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

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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.

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 thermalolability [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 isoforms [39]. 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 characteristics has been investigated.

ESR1 has also been suggested to lead to amplification of ESR1 isoforms [38]. More precisely, the C variant, also denoted as the P allele, is suggested to lead to the amplification of ESR1 isoforms. Previous reports, no association of this polymorphic site with isometric muscle strength has been found [39,40].

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 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 Bonalyste 1.3 (Commit; Ltd, Espoo, Finland). CV for mCSA in our laboratory was 1 %.

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 Bonalyste 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 angle 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, Mettiur, 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 [32] 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 ACCATG GAGATC AA) and reverse primers (5'-CCAGGG CTGACA ACGGGT CA) [54]. PCR reaction mixture of 15 µl contained 35 ng of DNA, 0.13 µM 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 for two hours at 37°C. The resulting fragments 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 generalised 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 lower 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 (χ²=0.004, p =0.95) suggesting that the subjects represented a homogenous 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.
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 (χ² = 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

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**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/m²)               | 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 |
| Muscle CSA (mm²)*         | 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

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**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)     | 66.9 (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/m²)               | 28.8 (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 |
| Muscle CSA (mm²)*         | 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

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Table 3. Mean difference (Mdf), standard error (SE), p-value and partial correlations (r_Y) 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                          | Mdf  | SE   | Mdf  | SE   | Mdf  | SE   | Mdf  | SE   | Mdf  | SE   |
|--------------------------------|------|------|------|------|------|------|------|------|------|------|
| Muscle CSA                     |      |      |      |      |      |      |      |      |      |      |
| Val158Met main                 | 0.651| 0.039| 0.76 | 0.048| 0.168| 0.046| 0.18 | 0.052| 0.007| 0.014|
| Val158Met main                 |      |      |      |      |      |      |      |      |      |      |
| ESR1 main effect               |      |      |      |      |      |      |      |      |      |      |
| PvuII main effect              |      |      |      |      |      |      |      |      |      |      |
| Interaction effect             |      |      |      |      |      |      |      |      |      |      |
| Knee extension strength        |      |      |      |      |      |      |      |      |      |      |
| Val158Met main                 |      |      |      |      |      |      |      |      |      |      |
| ESR1 main effect               |      |      |      |      |      |      |      |      |      |      |
| PvuII main effect              |      |      |      |      |      |      |      |      |      |      |
| Interaction effect             |      |      |      |      |      |      |      |      |      |      |
| Hand grip strength             |      |      |      |      |      |      |      |      |      |      |
| Val158Met main                 |      |      |      |      |      |      |      |      |      |      |
| ESR1 main effect               |      |      |      |      |      |      |      |      |      |      |
| PvuII main effect              |      |      |      |      |      |      |      |      |      |      |
| Interaction effect             |      |      |      |      |      |      |      |      |      |      |
| Leg extension power            |      |      |      |      |      |      |      |      |      |      |
| Val158Met main                 |      |      |      |      |      |      |      |      |      |      |
| ESR1 main effect               |      |      |      |      |      |      |      |      |      |      |
| PvuII main effect              |      |      |      |      |      |      |      |      |      |      |
| Interaction effect             |      |      |      |      |      |      |      |      |      |      |

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 COMTVal158Met (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≤0.004 for all comparisons), but the effect on mCSA was less clear (p=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≤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≤0.004, Table 5), but this effect 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 interindividual 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 (Md±), standard errors (SE), p values and partial correlations ($r_{p}$) 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 | Muscle CSA | Hand grip strength | Knee extension strength | Leg extension power |
|--------|------------|--------------------|------------------------|---------------------|
|        | Mdf | SE | p value | $r_{p}$ | Mdf | SE | p value | $r_{p}$ | Mdf | SE | p value | $r_{p}$ | Mdf | SE | p value | $r_{p}$ |
| Val158Met main |        |     |         |       |     |     |         |       |     |     |         |       |     |     |         |       |
| HL     | 47.79 | 123.75 | 0.699   | 0.028 | 4.411 | 7.16 | 0.538   | 0.044 | 4.87 | 10.62 | 0.646  | 0.034 | 0.01 | 4.34 | 0.997  | 0.000  |
| LL     | 340.29 | 147.30 | 0.021   | 0.164 | 12.311 | 8.69 | 0.157   | 0.101 | 5.42 | 11.93 | 0.649  | 0.034 | 1.99 | 5.18 | 0.701  | 0.028  |
| Physical activity mod | |     |         |       |     |     |         |       |     |     |         |       |     |     |         |       |
| 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 | 0.323 |
| main effect (sed) act | |     |         |       |     |     |         |       |     |     |         |       |     |     |         |       |
| act    | 223.79 | 127.19 | 0.078   | 0.126 | 24.22 | 7.12 | 0.001   | 0.237 | 60.91 | 10.67 | 0.000  | 0.390 | 0.001 |
| Val158Met phy physical HL mod | |     |         |       |     |     |         |       |     |     |         |       |     |     |         |       |
| HL mod | -849.69 | 260.99 | 0.017   | -0.169 | -367.6 | 14.52 | 0.011   | -0.178 | -65.69 | 23.74 | 0.006  | -0.201 | -23.76 | 7.82 | 0.002  |
| activity interaction HAct | |     |         |       |     |     |         |       |     |     |         |       |     |     |         |       |
| HAct   | -260.30 | 316.71 | 0.411   | -0.059 | -253.5 | 16.40 | 0.122   | -0.110 | -61.37 | 26.53 | 0.021  | -0.169 | -27.57 | 10.24 | 0.007  |
| effect (HHsed) act | |     |         |       |     |     |         |       |     |     |         |       |     |     |         |       |
| act    | -644.78 | 331.07 | 0.051   | -0.139 | -21.59 | 17.69 | 0.222   | -0.087 | -62.35 | 28.02 | 0.026  | -0.163 | -25.42 | 11.26 | 0.024  |

sed = sedentary
mod = moderately active
act = active

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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. 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

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.

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

| Effect                  | Muscle CSA | Hand grip strength | Knee extension strength | Leg extension power |
|-------------------------|------------|--------------------|-------------------------|---------------------|
|                         | Mdf        | SE                 | p value                | r_{YX}              | Mdf         | SE        | p value         | r_{YX}              | Mdf         | SE        | p value       | r_{YX}             | Mdf         | SE        | p value        | r_{YX}             |
| PvulI main effect Pp    | –13.08     | 127.74             | 0.918                  | −0.007               | −4.90       | 7.41      | 0.508            | 0.047               | −10.16      | 10.02     | 0.311         | −0.075             | −5.63       | 4.49      | 0.210          | −0.090             |
| (pp)                    | 170.25     | 162.36             | 0.294                  | 0.075                | −2.81       | 8.78      | 0.749            | 0.023               | −2.82       | 10.45     | 0.787         | −0.020             | −3.23       | 5.23      | 0.537          | −0.045             |
| Physical activity mod   | 27.06      | 116.03             | 0.816                  | 0.017                | 17.05       | 5.91      | **0.004**       | 0.202               | 37.90       | 9.40      | **0.001**    | 0.286              | 11.95       | 3.32      | **<0.001**    | 0.252              |
| main effect (sed) act   | 115.03     | 132.59             | 0.386                  | 0.062                | 26.27       | 6.87      | **<0.001**      | 0.264               | 50.63       | 9.67      | **<0.001**   | 0.362              | 12.53       | 3.80      | **0.001**      | 0.232              |
| PvuI* physical activity | Ppmod      | –214.46            | 217.09                 | 0.323                | −0.071      | −8.96     | 13.05           | 0.493               | −13.22      | 19.57     | 0.499         | −0.050             | −9.99       | 7.44      | 0.179          | −0.097             |
| interaction             | Ppact      | 133.93             | 234.63                 | 0.568                | 0.041       | −19.71    | 15.79           | 0.212               | 0.089       | 8.30      | 0.725         | 0.026              | −7.58       | 9.25      | 0.412          | −0.059             |
| effect (psed)           | PPmod      | –270.12            | 316.90                 | 0.394                | −0.061      | 20.86     | 16.28           | 0.200               | 0.091       | −22.52    | 23.62         | 0.430              | −0.070      | 8.07      | 8.90           | 0.364              | −0.065       |
| PPact                  | −129.81    | 369.17             | 0.725                  | −0.025               | 12.89       | 18.20     | 0.479            | 0.051               | −0.90       | 23.36     | 0.969         | −0.003             | 1.32        | 10.05     | 0.896          | 0.010              |

sed = sedentary
mod = moderately active
act = active
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intervention trial to see if the response to training is actually 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.

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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.

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