0
	168	0	0	**2.37**	0
	169	0	0	**2.44**	0
	170	0	0	**2.51**	0
	171	0	0	**2.56**	0
	172	0	0	**2.61**	0
	173	0	0	**2.65**	0
	174	0	0	**2.65**	0
	175	0	0	**2.58**	0
	176	0	0	**2.50**	0
	177	0	0	**2.41**	0
	178	0	0	**2.32**	0
	179	0	0	**2.22**	0
	180	0	0	**2.12**	0
	181	0	0	**2.01**	0.01

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Table 2 (CONTINUED)

Chromosomes and Chromosome Locations (cM) with LOD Score ≥ 2.0 in Multipoint Linkage Analyses for Isometric Knee Extensor Strength, Leg Extensor Power, Lower Leg Muscle CSA and Maximal Walking Speed

Chromosome	Chromosome location (cM)	LOD scores for			
		Isometric muscle strength	Leg extensor power	Lower leg muscle CSA	Maximal walking speed
13	59	0	0.3	0	**2.03**
	60	0	0.34	0	**2.09**
	61	0	0.35	0	**2.12**
	62	0	0.37	0	**2.16**
	63	0	0.39	0	**2.20**
	64	0	0.41	0	**2.24**
	65	0	0.42	0	**2.29**
	66	0	0.44	0	**2.32**
	67	0	0.46	0	**2.36**
	68	0	0.47	0	**2.38**
	69	0	0.48	0	**2.40**
	70	0	0.50	0	**2.41**
	71	0	0.51	0	**2.40**
	72	0	0.51	0	**2.39**
	73	0	0.52	0	**2.36**
	74	0	0.52	0	**2.32**
	75	0	0.52	0	**2.27**
	76	0.01	0.51	0	**2.21**
	77	0.01	0.50	0	**2.13**
	78	0.02	0.50	0	**2.05**
15	30	**2.03**	0.8	0	0.20
	31	**2.08**	0.78	0	0.19
	32	**2.11**	0.76	0	0.18
	33	**2.13**	0.73	0	0.16
	34	**2.14**	0.69	0	0.14
	35	**2.13**	0.65	0	0.12
	36	**2.10**	0.61	0	0.10
	37	**2.07**	0.56	0	0.08
	38	**2.02**	0.51	0	0.06
20	79	0.08	0.11	**2.04**	0
	80	0.05	0.07	**2.14**	0
	81	0.03	0.03	**2.24**	0
	82	0.01	0.01	**2.32**	0
	83	0	0	**2.39**	0
	84	0	0	**2.45**	0
	85	0	0	**2.50**	0
	86	0	0	**2.62**	0
	87	0	0	**2.74**	0
	88	0	0	**2.82**	0
	89	0	0	**2.87**	0
	90	0	0.01	**2.91**	0
	91	0	0.03	**2.93**	0
	92	0	0.04	**2.92**	0
	93	0	0.06	**2.78**	0
	94	0	0.08	**2.63**	0
	95	0	0.11	**2.46**	0
	96	0.01	0.14	**2.27**	0
	97	0.02	0.17	**2.05**	0

Note: LOD score ≥ 2.0 for each trait is in bold.

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Discussion

In the present study we performed a multivariate quantitative genetic modeling followed by multipoint genome-wide linkage analysis for maximal walking speed and three skeletal muscle characteristics in older women. Although genetic modeling showed additive genetic effect in common for lower leg muscle CSA, maximal isometric knee extensor strength, leg extensor power and maximal walking speed, genome-wide linkage analysis could not find strong evidence for the chromosomal areas in common for these three muscle phenotypes and walking speed. For individual phenotypes, the strongest suggestive evidence of linkage to maximal walking speed was observed on chromosome 13, to isometric muscle strength on chromosome 15, to leg extensor power on chromosome 8 and to lower leg muscle CSA on chromosome 20. LOD scores were expected to remain fairly low. To our knowledge this is the first study to attempt a genome-wide approach to assessing the genetic component of skeletal muscle phenotypes partly determining functional independence in elderly people.

The phenotypes selected for this analysis were known to be multifactorial and accordingly it is not unexpected that the LOD scores we found only meet the criteria for a suggestive linkage. However, our results are supported by previous genome-wide linkage studies of phenotypes related to the muscle characteristics and functional performance that we investigated. In these studies, chromosomal regions that partly overlap with the regions showing suggestive linkage in the present study have been implicated (Bouchard et al., 2000; Chagnon et al., 2001). Bouchard et al. (2000) found a weak linkage between oxygen uptake (VO_2max) in the sedentary state and the region on chromosome 8q that showed a suggestive linkage to leg extension power in our study. This overlapping area suggests interesting, hypothetical aspect that chromosome 8 might harbor genes which have an effect on functional performance.

All regions displaying a suggestive linkage in the present study contain a large number of potentially interesting candidate genes. In the chromosomes 8, 9 and 20 are located, for example, patterns of genes related to ubiquitination/deubiquitination of proteins. Ubiquitination/deubiquitination is an essential regulatory mechanism in intracellular degradation of proteins as well as in many other biological processes, including cell cycle progression, DNA repair, organelle biogenesis, vesicular trafficking, transcriptional activation and signal transduction (D'Andrea & Pellman, 1998; Quesada et al., 2004). Several extracellular matrix genes are also found on these chromosomal areas. Additionally, the areas of interest harbor several transcription factor genes. The highest LOD score for muscle CSA was observed on chromosome 20 (marker D20S100). On that area is located, for example, the OZZ gene, a developmentally regulated striated muscle specific E3-ubiquitin ligase which contributes to myofibrillogenesis and myofiber differentiation via interacting with β-catenin (Nastasi et al., 2004). Another muscle specific E3-ubiquitin ligase gene, atrogin-1, also related to muscle CSA, is located on chromosome 8 (marker D8S514). Atrogin-1 is one of the few examples of F-box proteins, and is strongly involved in many catabolic states such as neuromuscular disorders (Léger et al., 2006) and it is likely to play an important role in the generation of muscle atrophy (Gomes et al., 2001).

Within the chromosome 15, in the area with LOD scores of 2.0 and higher for isometric muscle strength, are located genes related to the signal transduction and function of synapses and neurons the genes being expressed especially in the cells of the central nervous system (LeBleau et al., 2003). One of the interesting genes, Connexin36 (marker D15S1007) is an integral membrane protein of neuronal gap junction channels, which provides the structural bases of electrical synapses between neurons (Söhl et al., 2005) and is also present in microglial cells (Dobrenis et al., 2005).

The highest LOD score for walking speed was found on chromosome 13. This chromosomal area harbors genes involved in the regulation of synapse formation as well as dynamics and signaling via synapses. Some of these genes are highly expressed in the cells of the central nervous system (Junghans et al., 2005). In that area (marker D13S153) are also located retinoblastoma (Rb1) and TRIM32 genes. Rb1 has an important role in myogenesis by regulating myoblast proliferation, mediating terminal cell cycle exit and differentiation, and is in control of cell survival (DeCaprio et al., 1992). Rb1 is also an important factor in three pathways of muscle cell signaling (Langley et al., 2002; Thomas et al., 2000). Huygens et al. (2004, 2005) suggested that variants in or near Rb1 can explain part of the interindividual variance of muscle strength among young male adults. In our previous study, genetic modeling (Tiainen et al., 2007) showed that muscle strength, power and maximal walking speed shared a genetic effect in common, and this was shared with muscle CSA as well as in the present analysis. Although all these skeletal muscle-related phenotypes were phenotypically correlated according to the genetic modeling partly common genetic background, the present study could not identify chromosomal areas in common for these three phenotypes. Walking speed is a multifactorial trait and muscle strength is only one factor, even though an important one, which has an effect on walking speed. The limited statistical power of the present study may be the reason for the missing linkage between muscle strength, power and Rb1 in our study. TRIM32, a member of the tripartite motif (TRIM) proteins with E3-ubiquitin ligase activity, is expressed in skeletal muscle and contributes to several cellular processes, for example, cell proliferation, differentiation and development (Nisole et al., 2005) and ubiqutinates actin (Kudryashova et al., 2005). Limb girdle muscular dystrophy type 2H is connected to

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mutated TRIM32 and Schoser et al. (2005) suggested that TRIM32 is likely involved in the generation of the sarcoplasmic reticulum system or in maintaining its structural integrity in the skeletal muscle.

Previously, the walking speed phenotype has not been addressed in candidate gene studies, but the genetic component of muscle strength has been addressed in several association studies. In their reviews, Rankinen et al. (2006) and Beunen and Thomis (2004) summarized the candidate genes, found in association studies, for muscle strength, which are located on chromosomes 1 (DIO1), 2 (GDF8), 3 (MYLK), 5 (NR3C1), 6 (TNF), 7 (CFTR), 11 (IGF2, CNTF, ACTN3), 12 (VDR), and 17 (COL1A1, ACE). All the reported associations were weak to moderate and typically reported only once. Therefore, the contribution of these candidate genes to the genetic variance of muscle strength remains to be elucidated. None of these genes are located in the chromosomal regions of a suggestive linkage in the present study.

In our previous study (Tiainen et al., 2007), genetic modeling showed that muscle strength, power and maximal walking speed shared a genetic effect in common, and the present analysis extended this to include muscle CSA. The present model and the earlier trivariate model (isometric muscle strength-leg extensor power-maximal walking speed, Tiainen et al., 2007) are almost identical. In the earlier trivariate model, the proportion of the genetic effect for isometric muscle strength was 52%, for leg extensor power 36%, and for maximal walking speed 34%. Also the proportions for nonshared environmental effects were quite similar, 48% for isometric muscle strength, 36% for leg extensor power and 38% for maximal walking speed. The most significant difference between these two models is that in the trivariate model the leg extensor power has the specific genetic effect (11%) and in the present model this effect is missing.

Despite the showed genetic effect in common, in the present study no overlapping chromosomal areas with LOD scores higher than 2.0 for different phenotypes were found. Though the highest phenotypic correlation (.56) was observed between isometric muscle strength and lower leg muscle power, these muscle characteristics were only weakly linked to the same chromosomal area on chromosome 15 (Figure 3). The results suggest that the investigated traits are affected by many different chromosomal areas and genes. Walking speed is a multifactorial trait and muscle strength is only one factor, albeit an important one, which has an effect on walking speed.

Our subjects were older women, and it is known that all the genes which have an effect on a trait are not similarly active throughout the lifespan. Gender differences are not always clear, but it is possible that different genes play a more prominent role in males than in females to determine muscle strength. Age as such has effect on skeletal muscle characteristics and walking speed. Accordingly, it is possible that these observed interesting chromosomal regions are not only related to skeletal muscle characteristics but also aging process in general.

The present study consisted of only limited number of female twin pairs and it is evident that the study is low in power. The most heritable trait for lower leg muscle CSA (LOD score 2.93) provided only 47% power for detecting a LOD score of 3 for a major locus explaining the additive genetic variance. On the other hand, the advantage of the sample was the relatively isolated Finnish population. This population is characterized by reduced environmental and genetic heterogeneity, resulting in restricted confounders and less genetic variability (Jorde et al., 2000; Peltonen et al., 2000).

In the present study isometric muscle strength, lower leg muscle power and lower leg muscle CSA were measured from different muscles. However, the measurements used in the present study have proved to be suitable and reliable methods among older persons (Arden & Spector, 1997; Bassey, 1998). As all studied muscles are essential muscles for functional performance such as walking, it is unlikely that environmental factors influence very differently on the gene expression in these muscles. So, genetic muscle characteristics can be assumed to be a similar.

Apart from the important role of multiple genes, environmental effects and interaction between genetic and environmental effects are also significant factors in explaining the differences between individuals in functional performance. Although some people may be more prone to functional limitation in old age due to their genetic disposition, many earlier studies have shown that adequate physical activity and training can maintain and increase muscle mass (Fiatarone et al., 1994; Sipilä & Suominen, 1995) and improve muscle strength and functional performance (de Vreede et al., 2005; Henwood & Taaffe, 2005; Hruda et al., 2003; Sipilä et al., 1996).

In conclusion, our study suggests that maximal walking speed and the related muscle strength, power and CSA of the lower extremities in elderly women have a complex and multifactorial background. We found a suggestive linkage of walking speed and muscle characteristics to several chromosomal regions, although the linked regions do not show significant overlap between the studied phenotypes. However, the study sample size is small and it must be noted that both type I and II errors are prevalent in such samples and the results should be considered with that in mind – replication of the results is very much in need. Also, the phase information in sibpairs with no parental data and a relatively sparse genetic map is limited. Interestingly, two of the identified regions, chromosome 8q24 (Bouchard et al., 2000) and 9q34 (Chagnon et al., 2001) have previously been implicated in studies of related phenotypes in much younger individuals, suggesting that our findings may not be limited solely to changes in the muscular characteristics of elderly

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people. Further studies are required to confirm whether the chromosomal regions found to be linked to the studied phenotypes in older Finnish women are in range with other populations and samples, including younger subjects and males.

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References

Abecasis, G. R., Cherny, S.S., Cookson, W.O., & Cardon, L.R. (2002). Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. *Nature Genetics, 30*, 97–101.

Almasy, L., & Blangero, J. (1998). Multipoint quantitative trait linkage analysis in general pedigrees. *American Journal of Human Genetics, 62,* 1198–1211.

Arden, N. K., & Spector, T. D. (1997). Genetic influences on muscle strength, lean body mass and mineral density: A twin study. *Journal of Bone and Mineral Research, 12*, 2076- 2081.

Bassey, E. J. (1998). Longitudinal changes in selected physical capabilities: Muscle strength, flexibility and body size. *Age and Ageing, 27*(Suppl.), 12–16.

Bassey, E. J., & Short, A., H. (1990). A new method for measuring power output in a single leg extension: Feasibility, reliability and validity. *European Journal of Applied Physiology, 60,* 385–390.

Bean, J. F., Leveille, S. G., Kiely, D. K., Bandinelli, S., Guralnik, J. M., & Ferrucci, L. A. (2003). Comparison of leg power and leg strength within the InCHIANTI study: Which influences mobility more? *Journals of Gerontology: A Biological Sciences and Medical Sciences, 58A*, 728–733, 2003.

Beunen, G., & Thomis, M. (2004). Gene powered? Where to go from heritability (h^2) in muscle strength and power? *Exercise and Sport Sciences Reviews, 4,* 148–154.

Bohannon, R. W. (1997). Comfortable and maximum walking speed of adults aged 20–79 years: Reference values and determinants. *Age and Ageing, 26,* 15–19.

Bohannon, R. W., Addrews, A. W., & Thomas, M. W. (1996). Walking speed: Reference values and correlates for older adults. *Journal of Orthopaedic and Sports Physical Therapy, 2,* 86–90.

Bouchard, C., Rankinen, T., Chagnon, Y. C., Rice, T., Pérusse, L., Gagnon, J., Borecki, I., An, P., Leon, A. S., Skinner, J. S., Wilmore, J. H., Province, M., & Rao, D. C. (2000). Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE family study. *Journal of Applied Physiology, 88,* 551–559.

Chagnon, Y. C., Rice, T., Pérusse, L., Borecki, I. B., Ho-Kim, M.-A., Lacaille, M., Pare, C., Bouchard, L., Gagnon, J., Leon, A. S., Skinner, J. S., Wilmore, J. H., Rao, D. C., & Bouchard, C. (2001). Genomic scan for genes affecting body composition before and after training in Caucasian from HERITAGE. *Journal of Applied Physiology, 90*, 1777–1787.

D'Andrea, A., & Pellman, D. (1998). Deubiquitinating enzymes. A new class of biological regulators. *Critical Review in Biochemistry and Molecular Biology, 33,* 337–352.

DeCaprio, J. A., Furukawa, Y., Ajchenbaum, F., Griffin, J. D., & Livingston, D. M. (1992). The retinoblastoma-susceptibility gene product becomes phosphorylated in multiple stages during cell cycle entry and progression. *Proceedings of the National Academics of Sciences of the USA, 89,* 1795–1798.

de Vreede, P. L., Samson, M. M., van Meeteren, N. L., Duursma, S. A., & Verhaar, H. J. J. (2005). Functional-task exercise versus resistance stength exercise to improve daily functional in older women: A randomized, controlled trail. *Journal of American Geriatrics Society, 53*, 2–10.

Dobrenis, K., Chang, H.-Y., Pina-Benabou, M. H., Woodroffe, A., Lee, S. C., Rozental, R., Spray, D. C., & Scemes, E. (2005). Human and mouse microglia express connexin36, and functional gep junctions are formed between rodent microglia and neurons. *Journal of Neuroscience Research, 82*, 306–315.

Enoka, R. M. (1994). *Neuromechanical basis of kinesiology* (2nd ed., pp. 203–209). Champaign (Illinois): Human Kinetics.

Fiatarone, M. A., O'Neill, E. F., Ryan, N. D., Clements, K. M., Solares, G. R., Nelson, M. E., Roberts, S. B., Kehayias, J. J., Lipsitz, L. A., & Evans, W. J. (1994). Exercise training and nutritional supplementation for physical frailty in very elderly people. *New England Journal of Medicine, 330*, 1769–1775.

Foldvari, M., Clark, M., Laviolette, L. C., Berstein, M. A., Kaliton, D., Castaneda, C., Pu, C. T., Hausdorff, J. M., Fielding, R. A., & Singh, M. A. (2000). Association of muscle power with functional status in community-dwelling elderly women. *Journals of Gerontology Biological Sciences and Medical Sciences, 55A,* M192–199.

Frontera, W. R., Hughes, V. A., Fielding, R. A., Fiatarone, M. A., Evans, W. J., & Roubenoff, R. (2000). Aging of skeletal muscle a 12-yr longitudinal study. *Journal of Applied Physiology, 88,* 1321–1326.

Frontera, W. R., Hughes, V. A., Lutz, K. J., & Evans, W. J. (1991). A cross-sectional study of muscle strength

and mass in 45- to 78-yr-old men and women. *Journal of Applied Physiology, 2,* 644–650.

Gomes, N. D., Lecker, S. H., Jagoe, R. T., Navon, A., & Goldberg, A. L. (2001). Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proceedings of the National Academy of Sciences USA, 25,* 14440–14445.

Guralnik, J. M., Ferrucci, L., Pieper, C. F., Leveille, S. G., Markides, K. S., Ostir, G. V., Studenski, S., Berkman, L. F., & Wallace, R. B. (2000). Lower extremity function and subsequent disability: Consistency across studies compared with the short physical performance battery. *Journals of Gerontology Medical Sciences, 55A,* M221-M231.

Guralnik, J. M., Ferrucci, L., Simonsick, E. M., Salive, M. E., & Wallace, R. B. (1995). Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *New England Journal of Medicine, 332*, 556–561.

Henwood, T. R., & Taaffe, D. R. (2005). Improved physical performance in older adults undertaking a short-term programme of high-velocity resistance training. *Gerontology, 51,* 108–115.

Hruda, K. V., Hicks, A. L., & McCartney, N. (2003). Training for muscle power in older adults: Effects on functional abilities. *Canadian Journal of Applied Physiology, 28,* 178–189.

Huygens, W., Thomis, M. A., Peeters, M. W., Aerssens, J., Janssen, R. G. J. H., Vlietinck, R. F., & Beunen, G. (2004). A quantitative trait locus on 13q14.2 for trunk strength. *Twin Research and Human Genetics*, *6*, 603–606.

Huygens, W., Thomis, M. A. I., Peeters, M. W., Aerssens, J., Vlietinck, R., & Beunen, G. P. (2005). Quantitative trait loci for human strength: Linkage analysis of myostatin pathway genes. *Physiological Genomics, 22*, 390–397.

Jorde, L. B., Watkins, W. S., Kere, J., Nyman, D., & Eriksson, A.W. (2000). Gene mapping in isolated populations: New roles for old friends? *Human Heredity, 50,* 57–65.

Junghans, D., Haas, I. G., & Kemier, R. (2005). Mammalian cadherins and protocadherins: About cell death, synapses and processing. *Current Opinion in Cell Biology*, *17,* 446–452.

Kaprio, J., & Koskenvuo, M. (2002). Genetic and environmental factors in complex diseases: The older Finnish twin cohort. *Twin Research and Human Genetics, 5,* 358–365.

Kaprio, J., Sarna, S., Koskenvuo, M., & Rantasalo, I. (1978). The Finnish Twin Registry: Formation and compilation, questionnaire study, zygosity determination procedures, and research program. *Progress in Clinical and Biological Research, 24 Pt B,* 179–184.

Kong, A., Gudbjartsson, D. F., Sainz, J., Jonsdottir, G. M., Gudjonsson, S. A., Richardsson, B., Sigurdardottir, S., Barnard, J., Hallbeck, B., Masson, G., Shlien, A., Palsson, S. T., Frigge, M. L., Thorgeirsson, T. E., Gulcher, J. R., & Stefansson, K. (2002). A high resolution recombination map of the human genome. *Nature Genetics, 31,* 241–247.

Kudryashova, E., Kudryashov, D., Kramerova, I., & Spencer, M. J. (2005). Trim32 is a ubiquitin ligase mutated in limb girdle muscular dystrophy type 2H that binds to skeletal muscle myosin and ubiquitinates actin. *Journal of Molecular Biology, 354,* 413–424.

Langley, B., Thomas, M., Bishop, A., Sharma, M., Gilmour, S., & Kambadur, R. (2002). Myostatin inhibits myoblast differentation by down-regulating MyoD Expression. *Journal of Biological Chemistry, 51*, 49831–49840.

Lauretani, F., Russo, C. R., Bandinelli, S., Bertali, B., Cavazzini, C., Iorio, A. D., Corsi, A. M., Rantanen, T., Guralnik, J. M., & Ferrucci, L. (2003). Age-associated changes in skeletal muscles and their effect on mobility: An operational diagnosis of sarcopenia. *Journal of Applied Physiology, 95,* 1851–60.

LeBleau, F. E. N., Traub, R. D., Monyer, H., Whittington, M. A., & Buhl. A. H. (2003). The role of electrical signalling via gap junctions in the generation of fast network oscillations. *Brain Research Bulletin, 62,* 3–13.

Léger, B., Vergani, L., Sorarù, G., Hespel, P., Derave, W., Gobelet, C., D'Ascenzio, C., Angelini, C., & Russell, A. P. (2006). Human skeletal muscle atrophy in amyotrophic lateral sclerosis reveals a reduction in Akt and an increase in atrogin-1. *Federation of American Societies for Experimental Biology, 20,* 583–585.

Loos, R., Thomis, M., Maes, H. H., Beunen, G., Claessens, A. L., Derom, C., Legius, E., Derom, R., & Vlietinck, R. (1997). Gender-specific regional changes in genetic structure of muscularity in early adolescence. *Journal of Applied Physiology, 6*, 1802–1810.

Nastasi, T., Bongiovanni, A., Campos, Y., Mann, L., Toy, J. N., Bostrom, J., Rottier, R., Hahn, C., Conaway, J. W., Harris, A. J., & d'Azzo, A. (2004). Ozz-E3, a muscle-specific ubiquitin ligase, regulates -catenin degradation during myogenesis. *Developmental Cell, 6,* 269–282.

Neale, M. C., Boker, S. M., Xie, G. H., & Maes, H. (2003). *Mx: Statistical modeling* (6th ed., pp. 1–185). Richmond, VA: Department of Psychiatry.

Neale, M. C., & Cardon, L. R. (1992). *Methodology for genetic studies of twins and families* (pp. 55–70). Dordrecht: Kluwer Academic Publishers.

Nisole, S., Stoye, J. P., & Saib, A. (2005). Trim family proteins: Retroviral restriction and antiviral defense. *Nature Reviews Microbiology, 3,* 799–808.

Onder, G., Penninx, B. W. J. H., Ferrucci, L., Fried, L. P., Guralnik, J. M., & Pahor, M. (2005). Measures of physical performance and risk for progressive and catastrophic disability: Results from the Women's Health and Aging Study. *Journal of Gerontology Medical Sciences, 60A,* 74–79.

https://doi.org/10.1375/twin.11.3.321 Published online by Cambridge University Press

Pajala, S., Era, P., Koskenvuo, M., Kaprio, J., Alén, M., Tolvanen, A., Tiainen, K., & Rantanen, T. (2005). Contribution of genetic and environmental factors to individual differences in maximal walking speed with or without second task in older women. *Journals of Gerontology Medical Sciences, 60A,* 1299–1303.

Peltonen, L., Palotie, A., & Lange, K. (2000). Use of population isolates for mapping complex traits. *Nature Reviews Genetics, 1,* 182–190.

Posthuma, D., Beem, A. L., de Geus, E. J. C., van Baal, G. C. M., von Hjelmborg, J. B., Iachine, I., & Boomsma, D. (2003). Theory and practice in quantitative genetics. *Twin Research and Human Genetics*, *6,* 361–376.

Quesada, V., Diaz-Perales, A., Gutierrez-Fernandez, A., Garabaya, C., Cal, S., & Lopex-Optin, C. (2004). Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. *Biochemical and Biophysical Research Communications, 314,* 54–62.

Rankinen, T., Bray, M. S., Hagberg, J. M., Pérusse, L., Roth, S. M., Wolfarth, B., & Bouchard, C. (2006). The human gene map for performance and heath-related fitness phenotypes: The 2005 update. *Medicine and Science in Sports and Exercise, 38,* 1863–1888.

Rantanen, T., & Avela, J. (1997). Leg extension power and walking speed in very old people living independently. *Journals of Gerontology Biological Sciences and Medical Sciences, 52A,* M225–M231.

Rantanen, T., Era, P., & Heikkinen, E. (1997). Physical activity and the changes in maximal isometric strength in men and women from the age of 75 to 80 years. *Journal of the American Geriatrics Society, 5,* 1439–1445.

Rantanen, T., Guralnik, J. M., Izmirlian, G., Williamson, J. D., Simonsick, E. M., Ferrucci, L., & Fried, L. P. (1998). Association of muscle strength with maximum walking speed in disabled older women. *American Journal of Physical Medicine & Rehabilitation, 77,* 299–305.

Rantanen, T., Masaki, K., Foley, D., Izmirlian, G., White, L., & Guralnik, J. M. (1998). Grip strength changes: Over 27 years in Japanese–American men. *Journal of Applied Physiology, 85,* 2047–2053.

Rosenberg, I. H. (1989). Epidemiologic and methodologic problems in determining nutritional status of older persons (Summary comments). *American Journal of Clinical Nutrition, 50*(Suppl.), 1231–1233.

Roubenoff, R. (2000). Sarcopenia and its implications for the elderly. *European Journal of Clinical Nutrition, 54*(Suppl.), S40-S47.

Sammalisto, S., Hiekkalinna, T., Suviolahti, E., Sood, K., Metzidis, A., Pajukanta, P., Lilja, H. E., Soro-Paavonen, A., Taskinen, M-R., Tuomi, T., Almgren, P., Orho-Melander, M., Groop, L., Peltonen, L., & Perola, M. (2005). A male-specific quantitative trait locus on 1p21 controlling human stature. *Journal of Medical Genetics, 42,* 932–939.

Sarna, S., & Kaprio, J. (1980). Use of multiple logistic analyses in twin zygosity diagnosis. *Human Heredity, 30,* 71–80.

Sarna, S., Kaprio, J., Sistonen, P., & Koskenvuo, M. (1978). Diagnosis of twin zygosity by mailed questionnaire. *Human Heredity, 28,* 241–254

Schoser, B. G. H., Frosk, P., Engel, A. G., Klutzny, U., Lochmuller, H., & Wrogemann, K. (2005). Commonality of TRIM32 mutation in causing sarcotubular myopathy and LGMD2H. *Annals of Neurology, 57,* 591–595.

Sipilä, S., Multanen, J., Kallinen, M., Era, P., & Suominen, H. (1996). Effects of strength and endurance training on isometric muscle strength and walking speed in elderly women. *Acta Physiologica Scandinavica, 156,* 457–464.

Sipilä, S., & Suominen, H. (1995). Effects of strength and endurance training on thigh and leg muscle mass and composition in elderly women. *Journal of Applied Physiology, 78,* 334–340

SPSS Inc. (2001). SPSS for Windows (Release 11.0.1) [Computer software]. Chicago, IL: SPSS, Inc.

Söhl, G., Maxeiner, S., & Willecke, K. (2005). Expression and functions of neuronal gap junctions. *Nature Reviews Neuroscience, 6,* 191- 200.

Thomas, M., Langley, B., Berry, C., Sharma, M., Kirk, S., Bass, J., & Kambadurs, R. (2000). Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *Journal of Biological Chemistry, 51,* 40235–40242.

Thomis, M. A., van Leemputte, M., Maes, H. H., Blimkie, C. J. R., Claessens, A. L., Marchal, G., Willems, E., Vlietinck, R. F., & Beunen, G. P. (1997). Multivariate genetic analysis of maximal isometric muscle force at different elbow angles. *Journal of Applied Physiology, 82,* 959–967.

Tiainen, K., Sipilä, S., Alén, M., Heikkinen, E., Kaprio, J., Koskenvuo, M., Tolvanen, A., Pajala, S., & Rantanen, T. (2004). Heritability of isometric muscle strength in older female twins. *Journal of Applied Physiology, 96,* 173–180.

Tiainen, K., Sipilä, S., Alén, M., Heikkinen, E., Kaprio, J., Koskenvuo, M., Tolvanen, A., Pajala, S., & Rantanen, T. (2005). Shared genetic and environmental effects on strength and power in older female twins. *Medicine and Science in Sports and Exercise, 1,* 72–78.

Tiainen, K., Pajala, S., Sipilä, S., Kaprio, J., Koskenvuo, M., Alén, M., Heikkinen, E., Tolvanen, A., & Rantanen, T. (2007). Genetic effects in common on maximal walking speed and muscle performance in older women. *Scandinavian Journal of Medicine and Science in Sports, 17,* 274–280.

Wang, F.-C., de Pasqua, V., & Delwaide, P. J. (1999). Age-related changes in fastest and slowest conducting axons of thenar motor units. *Muscle Nerve, 22,* 1022–1029.