

**This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.**

Author(s): Ronkainen, Paula; Laakkonen, Eija; Törmäkangas, Timo; Tiainen, Kristina; Koskenvuo, Markku; Kaprio, Jaakko; Rantanen, Taina; Sipilä, Sarianna; Kovanen, Vuokko

Title: Catechol-O-Methyltransferase Gene Polymorphism Is Associated with Skeletal Muscle Properties in Older Women Alone and Together with Physical Activity

Year: 2008

Version:

Please cite the original version:

Ronkainen, P., Laakkonen, E., Törmäkangas, T., Tiainen, K., Koskenvuo, M., Kaprio, J., Rantanen, T., Sipilä, S., & Kovanen, V. (2008). Catechol-O-Methyltransferase Gene Polymorphism Is Associated with Skeletal Muscle Properties in Older Women Alone and Together with Physical Activity. *PLoS ONE*, 3(3).
<https://doi.org/10.1371/journal.pone.0001819>

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.

Catechol-*O*-Methyltransferase Gene Polymorphism Is Associated with Skeletal Muscle Properties in Older Women Alone and Together with Physical Activity

Paula H. A. Ronkainen^{1,2*}, Eija Pöllänen^{1,2}, Timo Törmäkangas^{1,2}, Kristina Tiainen^{1,2}, Markku Koskenvuo³, Jaakko Kaprio^{4,5}, Taina Rantanen^{1,2}, Sarianna Sipilä^{1,2}, Vuokko Kovanen¹

1 Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, **2** The Finnish Centre for Interdisciplinary Gerontology, University of Jyväskylä, Jyväskylä, Finland, **3** Department of Public Health, University of Turku, Turku, Finland, **4** Department of Public Health, University of Helsinki, Helsinki, Finland, **5** Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland

Abstract

Background: Muscle strength declines on average by one percent annually from midlife on. In postmenopausal women this decrement coincides with a rapid decline in estrogen production. The genetics underlying the effects of estrogen on skeletal muscle remains unclear. In the present study, we examined whether polymorphisms within *COMT* and *ESR1* are associated with muscle properties and assessed their interaction and their combined effects with physical activity.

Methodology/Principal Findings: A cross-sectional data analysis was conducted with 434 63–76-year-old women from the population-based Finnish Twin Study on Aging. Body anthropometry, muscle cross-sectional area (mCSA), isometric hand grip and knee extension strengths, and leg extension power were measured. *COMT* Val158Met and *ESR1* PvuII genotypes were determined by the RFLP method. mCSA differed by *COMT* genotypes ($p = 0.014$) being significantly larger in LL than HL individuals in unadjusted ($p = 0.001$) and age- and height-adjusted model ($p = 0.004$). When physical activity and age were entered into GEE model, *COMT* genotype had a significant main effect ($p = 0.038$) on mCSA. Furthermore, sedentary individuals with the HH genotype had lower muscle mass, strength and power, but they also appeared to benefit the most from physical activity. No association of *ESR1* PvuII polymorphism with any of the muscle outcomes was observed.

Conclusions/Significance: The present study suggests that the *COMT* polymorphism, affecting the activity of the enzyme, is associated with muscle mass. Furthermore, sedentary individuals with potential high enzyme activity were the weakest group, but they may potentially benefit the most from physical activity. This observation elucidates the importance of both environmental and genetic factors in muscle properties.

Citation: Ronkainen PHA, Pöllänen E, Törmäkangas T, Tiainen K, Koskenvuo M, et al. (2008) Catechol-*O*-Methyltransferase Gene Polymorphism Is Associated with Skeletal Muscle Properties in Older Women Alone and Together with Physical Activity. *PLoS ONE* 3(3): e1819. doi:10.1371/journal.pone.0001819

Editor: A. Cecile J. W. Janssens, Erasmus University Medical Center, Netherlands

Received: June 21, 2007; **Accepted:** February 13, 2008; **Published:** March 19, 2008

Copyright: © 2008 Ronkainen et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by the Academy of Finland, Finnish Ministry of Education and the Lapland Fund of the Finnish Cultural Foundation. The Finnish Twin Cohort is part of the Academy of Finland Center of Excellence in Complex Disease Genetics for 2006–2011.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: paula.ronkainen@sport.jyu.fi

Introduction

One of the most important functions of skeletal muscle is voluntary movement. Muscle also serves as an amino acid reservoir and a site for various metabolic activities participating e.g. to the glucose metabolism of the whole body [1,2]. Absolute muscle mass is again critical, when the body has to recover from critical illnesses or traumatic injuries [3]. From midlife on, muscle strength declines, approximately one percent annually [4]. This decline may eventually predispose people to mobility limitation, falls and bone fractures [5–7]. Individual differences in muscle phenotypes in old age may be explained by both environmental and genetic factors [8–12].

In women, menopause is characterized by rapid decline in the production of estrogen, an anabolic female sex hormone, and coincides with an accelerated deterioration in muscle performance [13,14]. At phenotype level hormone replacement therapy (HRT)

exerts positive effects on skeletal muscle in some of the randomized, controlled trials reported [15–18], whereas others have documented contradictory results [19–21]. Moreover, a combination of high-impact training and HRT usage has been reported to exceed the beneficial effects of HRT alone [15]. Furthermore, in animal studies estrogen has been shown to contribute to skeletal muscle growth via specific receptors [22,23]. Given this putative role of estrogen in muscle function, genes related to estrogen action and metabolism are likely candidates contributing to the genetic component of muscle properties.

The synthesis and degradation of estrogens are mediated by several enzymes involved in multiple and complex metabolic pathways. After initial hydroxylation of estrogens by isoenzymes belonging to the cytochrome P450 family, they are further metabolized by catechol-*O*-methyltransferase (*COMT*) into more inactive methoxyestrogens. These *O*-methylated metabolites no longer bind to estrogen receptors (*ESRs*) [24], which mediate the

effects of estrogen in target tissues [25]. A functional G to A polymorphism in the fourth exon of *COMT* gene results in a valine to methionine amino acid transition at codon 158 (COMT Val158Met polymorphism) leading to thermolability [26,27] and lower activity of the enzyme. This could hence increase the availability of estrogen and induce the anabolic effects of this hormone on target tissues such as skeletal muscle. COMT represents an intracellular enzyme present in a variety of tissues [28]. Previous studies with men [29,30], early pubertal girls [31], as well as premenopausal [32] or postmenopausal women [33–35] have reported contradictory results, whether this polymorphism is actually associated with estrogen levels or not. To our knowledge, no studies have been published, in which the relationship of this polymorphism with skeletal muscle characteristics has been investigated.

ESR1 was recently shown to be expressed in human skeletal muscle [36] implying that skeletal muscle is sensitive to estrogen signaling, albeit to date the overall effects of estrogen on skeletal muscle remain poorly understood. PvuII polymorphism in the first intron of *ESR1* gene (ESR1 PvuII polymorphism) identifies a nucleotide T to C transition resulting in the loss of PvuII restriction site [37], which has been suggested to affect the magnitude of ESR1 transcription or production of various ESR1 isoforms [38]. More precisely, the C variant, also denoted as the P allele, is suggested to lead to the amplification of ESR1 transcription. In previous reports, no association of this polymorphic site with isometric muscle strength has been found [39,40].

Theoretically, polymorphisms residing in genes related to estradiol metabolism and action, in this case *COMT* and *ESR1*, may have shared effects on estradiol signaling in target tissues. More precisely, a polymorphism affecting the activity of COMT may directly or indirectly modulate the amount of estradiol available to be bound by membrane-bound or intracellular estrogen receptors, whereas a polymorphism potentially modulating the amount of ESR1 transcript may further affect the availability of these receptors. On the other hand, the two estrogen-related polymorphisms under investigation may act in conjunction with physical activity resulting in a specific muscle phenotype. Our aim was to determine whether the two estrogenic polymorphisms, COMT Val158Met and ESR1 PvuII, are associated with serum estradiol levels and skeletal muscle phenotypes or not. Furthermore, we studied the interaction of these polymorphisms and their interaction with physical activity.

Methods

Study subjects

This study is a part of the Finnish Twin Study on Aging (FITSA), which investigates the genetic and environmental effects on the disablement process in older female twins. The detailed study design, including selection procedures, determination of zygosity and description of the participants, has been reported elsewhere [10]. Briefly, participants were recruited from the Finnish Twin Cohort [41,42] consisting of 13 888 twin pairs. 103 monozygotic and 114 dizygotic female twin pairs (total n = 434 subjects) aged 63–76 years (mean 68.6 years, SE 0.16) were invited to laboratory investigations performed in Jyväskylä during 2000–2001. Shared willingness to participate obtained from both sisters was a prerequisite for recruiting. Zygosity was determined with a battery of ten highly polymorphic gene markers using DNA extracted from a venous blood sample. The Ethics Committee of the Central Finland Hospital approved the study and it was conducted according to the guidelines in The Declaration of

Helsinki. The subjects signed an informed consent before participating in the measurements.

In the whole study sample the prevalence of various diseases potentially affecting muscle properties were: 12 % for coronary heart disease, 8 % for asthma, 7 % for cerebrovascular disease, 6 % for type 2 diabetes, 4 % for rheumatoid arthritis, 29 % for knee, 14 % for hip and 12 % for foot and ankle osteoarthritis. 67 % of all the subjects had never used HRT, whereas 14 % were former users and 19 % current users. Moreover, 3 % of the subjects in the entire population were current cortisol users and 12 % former users, whereas 85 % had never been under long-term cortisol treatment. Subjects with the above-mentioned diseases and different HRT or cortisol status were equally distributed between genotypes.

Phenotyping

Hormone measurements. Nonfasting blood samples were collected between 0830 and 0930 hours and sera stored at -70°C for later analysis. Total serum 17β -estradiol was determined by competitive immunoenzymatic colorimetric assay (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) and serum sex hormone binding globulin (SHBG) levels using solid-phase, chemiluminescent immunometric assay (Immulite[®] 1000 SHBG, DPC [Diagnostic Products Corporation], Los Angeles, USA). Free estradiol levels were calculated from 17β -estradiol and SHBG levels according to a previously presented method [43,44]. In our laboratory, intra- and interassay coefficient of variations (CVs) were 3.2 % and 3.2 % for 17β -estradiol and 2.3 % and 6.5 % for SHBG, respectively.

Body anthropometry. Body mass and body height were measured using standard procedures. Lean body mass and total body fat were assessed by bioelectrical impedance (Spectrum II; RJL Systems, Detroit, MI, USA). The CV in our laboratory has been less than 2 % for lean body mass and less than 3 % for body fat [45]. Lower leg muscle cross-sectional area (mCSA), which gives an estimate of muscle mass, from the dominant hand side was assessed by peripheral quantitative computed tomography (pQCT, XCT-2000, Stratec Medizintechnik, Pforzheim, Germany). Tomography slices of two millimeters were obtained at 55 % upwards from the joint surface of the distal tibia. The whole mCSA of lower leg, i.e. the tissue area excluding subcutaneous fat and bones, was determined by Bonalyse 1.3 (Commit; Ltd, Espoo, Finland). CV for mCSA in our laboratory was 1 %.

Muscle strength and power. Maximal isometric strength, defined as the maximum voluntary contraction performed at a specific joint ankle against unyielding resistance, was measured for knee extension and hand grip. Maximal isometric hand grip and knee extension strengths were measured on the dominant side in a sitting position using an adjustable dynamometer chair (Good Strength, Metitur, Jyväskylä, Finland). After familiarization with the test, three to five maximal efforts separated by a one-minute interval were conducted. Mid-life maximal isometric hand grip strength, strength of a non-bearing limb, has been reported to correlate with strength of other muscle groups thus being a good indicator of overall strength [46], and also to be highly predictive of mortality and functional disability in later life [47,48]. Knee extension strength, on the other hand, represents the strength of a bearing limb, potentially prone to physical exercise or disuse. Knee extension strength is important for functional capacity as decline in strength together with poor balance is reported to predict severe walking disability [49]. Leg extensor power of single leg was assessed according to published guidelines [50] using the Leg Extensor Power Rig (Nottingham, UK). Leg extension power

measures the ability of the neuromuscular system to produce the greatest possible force as fast as possible. After two to three practice trials, five to nine maximal efforts were conducted. In all measurements, the best performance with the highest value was accepted as the result for each subject. CVs between two consecutive measurements have in our laboratory been 6 % for knee extension and hand grip strengths [51] and was 8 % for leg extension power in the present study. Physician evaluated possible contraindications for muscle strength and power measurements. All the subjects were able to perform at least one of the above-mentioned measurements.

Physical activity. Information concerning physical activity was collected using the scale of Grimby [52] with slight modifications. The participants were categorized on the basis of their self-reported physical activity as sedentary group (no other activities, but at the most light walking ≤ 2 times/week), moderately active (walking or other light exercise at least 3 times/week, but no other more intensive activities) and active (moderate or vigorous exercise at least 3 times/week). Of the entire population, 32 % were sedentary (sed), 48 % moderately active (mod) and 20 % active (act) according to this division. Subjects with various physical activity levels were equally distributed within the genotypes.

Genotyping

Genomic DNA was extracted from EDTA-anticoagulated whole blood according to standard procedures (PUREGENE® Kit, Gentra Systems Inc., Minneapolis, USA). Genotyping for Val158Met and PvuII polymorphisms was performed using PCR (thermal cycler: Eppendorf® Mastercycler® gradient, Eppendorf, Boulder, CO, USA) followed by restriction fragment length polymorphism (RFLP) analysis [37,53].

COMT Val158Met. The G to A transition at the 158th codon in the *COMT* gene was determined by copying a 109-bp fragment with a primer pair (Oligomer Oy, Helsinki, Finland) including forward (5'-CTCATC ACCATC GAGATC AA) and reverse primers (5'-CCAGGT CTGACA ACGGGT CA) [54]. PCR reaction mixture of 15 μ l contained 35 ng of DNA, 0.13 μ M of both primers, 0.4 mM of dNTP mix (Eppendorf, Boulder, CO, USA) and 1.5 U of HotMaster Taq polymerase (Eppendorf, Boulder, CO, USA). PCR conditions included a pre-incubation period of 2 min at 95°C after which the DNA was subjected to 40 cycles of 95°C for 1 min, 54°C for 1 min and 72°C for 1 min followed by final extension step of 5 min at 72°C. The resulting PCR product was digested by adding 5 U of NlaIII restriction endonuclease (New England Biolabs, Ipswich, MA, USA) and incubated overnight at 37°C. The resulting fragments were separated in a 4.5 % agarose gel and the genotypes determined. Genotypes were coded as HH, HL and LL, in which capital H denotes the presence of valine and thus high activity allele, whereas L refers to the presence of methionine and the low activity allele.

ESR1 PvuII. In ESR1 PvuII genotyping a 373-bp PCR fragment was produced using a primer pair (Oligomer Oy, Helsinki, Finland) consisting of forward (5'-GATATC CAGGGT TATGTG GCA) and reverse primers (5'-TTACCT CTTGCC GTCTGT TGC) in a 10 μ l reaction mixture containing 20 ng of DNA, 0.1 μ M of both primers, 0.4 mM of dNTP mix (Eppendorf, Boulder, CO, USA) and 0.35 U of HotMaster Taq polymerase (Eppendorf, Boulder, CO, USA). PCR reaction was carried out with the following steps: preheating at 95°C for 2 min followed by 36 cycles, in which denaturation was performed at 94°C for 45 s, annealing for 45 s with gradually declining temperature (36 cycles with declining temperature from 67°C to 60°C) and extension at

72°C for 45 s. The PCR product was digested by adding 5 U of PvuII restriction endonuclease (Invitrogen™, Carlsbad, CA, USA) and incubated for two hours at 37°C. The digested products were further separated by electrophoresis in a 3 % agarose gel. Genotypes were determined due to resulting fragments and coded as PP, Pp and pp. Uppercase letters indicate the absence and lowercase letters the presence of a restriction site.

RFLP identification was carried out by two independent investigators from whom data on phenotypes was concealed. Genotyping was successfully performed in 423 for COMT Val158Met and 421 for ESR1 PvuII site out of 434 subjects. Reasons for missing determinations include insufficient amount of DNA or contamination of the blood sample.

Statistical analyses

Hardy-Weinberg equilibrium was tested using the likelihood ratio test. Allele frequencies were determined by gene counting. All statistical models were constructed in SAS, version 9.1 using the generalized estimating equations approach (GEE), which allows taking into account the correlation between sisters within a twin pair. All outcome variables were normally distributed except for estradiol concentrations, which were skewed towards low concentrations and were considered to follow the gamma-distribution. Two types of single genotype models were constructed, one including the unadjusted main effects of the genotypes, and the other adjusted for age and height. To assess genotype-genotype and genotype-physical activity interactions a reference category was selected for the categorical predictor variables of physical activity (sedentary level), COMT (the HH genotype) and ESR1 PvuII (the pp genotype). Planned contrasts were used in comparing mean levels of each outcome variable between the predictor variable levels and their interactions against the reference category. Test-wise type I error rate was set at 0.05 in all analyses and partial correlation coefficients from the GEE model contrasts [55] were computed as estimates of effect size.

We hypothesized that subjects with assumed lower amount of circulating estradiol (HH genotype) and potential lower levels of ESR1 transcript (pp genotype) would be less responsive to estradiol and thus have worse muscle properties in comparison with other combinations. In the models including physical activity, subjects with potential low amount of circulating estradiol (HH genotype) or suggested low amount of ESR1 transcript (pp genotype) combined with sedentary life-style, were assumed to be weaker and have smaller muscles than other combinations. In our approach, reference groups were chosen according to these initial hypothesis and the mean values of other groups compared to that of the reference groups. The reference groups for the interaction effect were formed based on the combination of the main effect reference categories. The main effects of the two components of interest are always presented in contrast to the reference group.

Results

COMT Val158Met genotype

In our study population 79 subjects (18.0 %) were homozygous for the high activity allele (HH), 208 (47.9 %) heterozygotes (HL) and 137 (31.6 %) homozygous for the low active allele (LL). The allele frequencies were 0.43 for the H and 0.57 for the L allele. The genotype distribution of the entire cohort was in Hardy-Weinberg equilibrium ($\chi^2 = 0.004$, $p = 0.95$) suggesting that the subjects represented a homogeneous genetic background. Subject characteristics according to Val158Met genotypes are presented in Table 1. Physical characteristics and estradiol levels were similar in all Val158Met genotypes. However, mCSA was significantly larger

Table 1. Body composition, hormone levels and muscle properties categorized according to COMT Val158Met genotypes.

Variable	COMT genotypes			p for trend
	HH (n = 79)	HL (n = 208)	LL (n = 136–137)	
Weight (kg)	70.0 (1.7)	70.3 (1.0)	70.0 (1.2)	0.972
Height (cm)	157.5 (0.87)	158.2 (0.53)	159.7 (0.66)	0.085
Body fat (kg)	24.0 (1.1)	24.5 (0.8)	23.6 (0.87)	0.763
Lean body mass (kg)	46.1 (0.68)	45.6 (0.36)	46.5 (0.47)	0.194
BMI (kg/m ²)	28.2 (0.7)	28.2 (0.4)	27.6 (0.5)	0.565
Estradiol (nmol/l)	0.29 (0.051)	0.35 (0.054)	0.39 (0.069)	0.462
Free estradiol (nmol/l)	0.0059 (0.0008)	0.0073 (0.0010)	0.0083 (0.0015)	0.242
	(n = 74–79)	(n = 193–208)	(n = 131–136)	
Muscle CSA (mm ²)*	5950.9 (109.8)	5880.6 (73.7)	6199.8 (91.5)	0.014 [§]
Hand grip strength (N)*	190.6 (6.0)	189.3 (3.8)	194.0 (5.7)	0.763
Knee extension strength (N)*	352.5 (17.2)	322.5 (14.7)	343.4 (16.7)	0.092
Leg extension power (W)*	101.0 (4.2)	99.1 (2.6)	100.9 (3.4)	0.858

Data are mean (SE).

*Adjusted for age and height

[§]Contrasts: COMT^{LL} vs. COMT^{HL} (p = 0.004); COMT^{LL} vs. COMT^{HH} (p = 0.078); COMT^{HL} vs. COMT^{HH} (p = 0.569)

doi:10.1371/journal.pone.0001819.t001

in LL than HL individuals both in the unadjusted model (p = 0.001, data not shown) and after adjusting with age and height (p = 0.004, Table 1). No statistically significant association between Val158Met genotype and hand grip strength, knee extension strength or leg extension power was found.

ESR1 PvuII genotype

The most common genotype was Pp (n = 187, 43.1 %), whereas pp genotype was more frequent (n = 144, 33.2 %) than PP (n = 90, 20.7 %). The allele frequencies were 0.44 and 0.56 for the P and p alleles, respectively. The genotypes were slightly out of Hardy-Weinberg equilibrium ($\chi^2 = 3.943$, p = 0.047) suggesting that our study sample may not be representative of the target population. Physical characteristics, including hormone levels, were similar in

all PvuII genotypes. Furthermore, PvuII polymorphism was not associated with any of the measured muscle variables (Table 2).

Interaction of COMT and ESR1 polymorphisms with respect to muscle properties

We further studied whether ESR1 modified the effects of COMT. The results of age-adjusted models are shown in Table 3. In the model including COMT and ESR1 genotypes COMT-Val158Met polymorphic site had a main effect on mCSA. More precisely, individuals with the HH genotype had significantly smaller muscle mass than LL subjects (p = 0.038). Furthermore, a significant interaction was present in knee extension strength between HH and LL subjects (p = 0.031). Here, the mean difference between the comparison and reference groups was

Table 2. Body composition, hormone levels and muscle properties categorized according to ESR1 PvuII genotypes.

Variable	ESR1 genotypes			p for trend
	PP (n = 90)	Pp (n = 187)	pp (n = 144)	
Weight (kg)	70.3 (1.4)	69.6 (1.1)	70.7 (1.3)	0.797
Height (cm)	159.2 (0.88)	158.3 (0.58)	158.5 (0.63)	0.669
Body fat (kg)	24.3 (1.1)	23.7 (0.7)	24.6 (1.0)	0.664
Lean body mass (kg)	46.3 (0.52)	45.7 (0.44)	46.2 (0.44)	0.573
BMI (kg/m ²)	27.8 (0.6)	27.9 (0.4)	28.3 (0.5)	0.813
Estradiol (nmol/l)	0.36 (0.08)	0.31 (0.04)	0.40 (0.08)	0.584
Free estradiol (nmol/l)	0.0075 (0.0017)	0.0062 (0.0006)	0.0086 (0.0017)	0.375
	(n = 81–90)	(n = 177–187)	(n = 136–144)	
Muscle CSA (mm ²)*	6124.6 (121.5)	5927.3 (81.8)	6002.5 (97.4)	0.393
Hand grip strength (N)*	192.0 (6.3)	190.3 (4.4)	192.3 (5.2)	0.950
Knee extension strength (N)*	347.7 (17.8)	337.2 (14.8)	323.2 (15.1)	0.416
Leg extension power (W)*	99.2 (3.5)	98.3 (2.5)	103.5 (3.7)	0.494

Data are mean (SE).

*Adjusted for age and height

doi:10.1371/journal.pone.0001819.t002

Table 3. Mean differences (Mdf), standard errors (SE), p values and partial correlations ($r_{y.e}$) for genetic effects in age-adjusted models including COMT Val158Met and ESR1 PvuII polymorphisms for muscle CSA, hand grip strength, knee extension strength and leg extension power.

Effect (reference group)	Muscle CSA			Hand grip strength			Knee extension strength			Leg extension power						
	Mdf	SE	p value	$r_{y.e}$	Mdf	SE	p value	$r_{y.e}$	Mdf	SE	p value	$r_{y.e}$				
Val158Met main	-64.46	136.44	0.637	-0.034	0.76	7.37	0.918	0.007	-18.89	11.16	0.090	-0.124	-1.39	4.34	0.749	-0.023
effect (HH)	307.15	147.78	0.038	0.148	6.94	9.11	0.446	0.054	-6.80	12.11	0.574	-0.041	1.86	5.16	0.718	0.026
PvuII main effect	-60.34	125.72	0.631	-0.034	-7.92	7.83	0.312	-0.072	-15.32	11.74	0.192	-0.096	-4.48	4.79	0.349	-0.067
(pp)	165.45	151.92	0.276	0.078	-4.81	9.17	0.600	-0.037	-2.78	11.01	0.801	-0.019	-2.97	5.37	0.581	-0.040
Val158Met*PvuII	-256.27	318.00	0.420	-0.058	16.13	18.57	0.385	0.062	14.90	28.24	0.598	0.039	-0.59	10.86	0.957	-0.004
interaction effect	-304.91	381.91	0.425	-0.057	4.09	19.41	0.833	0.015	-15.94	26.63	0.549	-0.044	-6.98	11.41	0.540	-0.044
(HHpp)	-572.52	324.83	0.078	-0.126	-17.25	20.40	0.398	-0.060	-9.03	31.12	0.772	-0.021	0.70	12.39	0.955	0.004
LLPP	-302.04	380.25	0.427	-0.057	-44.14	25.18	0.080	-0.124	-61.44	28.44	0.031	-0.158	-18.20	13.14	0.166	-0.099

doi:10.1371/journal.pone.0001819.t003

-61.44, suggesting that an addition of two P alleles to LLpp genotype (LLpp→LLPP) leads to a lesser increase in knee extension strength in comparison to the HH genotype (HHpp→HHPP). Other interaction effects between COMT Val158Met and ESR1PvuII polymorphisms were not significant.

Interaction of COMT or ESR1 polymorphism and physical activity with respect to muscle properties

In further analyses we examined whether physical activity level modulates the effects of COMT Val158Met (Table 4 and Figure 1) or ESR1PvuII (Table 5) polymorphism on muscle properties. In the model including the COMT genotype, physical activity and age as explanatory variables, the genotype had a statistically significant main effect on mCSA; LL subjects were greater than HH subjects in their muscle size ($p = 0.021$). As expected, physical activity had a significant main effect on all the muscle strength and power variables (sedentary subjects were weaker than moderately active or active individuals, $p \leq 0.004$ for all comparisons), but the effect on mCSA was less clear ($p \geq 0.078$ for all comparisons). Significant interaction effects of the COMT genotype and physical activity were present in all muscle variables. In knee extension strength and leg extension power, all the interaction effects were statistically significant ($p < 0.05$ for all comparisons). The mean differences imply that in all these comparisons, an increase in physical activity from sedentary to moderate or from sedentary to active level within the HH genotype, creates a larger increase in both knee extension strength and leg extension power than among HL or LL individuals ($p \leq 0.045$). This trend was also evident in mCSA, although the effect between HH and HL subjects was not statistically significant when sedentary and active individuals were compared ($p = 0.41$). In hand grip strength a significant interaction effect was observed only between HH and HL individuals, when sedentary subjects were compared to their moderate active counterparts (Mdf = -36.76, $p = 0.011$). In general, the mean values of sedentary HH subjects in all the measured muscle outcomes were lower than subjects with other genotype and/or physical activity level (Figure 1). Moderately active or active subjects with HH genotype, however, had comparable values to those of other genotypes. The partial correlations for the main and interaction effects indicate that the effect sizes were small (0.1) or moderate (0.3) in all significant effects.

In the model including ESR1 genotype, physical activity and age as explanatory variables, physical activity had a main effect on muscle strength and power (sedentary subjects were weaker than moderately active or active individuals, $p \leq 0.004$, Table 5), but this effects was not observed in mCSA. Neither main effects of ESR1 nor interaction effects of ESR1 genotype and physical activity on any of the studied muscle properties were present.

Discussion

In the present study we examined the contribution of inter-individual variation in two candidate genes involved in estrogen metabolism and action, *COMT* and *ESR1*, to skeletal muscle properties in older women. We hypothesized that variation in these genes, essentially Val158Met polymorphism within *COMT* and PvuII polymorphism within *ESR1*, alone or together with physical activity may, at least partly, modulate muscle mass and performance phenotypes in older women. Our results suggest that COMT Val158Met polymorphism is associated with muscle mass in that subjects with the LL genotype have significantly larger muscles than heterozygotes. Furthermore, within the subjects with HH genotype – leading to the presumed higher COMT activity – and sedentary life-style, lower levels of muscle mass, strength and

Table 4. Mean differences (Mdf), standard errors (SE), p values and partial correlations ($r_{y.e}$) for genetic effects in age-adjusted models including COMT Val158Met polymorphism and physical activity for muscle CSA, hand grip strength, knee extension strength and leg extension power.

Effect (reference group)	Muscle CSA			Hand grip strength			Knee extension strength			Leg extension power			
	Mdf	SE	p value	$r_{y.e}$	Mdf	SE	p value	$r_{y.e}$	Mdf	SE	p value	$r_{y.e}$	
Val158Met main	HL 47.79	123.75	0.699	0.028	4.41	7.16	0.538	0.044	-4.87	10.62	0.646	-0.034	0.001
effect (HH)	LL 340.29	147.30	0.021	0.164	12.31	8.69	0.157	0.101	5.42	11.93	0.649	0.034	0.701
Physical activity	mod 135.35	105.35	0.199	0.092	17.58	6.05	0.004	0.204	48.19	9.97	< 0.001	0.337	< 0.001
main effect (sed)	act 223.79	127.19	0.078	0.126	24.22	7.12	0.001	0.237	60.91	10.67	< 0.001	0.390	< 0.001
Val158Met*physical	HLmod -588.22	246.92	0.017	-0.169	-36.76	14.52	0.011	-0.178	-65.69	23.74	0.006	-0.201	0.002
activity interaction	HLact -260.30	316.71	0.411	-0.059	-25.35	16.40	0.122	-0.110	-61.37	26.53	0.021	-0.169	0.007
effect (HHsed)	LLmod -849.69	260.99	0.001	-0.228	-25.24	16.59	0.128	-0.108	-53.31	26.58	0.045	-0.147	0.045
	LLact -644.78	331.07	0.051	-0.139	-21.59	17.69	0.222	-0.087	-62.35	28.02	0.026	-0.163	0.024

sed = sedentary

mod = moderately active

act = active

doi:10.1371/journal.pone.0001819.t004

power were observed than within other sedentary subjects or subjects with more active life-style.

Since the *O*-methylation of catechol estrogens by COMT occurs rapidly [24], Val158Met polymorphism within *COMT* may have significant contribution to circulating estrogen levels by mediating the activity of the enzyme. In the present study no statistically significant differences in total or free estradiol levels between different COMT genotypes were found, but there was a gradient, albeit not statistically significant, towards lower estradiol levels in HH individuals. Our subjects were postmenopausal women characterized by relatively low serum estradiol levels making their measurement with a direct assay difficult [56], thereby possibly affecting the accuracy of the results. From our data, the possibility that during the pre-menopausal years of our study subjects, i.e. the majority of their life-span, their estradiol levels may have been affected by COMT genotype, cannot be excluded. If so, their present muscle properties would also have been vulnerable to the effects of different hormone levels during these pre-menopausal years. Here, we can not rule out this period of their life, which certainly is evident in their present muscle phenotype. Thus far, contradictory results exist, whether this genotype can really affect free estradiol levels or not. In pubertal girls Val158Met genotype has been reported to affect free estradiol levels in that subjects with the low activity variant have higher levels of circulating estradiol [31]. Additionally, the same polymorphism has been demonstrated to result in higher bone mineral density in young men carrying the H allele compared to non-carriers [29] as well as increased non-vertebral fracture risk in elderly men with the L allele [30] without, however, an evident connection to free circulating estradiol levels. However, in postmenopausal women receiving an oral estradiol preparation, serum estradiol levels correlated significantly with Val158Met genotype [35], whereas no direct association between estradiol levels and Val158Met polymorphism was found elsewhere [32–34].

Our results suggest that Val158Met polymorphism affects muscle size such that LL genotype favors larger muscle cross-sectional area. This result is supported by a similar outcome in a study with early pubertal girls [31]. In previous studies muscle mass has been reported to be under rather strong genetic control [11,12], whereas genetic effects explained muscle strength and power to a lesser extent [9,10].

In our study sample, no association between PvuII polymorphism in *ESR1* and serum estradiol levels, or muscle structure or function was found. This supports the results from previous reports, in which no connection between this polymorphism and hand grip [39,40] or quadriceps isometric strength [39] has been found. The biological significance of PvuII polymorphism is hitherto unclear. Loss of PvuII restriction site has been reported to result in a potential binding site for B-myb transcription factor and in some settings this further led to induced transcription of a reporter gene [38]. In addition to this possibility to influence the mRNA levels of *ESR1*, the connections of PvuII polymorphism to e.g. bone phenotypes recognized so far may be due to some unknown polymorphism residing in close proximity of and being in linkage disequilibrium with PvuII locus within *ESR1*. This polymorphic site within *ESR1*, or less likely within an adjacent gene, may affect bone, but not muscle properties supporting our results together with the findings from previous studies [39,40].

In theory, polymorphisms within *COMT* and *ESR1* genes may act in concert by regulating the availability of estrogen and estrogen receptors, respectively. To the best of our knowledge, the present study is the first to examine, whether the genetic variation in *ESR1* modulates the effects of COMT Val158Met on muscle properties. According to our results, COMT genotype has a main

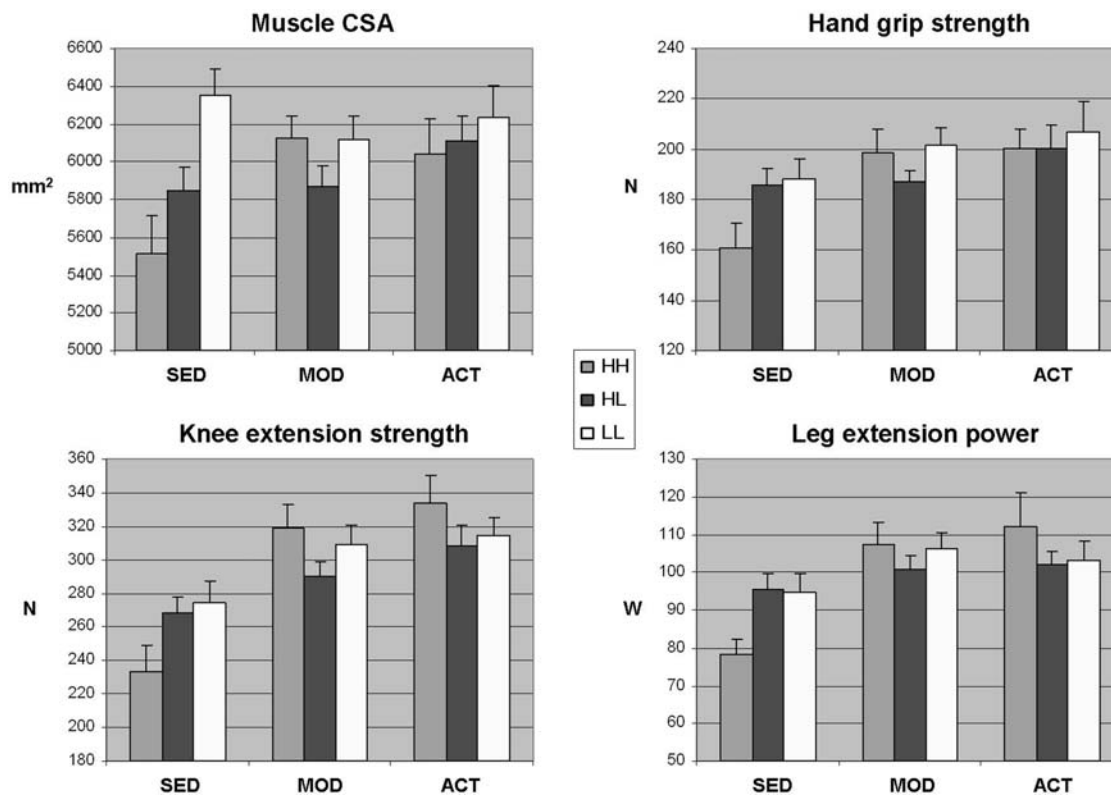


Figure 1. Muscle CSA, hand grip strength, knee extension strength and leg extension power according to COMT genotype and physical activity. Diagram presents the mean values (+SE) for CSA, hand grip strength, knee extension strength and leg extension power from GEE model according to COMT genotypes (HH, HL and LL) and physical activity (sed for sedentary, mod for moderately active and act for active). The model is adjusted with age. Results from statistical testing are shown in Table 4. doi:10.1371/journal.pone.0001819.g001

effect on muscle CSA regardless of the ESR1 genotype. Moreover, we observed an interaction effect of these polymorphisms on knee extension strength. This effect, however, seemed sporadic, given the small effect size and since other interaction effects were not present. Further studies are warranted in order to confirm this finding.

In the GEE model dissecting the interaction effects of COMT Val158Met polymorphism and physical activity on muscle properties a more clear gradient of the effects was observed. The HH subjects showed more variation in relation to physically active life-style compared to other genotypes as measured by knee extension strength and leg extension power. For example, the adjusted mean values in moderately active subjects with the HH genotype were 36.7 % higher in knee extension strength than in sedentary subjects with the same genotype, whereas within the HL genotype this difference was only 7.9 % in the favor of the moderately active subjects (Figure 1). In general, the sedentary subjects with the HH genotype were the weakest group, but those with the same genotype and more active life-style had comparable muscle properties to that of other genotypes with whichever level of activity. These data suggest that individuals with presumed low levels of circulating estradiol and thereby its minor effect on skeletal muscle can be prone to low muscle mass, strength and power, which may, however, be compensated for by physically active life-style. A clinical trial of muscle training among sedentary subjects with differing genotypes would be needed to confirm these observational data.

Physical activity had a significant main effect on muscle strength and power measures, in the models investigating the interaction

effects between COMT or ESR1 genotype and physically active life-style. Here, both moderately active and active individuals were stronger than sedentary subjects. In muscle mass, however, the effect was less clear or absent. This observation shows that our assessment of physical activity level with the modified scale of Grimby was in accordance with our expectations in muscle performance variables, but not in muscle mass. This notion seems reasonable taken into account the general mode of physical activity in older subjects; physical activity in the ages around 60 and 70 in general is not hypertrophying that would be evident as an increased muscle mass, but rather includes various types of aerobic everyday activities affecting the properties of muscle performance. Our assessment was clearly able to differentiate sedentary individuals from more active ones in the model investigating the interaction of physical activity with the COMT genotype.

A limitation of the present study is the relatively small sample size, which may have also been selected towards rather healthy women creating a possible healthy population bias. Moreover, we present results from various measurements describing muscle strength. Our test battery includes variables presenting both isometric (hand grip and knee extension strength) and dynamic (leg extension power) muscle performance as well as measures from both lower and upper limbs. Furthermore, during isometric testing, the speed of muscle contraction is not as essential as in muscle power measurements. On the other hand, these data provide a multifaceted estimate of the effects of the chosen genotypes on whole body musculature. The results provided by our cross-sectional data set should be further confirmed in a follow-up study and, if possible, with a larger sample and an

Table 5. Mean differences (Mdf), standard errors (SE), p values and partial correlations ($r_{y.e}$) for genetic effects in age-adjusted models including ESR1 PvuII polymorphism and physical activity for muscle CSA, hand grip strength, knee extension strength and leg extension power.

Effect (reference group)	Muscle CSA			Hand grip strength			Knee extension strength			Leg extension power			
	Mdf	SE	p value	$r_{y.e}$	SE	p value	Mdf	SE	p value	$r_{y.e}$	SE	p value	$r_{y.e}$
PvuII main effect Pp	-13.08	127.74	0.918	-0.007	7.41	0.508	-10.16	10.02	0.311	-0.075	4.49	0.210	-0.090
(pp)	170.25	162.36	0.294	0.075	8.78	0.749	-2.82	10.45	0.787	-0.020	5.23	0.537	-0.045
Physical activity mod	27.06	116.03	0.816	0.017	5.91	0.004	37.90	9.40	< 0.001	0.286	3.32	< 0.001	0.252
main effect (sed) act	115.03	132.59	0.386	0.062	6.87	< 0.001	50.63	9.67	< 0.001	0.362	3.80	0.001	0.232
PvuII* physical	-214.46	217.09	0.323	-0.071	13.05	0.493	-13.22	19.57	0.499	-0.050	7.44	0.179	-0.097
activity interaction	133.93	234.63	0.568	0.041	15.79	0.212	8.30	23.57	0.725	0.026	9.25	0.412	-0.059
effect (ppsed)	-270.12	316.90	0.394	-0.061	16.28	0.200	-22.52	23.62	0.340	-0.070	8.90	0.364	-0.065
PPact	-129.81	369.17	0.725	-0.025	18.20	0.479	-0.90	23.36	0.969	-0.003	10.05	0.896	0.010

sed = sedentary
mod = moderately active
act = active
doi:10.1371/journal.pone.0001819.t005

intervention trial to see, if the response to training is actually genotype-dependent.

In conclusion, the identification of the genetic susceptibility factors predisposing the elderly to impaired muscle performance could provide novel insights into the etiology of sarcopenia and would enable the recognition of those people at high risk of disability. Analysis of genetic variants, such as SNPs, represents a powerful approach to examine the role of candidate genes in the progression of this obviously multifactorial state. In the present study, we found an association between a polymorphism in the *COMT* gene and muscle mass. Furthermore, the interaction effect of this polymorphism and physical activity on muscle mass, strength and power elucidates the interplay of environmental and genetic factors in muscle properties and represents an example of how an unfavorable genetic background may perhaps be compensated for by healthy living habits, in this case physical

activity. Overall, our results imply that *COMT* gene, related to the metabolism of estrogens, may be connected with muscle properties, albeit the exact mechanisms remain unknown.

Acknowledgments

The authors want to thank all the women participating in this study and Kaisa-Leena Tulla, Erkki Helkala and Tuovi Nykänen for technical assistance in the lab.

Author Contributions

Conceived and designed the experiments: PR EP VK SS. Performed the experiments: PR EP KT. Analyzed the data: PR EP TT. Contributed reagents/materials/analysis tools: PR VK. Wrote the paper: PR. Other: Designed the original study: JK MK TR. Commented on the manuscript: EP TT KT JK MK TR SS VK.

References

- Nader GA (2005) Molecular determinants of skeletal muscle mass: Getting the "AKT" together. *Int J Biochem Cell Biol* 37: 1985–1996.
- Zurlo F, Larson K, Bogardus C, Ravussin E (1990) Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* 86: 1423–1427.
- Harridge SD (2007) Plasticity of human skeletal muscle: Gene expression to in vivo function. *Exp Physiol* 92: 783–797.
- Rantanen T, Masaki K, Foley D, Izmirlian G, White L, et al. (1998) Grip strength changes over 27 yr in japanese-american men. *J Appl Physiol* 85: 2047–2053.
- Doherty TJ (2003) Invited review: Aging and sarcopenia. *J Appl Physiol* 95: 1717–1727.
- Greenlund LJ, Nair KS (2003) Sarcopenia—consequences, mechanisms, and potential therapies. *Mech Ageing Dev* 124: 287–299.
- Roubenoff R, Castaneda C (2001) Sarcopenia—understanding the dynamics of aging muscle. *JAMA* 286: 1230–1231.
- Beunen G, Thomis M (2004) Gene powered? where to go from heritability (h²) in muscle strength and power? *Exerc Sport Sci Rev* 32: 148–154.
- Tiainen K, Sipilä S, Alen M, Heikkinen E, Kaprio J, et al. (2005) Shared genetic and environmental effects on strength and power in older female twins. *Med Sci Sports Exerc* 37: 72–78.
- Tiainen K, Sipilä S, Alen M, Heikkinen E, Kaprio J, et al. (2004) Heritability of maximal isometric muscle strength in older female twins. *J Appl Physiol* 96: 173–180.
- Loos R, Thomis M, Maes HH, Beunen G, Claessens AL, et al. (1997) Gender-specific regional changes in genetic structure of muscularity in early adolescence. *J Appl Physiol* 82: 1802–1810.
- Thomis MA, Van Leemputte M, Maes HH, Blimkie CJ, Claessens AL, et al. (1997) Multivariate genetic analysis of maximal isometric muscle force at different elbow angles. *J Appl Physiol* 82: 959–967.
- Phillips SK, Rook KM, Siddle NC, Bruce SA, Woledge RC (1993) Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clin Sci (Lond)* 84: 95–98.
- Samson MM, Meeuwse IB, Crowe A, Dessens JA, Duursma SA, et al. (2000) Relationships between physical performance measures, age, height and body weight in healthy adults. *Age Ageing* 29: 235–242.
- Sipilä S, Taaffe DR, Cheng S, Puolakka J, Toivanen J, et al. (2001) Effects of hormone replacement therapy and high-impact physical exercise on skeletal muscle in post-menopausal women: A randomized placebo-controlled study. *Clin Sci (Lond)* 101: 147–157.
- Taaffe DR, Sipilä S, Cheng S, Puolakka J, Toivanen J, et al. (2005) The effect of hormone replacement therapy and/or exercise on skeletal muscle attenuation in postmenopausal women: A yearlong intervention. *Clin Physiol Funct Imaging* 25: 297–304.
- Skelton DA, Phillips SK, Bruce SA, Naylor CH, Woledge RC (1999) Hormone replacement therapy increases isometric muscle strength of adductor pollicis in post-menopausal women. *Clin Sci (Lond)* 96: 357–364.
- Dobs AS, Nguyen T, Pace C, Roberts CP (2002) Differential effects of oral estrogen versus oral estrogen-androgen replacement therapy on body composition in postmenopausal women. *J Clin Endocrinol Metab* 87: 1509–1516.
- Kenny AM, Kleppinger A, Wang Y, Prestwood KM (2005) Effects of ultra-low-dose estrogen therapy on muscle and physical function in older women. *J Am Geriatr Soc* 53: 1973–1977.
- Ribom EL, Piehl-Aulin K, Ljunghall S, Ljunggren O, Naessen T (2002) Six months of hormone replacement therapy does not influence muscle strength in postmenopausal women. *Maturitas* 42: 225–231.
- Tanko LB, Movsesyan L, Svendsen OL, Christiansen C (2002) The effect of hormone replacement therapy on appendicular lean tissue mass in early postmenopausal women. *Menopause* 9: 117–121.
- Puah JA, Bailey CJ (1985) Effect of ovarian hormones on glucose metabolism in mouse soleus muscle. *Endocrinology* 117: 1336–1340.
- Rance NE, Max SR (1984) Modulation of the cytosolic androgen receptor in striated muscle by sex steroids. *Endocrinology* 115: 862–866.
- Zhu BT, Conney AH (1998) Functional role of estrogen metabolism in target cells: Review and perspectives. *Carcinogenesis* 19: 1–27.
- Deroo BJ, Korach KS (2006) Estrogen receptors and human disease. *J Clin Invest* 116: 561–570.
- Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, et al. (1995) Kinetics of human soluble and membrane-bound catechol O-methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 34: 4202–4210.
- Scanlon PD, Raymond FA, Weinshilboum RM (1979) Catechol-O-methyltransferase: Thermolabile enzyme in erythrocytes of subjects homozygous for allele for low activity. *Science* 203: 63–65.
- Mannisto PT, Kaakkola S (1999) Catechol-O-methyltransferase (COMT): Biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 51: 593–628.
- Lorentzon M, Eriksson AL, Mellstrom D, Ohlsson C (2004) The COMT val158met polymorphism is associated with peak BMD in men. *J Bone Miner Res* 19: 2005–2011.
- Stolk L, van Meurs JB, Jhamai M, Arp PP, van Leeuwen JP, et al. (2007) The catechol-O-methyltransferase Met158 "low activity" allele and association with non-vertebral fracture risk in elderly men. *J Clin Endocrinol Metab*.
- Eriksson AL, Suuriniemi M, Mahonen A, Cheng S, Ohlsson C (2005) The COMT val158met polymorphism is associated with early pubertal development, height and cortical bone mass in girls. *Pediatr Res* 58: 71–77.
- Lurie G, Maskarinec G, Kaaks R, Stanczyk FZ, Le Marchand L (2005) Association of genetic polymorphisms with serum estrogens measured multiple times during a 2-year period in premenopausal women. *Cancer Epidemiol Biomarkers Prev* 14: 1521–1527.
- Dunning AM, Dowsett M, Healey CS, Tee L, Luben RN, et al. (2004) Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst* 96: 936–945.
- TwoRoger SS, Chubak J, Aiello EJ, Ulrich CM, Atkinson C, et al. (2004) Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 13: 94–101.
- Worda C, Sator MO, Schneberger C, Jantschev T, Ferlitsch K, et al. (2003) Influence of the catechol-O-methyltransferase (COMT) codon 158 polymorphism on estrogen levels in women. *Hum Reprod* 18: 262–266.
- Lemoine S, Granier P, Tiffoche C, Rannou-Bekono F, Thieulant ML, et al. (2003) Estrogen receptor alpha mRNA in human skeletal muscles. *Med Sci Sports Exerc* 35: 439–443.
- Castagnoli A, Maestri I, Bernardi F, Del Senno L (1987) PvuII RFLP inside the human estrogen receptor gene. *Nucleic Acids Res* 15: 866.
- Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, et al. (2002) Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation* 105: 1879–1882.
- Vandevyver C, Vanhoof J, Declercq K, Stinissen P, Vandervorst C, et al. (1999) Lack of association between estrogen receptor genotypes and bone mineral density, fracture history, or muscle strength in elderly women. *J Bone Miner Res* 14: 1576–1582.
- Salmen T, Heikkinen AM, Mahonen A, Kroger H, Komulainen M, et al. (2002) Relation of estrogen receptor-alpha gene polymorphism and hormone replacement therapy to fall risk and muscle strength in early postmenopausal women. *Ann Med* 34: 64–72.

41. Kaprio J, Pulkkinen L, Rose RJ (2002) Genetic and environmental factors in health-related behaviors: Studies on Finnish twins and twin families. *Twin Res* 5: 366–371.
42. Kaprio J, Sarna S, Koskenvuo M, Rantasalo I (1978) The Finnish twin registry: Formation and compilation, questionnaire study, zygosity determination procedures, and research program. *Prog Clin Biol Res* 24 Pt B. pp 179–184.
43. Bjornerem A, Straume B, Midtby M, Fonnebo V, Sundsfjord J, et al. (2004) Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: The Tromsø study. *J Clin Endocrinol Metab* 89: 6039–6047.
44. Sodergard R, Backstrom T, Shanbhag V, Carstensen H (1982) Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem* 16: 801–810.
45. Sipila 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 Physiol Scand* 156: 457–464.
46. Rantanen T, Era P, Kauppinen M, Heikkinen E (1994) Maximal isometric muscle strength and socio-economic status, health and physical activity in 75-year-old persons. *J Aging Phys Activity* 2: 206–220.
47. Fujita Y, Nakamura Y, Hiraoka J, Kobayashi K, Sakata K, et al. (1995) Physical-strength tests and mortality among visitors to health-promotion centers in Japan. *J Clin Epidemiol* 48: 1349–1359.
48. Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, et al. (1999) Midlife hand grip strength as a predictor of old age disability. *JAMA* 281: 558–560.
49. Rantanen T, Guralnik JM, Ferrucci L, Penninx BW, Leveille S, et al. (2001) Coimpairments as predictors of severe walking disability in older women. *J Am Geriatr Soc* 49: 21–27.
50. Bassey EJ, Short AH (1990) A new method for measuring power output in a single leg extension: Feasibility, reliability and validity. *Eur J Appl Physiol Occup Physiol* 60: 385–390.
51. 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. *J Am Geriatr Soc* 45: 1439–1445.
52. Grimby G (1986) Physical activity and muscle training in the elderly. *Acta Med Scand Suppl* 711: 233–237.
53. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, et al. (1996) Human catechol-O-methyltransferase pharmacogenetics: Description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 6: 243–250.
54. Al-Hendy A, Salama SA (2006) Catechol-O-methyltransferase polymorphism is associated with increased uterine leiomyoma risk in different ethnic groups. *J Soc Gynecol Investig* 13: 136–144.
55. Natarajan S, Lipsitz S, Parzen M, Lipshultz S (2007) A measure of partial association for generalized estimating equations. *Statistical modelling* 7: 175–190.
56. Lee JS, Ettinger B, Stanczyk FZ, Vittinghoff E, Hanes V, et al. (2006) Comparison of methods to measure low serum estradiol levels in postmenopausal women. *J Clin Endocrinol Metab* 91: 3791–3797.