Role of selected polymorphisms in determining muscle fiber composition in Japanese men and women

Hiroshi Kumagai,1,2 Takuro Tobina,3 Noriko Ichinoseki-Sekine,1,4 Ryo Kakigi,5 Takamasa Tsuzuki,1 Hirofumi Zempo,4 Keisuke Shiose,7,11 Eiichi Yoshimura,8 Hideaki Kumahara,9 Makoto Ayabe,10 Yasuki Higaki,11 Ryo Yamada,12 Hiroyuki Kobayashi,13 Akira Kiyonaga,11 Hisashi Naito,1 Hiroaki Tanaka,11 and Noriyuki Fuku1

1Graduate School of Health and Sports Science, Juntendo University, Chiba, Japan; 2Japanese Society for the Promotion of Science, Tokyo, Japan; 3Faculty of Nursing and Nutrition, University of Nagasaki, Nagasaki, Japan; 4Faculty of Liberal Arts, The Open University of Japan, Chiba, Japan; 5Faculty of Medicine, Juntendo University, Tokyo, Japan; 6Faculty of Health and Nutrition, Tokyo Seiei College, Tokyo, Japan; 7Faculty of Medicine, Nagasaki University, Nagasaki, Japan; 8Department of Food and Health Sciences, Prefectural University of Kumamoto, Kumamoto, Japan; 9Faculty of Nutritional Sciences, Nakamura Gakuen University, Fukuoka, Japan; 10Faculty of Computer Science and Systems Engineering, Okayama Prefectural University, Okayama, Japan; 11Faculty of Sports and Health Science, Fukuoka University, Fukuoka, Japan; 12Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; and 13Department of General Medicine, Mito Medical Center, Tsukuba University Hospital, Ibaraki, Japan

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Address for reprint requests and other correspondence: N. Fuku, Graduate School of Health and Sports Science, Juntendo Univ., 1-1 Hiraka-gakuendai, Inzai-city, Chiba 270-1695, Japan (e-mail: noriyuki.fuku@nifty.com).

Kumagai H, Tobina T, Ichinoseki-Sekine N, Kakigi R, Tsuzuki T, Zempo H, Shiose K, Yoshimura E, Kumahara H, Ayabe M, Higaki Y, Yamada R, Kobayashi H, Kiyonaga A, Naito H, Tanaka H, Fuku N. Role of selected polymorphisms in determining muscle fiber composition in Japanese men and women. J Appl Physiol 124: 1377–1384, 2018. First published January 18, 2018; doi:10.1152/japplphysiol.00953.2017.—Genetic polymorphisms and sex differences are suggested to affect muscle fiber composition; however, no study has investigated the effects of genetic polymorphisms on muscle fiber composition with respect to sex differences. Therefore, the present study examined the effects of genetic polymorphisms on muscle fiber composition with respect to sex differences in the Japanese population. The present study included 211 healthy Japanese individuals (102 men and 109 women). Muscle biopsies were obtained from the vastus lateralis to determine the proportion of myosin heavy chain (MHC) isoforms (MHC-I, MHC-IIa, and MHC-IIx). Moreover, we analyzed polymorphisms in α-actinin-3 gene (ACTN3; rs1815739), angiotensin-converting enzyme gene (ACE; rs4341), hypoxia-inducible factor 1 α gene (rs11549465), vascular endothelial growth factor receptor 2 gene (rs1870377), and angiotensin II receptor, type 2 gene (rs11091046), by TaqMan single-nucleotide polymorphism genotyping assays. The proportion of MHC-I was 9.8% lower in men than in women, whereas the proportion of MHC-IIa and MHC-IIx was higher in men than in women (5.0 and 4.6%, respectively). Men with the R577X and ACTN3 RX genotype had a 4.7% higher proportion of MHC-I than those with the ACTN3 DD genotype. The ID + DD genotype had a 4.7% higher proportion of MHC-I than those with the ACE ID + DD genotype, and the ACE ID + DD genotype had a 4.7% higher proportion of MHC-I than those with the ACE II genotype. Furthermore, a combined genotype of the ACTN3 R577X and ACE insertion/deletion (I/D) was significantly correlated with the proportion of MHC-I (r = −0.23) and MHC-IIx (r = 0.27) in men. In contrast, no significant correlation was observed between the examined polymorphisms and muscle fiber composition in women. These results suggest that the ACTN3 R577X and ACE I/D polymorphisms independently affect the proportion of human skeletal muscle fibers MHC-I and MHC-IIx in men but not in women.

NEW & NOTEWORTHY In men, the RR + RX genotype of the α-actinin-3 gene (ACTN3) R577X polymorphism was associated with a higher proportion of myosin heavy chain (MHC-IIx). The ID + DD genotype of the angiotensin-converting enzyme gene (ACE) insertion/deletion (I/D) polymorphism, in contrast to a previous finding, was associated with a higher proportion of MHC-I in men. In addition, the combined genotype of these polymorphisms was correlated with the proportion of MHC-I and MHC-IIx in men. Thus ACTN3 R577X and ACE I/D polymorphisms influence the muscle fiber composition in Japanese men.

ACE; ACTN3; myosin heavy chain isoform; polymorphism; sex difference

INTRODUCTION

Human skeletal muscles are composed of two main fiber types, namely, types I and II; type II muscle fibers are further divided into subgroups IIA and IIx (8). Type I fibers show high resistance to fatigue and are suitable for endurance performance, type IIA fibers are suitable for medium-term anaerobic exercise, and type IIx fibers are suitable for short bursts of strength and speed (5, 14). Type I fibers contain high levels of oxidative enzymes and low levels of glycolytic enzymes, whereas type IIx fibers contain high levels of glycolytic enzymes and low levels of oxidative enzymes; in contrast, the properties of type IIA fibers are intermediate to those of types I and IIx fibers (11). Simoneau and Bouchard (38) reported large, interindividual differences in the fiber-type composition of human skeletal muscle (i.e., 15–85% type I fibers, 5–77% type IIa, 0–44% type IIx) in healthy individuals. This variation in the composition of skeletal muscle fibers partly explains the marked difference in the physical performance of individuals, such as endurance running performance (32, 49), and occur-
ence of lifestyle-related diseases, such as obesity, type 2 diabetes mellitus, and hypertension (7, 15).

Genetic factors are suggested to play an important role in determining human skeletal muscle fiber composition. Komi et al. (18) reported for the first time that heritability estimates for muscle fiber composition were 99.5% in men and 92.8% in women. However, a sample size of that study was relatively small. Results of a previous study performed by Simonneau and Bouchard (37) showed that genetic factors (~45%) contributed more to the determination of the muscle fiber composition than environmental factors (~40%), with the remaining 15% because of muscle sampling and technical variance. These findings indicate that genetic factors exert a greater effect than environmental factors or that both of these factors exert comparable effects in determining muscle fiber composition.

Several studies have reported that some genetic polymorphisms, such as R577X (rs1815739) in the hypoxia-inducible factor 1 gene (HIF1A) (2), Q472H (rs1870377) in the vascular endothelial growth factor receptor 2 gene (VEGFR2) (3), and C/A polymorphism (rs11091046) in the angiotensin II receptor, type 2 gene (AGTR2) (25), are associated with muscle fiber composition. However, these findings are yet to be confirmed by other studies. Moreover, a sex-based difference has only been considered in one previous study (28).

Although many studies have shown sex-based differences in muscle fiber composition, conflicting reports are available on the proportion of fast and slow muscle fibers in men and women. Several studies have reported a higher proportion of type I fibers in women than in men (21, 38–40), whereas several studies have reported a higher proportion of type I fibers in men than in women (12, 17). Furthermore, several studies have reported no difference in the proportion of type I fibers between men and women (29, 34). These conflicting results may be associated with differences in sample size, age, ethnicity, and genetic background of study subjects included in these studies. Therefore, further studies are necessary to confirm the association between genetic polymorphisms and muscle fiber composition in men and women separately.

Therefore, the present study investigated the effects of five previously published genetic polymorphisms, namely, ACTN3 R577X, ACE I/D, HIF1A C/T, VEGFR2 Q472H, and AGTR2 C/A, on muscle fiber composition with respect to sex-based differences in the general Japanese population.

MATERIALS AND METHODS

Subjects. The present study included 211 Japanese subjects. The subjects were recruited from Juntendo University (30 men and 22 women; age 20–43 yr) and Fukuoka University (72 men and 87 women; age 21–79 yr). All of the subjects provided written informed consent before their inclusion in this study. The study protocols were approved by the Ethics Committees of Juntendo University and Fukuoka University.

Aerobic fitness. Peak oxygen consumption (VO2peak) was measured using an incremental exercise test (15 W/min for men and 10 W/min for women) on a bicycle ergometer (Rehcor; Lode BV, Groningen, Netherlands). The test was continued until subjective exhaustion was achieved. We assumed that the participants had reached VO2peak when at least two of the following criteria were met: 1) a plateau in VO2 with an increase in the work load; 2) blood lactate levels ≥8.0 mM; 3) a respiratory exchange ratio ≥1.15; 4) heart rate within 10 beats of the predicted maximum heart rate; and 5) ratings of perceived exertion ≥19. Respiratory gas analysis was conducted using the mixing chamber method to evaluate the volume of expired air, and the O2 and CO2 fractions were analyzed by mass spectrometry (ARCO 1000 and 2000; Arco System, Chiba, Japan). Lactic acid was analyzed using the portable blood lactate analyzer (Lactate Pro; Arkay, Kyoto, Japan). To take into account individual differences in body weight, VO2 was expressed as kilograms of body weight.

Genotyping. Total DNA was isolated from the venous blood of the study subjects by using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. The total DNA content was measured using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Subsequently, DNA samples were adjusted to a concentration of 10 ng/µl with Tris-EpTDA buffer and were stored at 4°C. ACTN3 R577X (rs1815739), ACE C/G (I/D) (rs4341), HIF1A C/T (rs11549465), VEGFR2 Q472H (rs1870377), and AGTR2 C/A (rs11091046) polymorphisms were genotyped using a real-time thermocycler with an end-point analysis mode (LightCycler 480; Roche Applied Science, Mannheim, Germany) by using the TaqMan Single-Nucleotide Polymorphism (SNP) Genotyping Assay [assay identifications, ACTN3 R577X: C_590093_1_, HIF1A C/T: C_25473074_10, VEGFR2 Q472H: C_11895315_20, AGTR2 C/A: C_1841568_10]. A total of 5 µl of the genotyping mixture, containing 2.5 µl TaqMan GTXpress Master Mix (2×), 0.0625 µl TaqMan SNP Genotyping Assay (40×), and 1.4375 µl distilled water, was mixed with 1 µl genomic DNA (10 ng/µl) for each reaction. Four negative controls were included on each plate. Thermal cycling conditions included an initial denaturation at 95°C for 20 s, followed by 40 cycles of denaturation at 95°C for 3 s and annealing/extension at 60°C for 20 s. The ACE I/D genotype (rs4340) was determined using the ACE C/G genotype (rs4341), which is in perfect linkage disequilibrium with the I/D genotype as follows: C/C as II, C/G as ID, and G/G as DD (43). Allelic discrimination analysis was performed using LightCycler 480 software version 1.5.1.62 (Roche Applied Science). To confirm the accuracy of genotyping by the TagMan SNP Genotyping Assay, we subjected at least 96 DNA samples, for which each genetic polymorphism sequence had been determined by direct sequencing. In each instance, the genotypes determined by the TaqMan SNP Genotyping Assay were identical to that determined by direct sequencing.

Muscle biopsy. For this, 10–15 mg muscle samples were obtained from the belly of the vastus lateralis under sterile conditions and local anesthesia (1% lidocaine) by using a disposal needle-biopsy instrument (Max Core or Magnum; C.R. Bard, Covington, GA). The obtained muscle samples were frozen immediately in liquid nitrogen and were stored at −80°C until further analysis.

SDS-PAGE analysis of myosin heavy chain isoforms. We assessed myosin heavy chain (MHC) isoforms as markers of the muscle fiber

| Table 1. Characteristics of subjects |
|-------------------------------------|
|                                    |
| **Men (n = 102)**                   |
| Age, yr                            | 46.7 ± 17.8 |
| Height, cm                         | 169.7 ± 6.1 |
| Body mass, kg                      | 74.7 ± 11.5 |
| BML kg/m²                          | 25.9 ± 3.9 |
| VO2peak ml-min⁻¹kg⁻¹†              | 26.2 ± 6.1 |
| MHC-I, %                           | 40.5 ± 11.7 |
| MHC-II, %                          | 35.8 ± 8.3 |
| MHC-IX, %                          | 23.6 ± 9.2 |

**Women (n = 109)**

| Age, yr                            | 47.7 ± 16.5 |
| Height, cm                         | 156.7 ± 5.9*|
| Body mass, kg                      | 63.9 ± 9.9* |
| BML kg/m²                          | 25.5 ± 4.3 |
| VO2peak ml-min⁻¹kg⁻¹†              | 23.3 ± 5.2 |
| MHC-I, %                           | 50.3 ± 11.1*|
| MHC-II, %                          | 30.8 ± 8.2* |
| MHC-IX, %                          | 19.0 ± 8.3* |

BML, body mass index; MHC, myosin heavy chain; VO2peak, peak oxygen consumption. Data are expressed as means ± SD. *P < 0.001 vs. men. †Data are available in 53 and 64 in men and women, respectively. ‡P < 0.01 vs. men.
Table 2. Correlations (r) among each characteristic and muscle fiber composition in men and women

|                | MHC-I | MHC-IIa | MHC-IIx |
|----------------|-------|---------|---------|
| Men            |       |         |         |
| Age, yr        | 0.35† | −0.36*  | −0.13   |
| Height, cm     | −0.13 | 0.07    | 0.10    |
| Body mass, kg  | −0.08 | −0.05   | 0.15    |
| BMI, kg/m²     | −0.03 | −0.08   | 0.12    |
| VO₂peak, ml·min⁻¹·kg⁻¹ | 0.17 | −0.04   | −0.17   |
| Women          |       |         |         |
| Age, yr        | 0.22† | −0.18   | −0.12   |
| Height, cm     | −0.15 | 0.26*   | −0.06   |
| Body mass, kg  | −0.12 | −0.05   | 0.21†   |
| BMI, kg/m²     | −0.03 | −0.16   | 0.24†   |
| VO₂peak, ml·min⁻¹·kg⁻¹ | 0.01 | 0.05    | −0.06   |

BMI, body mass index; MHC, myosin heavy chain; VO₂peak, peak oxygen consumption. *P < 0.01. †P < 0.05.

Results

Men and women included in the present study did not show any significant difference with respect to age but showed significant differences in height body mass, body mass index (BMI), and VO₂peak (Table 1). The relative proportion of MHC-I was significantly lower in men than in women (40.5 ± 11.7 vs. 50.3 ± 11.1%, P < 0.001), whereas the relative proportion of MHC-IIa (35.8 ± 8.3 vs. 30.8 ± 8.2%, P < 0.001) and MHC-IIx (23.6 ± 9.2 vs. 19.0 ± 8.3%, P < 0.001) was significantly higher in men than in women. The correlations for each subject’s physical characteristics and muscle fiber composition in men and women are shown in Table 2. In men, age was significantly correlated with the proportion of MHC-I (r = 0.35, P < 0.001) and MHC-IIa (r = −0.36, P < 0.001). In women, age was significantly correlated with the proportion of MHC-I (r = 0.22, P = 0.023), height was significantly associated with the proportion of MHC-IIa (r = 0.26, P = 0.006), and body mass (r = 0.21, P = 0.032) and BMI (r = 0.24, P = 0.012) were significantly associated with the proportion of MHC-IIx.

All of the polymorphisms followed the Hardy-Weinberg equilibrium. The rate of genotyping success was 211/211 (100%) for ACTN3 R577X (rs1815739), 209/211 (99.1%) for ACE I/D (rs4340), 207/211 (98.1%) for HIF1A C/T. Covariates included in the multiple linear regression models were age, BMI, ACTN3 R577X genotype (rs1815739), ACE I/D genotype (rs4340), HIF1A C/T genotype (rs11549465), VEGFR2 Q472H genotype (rs1870377), and AGTR2 C/A genotype (rs11091046). Values in bold denote P < 0.05.
(rs11549465), 208/211 (98.6%) for VEGFR2 Q472H (rs1870377), and 209/211 (99.1%) for AGTR2 C/A (rs11091046). Table 3 shows independent determinants of the composition of each muscle fiber in men and women. In men, the ACE I/D genotype was significantly associated with MHC-I, and the ACTN3 R577X genotype was significantly associated with MHC-IIx. In contrast, no significant association was observed between muscle fiber composition and HIF1A C/T, VEGFR2 Q472H, and AGTR2 C/A polymorphisms. Moreover, no significant association was observed between muscle fiber composition and the analyzed genetic polymorphisms in women. Muscle fiber composition in each polymorphism, i.e., ACTN3 R577X, ACE I/D, HIF1A C/T, VEGFR2 Q472H, and AGTR2 C/A, is shown in Table 4. Men with the ACTN3 RR + RX genotype had a significantly higher proportion of MHC-IIx than men with the ACTN3 XX genotype (24.8 ± 9.1 vs. 20.4 ± 8.6%, P = 0.031). Moreover, men with the ACE ID + DD genotype had a significantly higher proportion of MHC-I than men with the ACE II genotype (42.2 ± 10.8 vs. 37.6 ± 12.5%, P = 0.049).

Figure 1 shows the most fitting models of combined effects of ACTN3 R577X and ACE I/D polymorphisms on muscle fiber composition in men. In men, the ACTN3 R577X and ACE I/D polymorphisms were significantly correlated with MHC-I (r = -0.23, P = 0.020; Fig. 1A) and MHC-IIx (r = 0.27, P = 0.007; Fig. 1C) but not MHC-IIa (r = 0.17, P = 0.090; Fig. 1B). Men with combined ACTN3 XX and ACE ID + DD genotypes had the highest proportion of MHC-I and the lowest proportion of MHC-IIx, whereas men with combined genotypes of the ACTN3 RR + RX and the ACE II had the lowest proportion of MHC-I and the highest proportion of MHC-IIx.

**DISCUSSION**

In the present study, we investigated the effects of genetic polymorphisms in ACTN3, ACE, HIF1A, VEGFR2, and AGTR2 on human skeletal muscle fiber composition with respect to sex-based differences in the general Japanese population. Our results showed that men with the ACTN3 RR + RX genotype had a significantly higher proportion of MHC-IIx compared to women with the same genotype.

| Table 4. Muscle fiber composition by each genetic polymorphism in men and women |
|---------------------------------------------|----------------|----------------|
| **Gene name (rs number)** | **Genotype** | **P** |
| **Men** | | |
| ACTN3 (rs1815739) | RR (n = 24) | RX (n = 51) |
| MHC-I, % | 41.2 ± 12.0 | 39.3 ± 12.0 |
| MHC-IIa, % | 35.2 ± 8.9 | 35.3 ± 8.1 |
| MHC-IIx, % | 23.6 ± 6.7 | 25.4 ± 10.1 |
| ACE (rs4340) | II (n = 40) | ID (n = 48) |
| MHC-I, % | 37.6 ± 12.5 | 41.6 ± 9.8 |
| MHC-IIa, % | 37.4 ± 8.9 | 35.1 ± 8.0 |
| MHC-IIx, % | 25.0 ± 9.7 | 23.3 ± 8.1 |
| HIF1A (rs1149465) | CC (n = 90) | CT (n = 96) |
| MHC-I, % | 40.0 ± 12.0 | 46.2 ± 7.1 |
| MHC-IIa, % | 36.2 ± 8.4 | 31.8 ± 5.9 |
| MHC-IIx, % | 23.8 ± 9.5 | 22.0 ± 5.9 |
| VEGFR2 (rs1870377) | AA (n = 17) | AT (n = 42) |
| MHC-I, % | 36.8 ± 9.9 | 41.4 ± 12.3 |
| MHC-IIa, % | 38.6 ± 8.0 | 34.4 ± 8.9 |
| MHC-IIx, % | 24.6 ± 9.4 | 24.3 ± 8.6 |
| AGTR2 (rs11091046) | CC (n = 66) | CA (n = 0) |
| MHC-I, % | 41.0 ± 11.4 | |
| MHC-IIa, % | 35.6 ± 8.2 | |
| MHC-IIx, % | 23.5 ± 9.2 | |
| **Women** | | |
| ACTN3 (rs1815739) | RR (n = 27) | RX (n = 48) |
| MHC-I, % | 51.6 ± 13.1 | 49.3 ± 10.2 |
| MHC-IIa, % | 30.6 ± 7.0 | 30.5 ± 7.2 |
| MHC-IIx, % | 17.8 ± 9.4 | 20.2 ± 7.8 |
| ACE (rs4340) | II (n = 43) | ID (n = 48) |
| MHC-I, % | 50.9 ± 12.0 | 49.5 ± 10.3 |
| MHC-IIa, % | 29.1 ± 7.8 | 32.9 ± 8.4 |
| MHC-IIx, % | 20.9 ± 9.9 | 17.7 ± 7.7 |
| HIF1A (rs1149465) | CC (n = 95) | CT (n = 13) |
| MHC-I, % | 49.9 ± 11.2 | 51.1 ± 9.8 |
| MHC-IIa, % | 30.5 ± 8.4 | 32.6 ± 7.0 |
| MHC-IIx, % | 19.6 ± 8.0 | 16.2 ± 8.8 |
| VEGFR2 (rs1870377) | AA (n = 16) | AT (n = 53) |
| MHC-I, % | 48.0 ± 10.0 | 48.9 ± 10.5 |
| MHC-IIa, % | 32.4 ± 7.2 | 31.4 ± 8.9 |
| MHC-IIx, % | 19.6 ± 7.3 | 19.7 ± 8.6 |
| AGTR2 (rs11091046) | CC (n = 39) | CA (n = 54) |
| MHC-I, % | 51.3 ± 12.4 | 50.3 ± 10.3 |
| MHC-IIa, % | 30.6 ± 8.1 | 30.5 ± 8.5 |
| MHC-IIx, % | 18.1 ± 9.3 | 19.2 ± 7.5 |

Data are expressed as means ± SD. Data are available in 102 men in ACTN3, 101 men in ACE, 99 men in HIF1A, 100 men in VEGFR2, and 101 men in AGTR2. Data are available in 109 women in ACTN3 and 108 women in ACE, HIF1A, VEGFR2, and AGTR2. Values in bold denote P < 0.05.
α-Actins are important structural components of the Z-membrane, and expression of ACTN3 is limited to fast-twitch skeletal muscle fibers. A common nonsense polymorphism in ACTN3 induces an amino acid substitution from arginine (R) to the stop codon (X) at position 577 (R577X), thus depleting the ACTN3 protein level in fast-twitch skeletal muscle fibers. Previous studies have shown that men with the ACTN3 RR + RX genotype showed superior sprinting performance (20, 30).

In the present study, we found that men with the ACTN3 RR + RX genotype had a significantly higher proportion of MHC-IIx than men with the ACTN3 XX genotype. This is consistent with the results of a study by Vincent et al. (47), which reported a higher proportion of MHC-IIx in the vastus lateralis of young, healthy men with the ACTN3 RR genotype than those with the ACTN3 XX genotype. However, this study did not assess the effect of the ACTN3 RX genotype. In the present study, the proportion of MHC-IIx was higher in subjects with the ACTN3 RX genotype than in those with the ACTN3 XX genotype (P = 0.03; data not shown). Taken together, our results indicate that R-allele carriers (i.e., subjects with the RR and RX genotypes) in the ACTN3 R577X polymorphism had a higher proportion of MHC-IIx compared with subjects with the XX genotype in men but not in women.

The mechanism underlying the association between the ACTN3 R577X polymorphism and muscle fiber composition may be associated with the signaling protein calcineurin. Calcineurin is a serine-threonine phosphatase activated by Ca^2+ -calmodulin, and calcineurin activation plays a key role in the determination and/or adaptation of slow-twitch muscle fibers (9, 10, 22, 23, 26). Previously, Seto et al. (36) have reported that calcineurin signaling was increased in ACTN3 knockout mice, and they also have demonstrated that human muscles of subjects with the XX genotype in the ACTN3 R577X polymorphism showed significantly increased calcineurin signaling compared with subjects with the RR genotype. In the present study, we found that men with the ACTN3 RR + RX genotype had a significantly higher proportion of MHC-IIx than those with the ACTN3 XX genotype, which may be associated with changes in calcineurin signaling.

The ACE I/D polymorphism is one of the most common polymorphisms associated with physical performance. Several studies involving the European population have shown that the ACE I allele is associated with endurance performance, and the ACE D allele is associated with sprint/power performance (6, 13, 24, 27). In addition, a meta-analysis showed that the ACE I/I genotype is associated with endurance performance (19). However, almost all studies involving the Asian population have reported contrasting results (4, 16, 45, 48). We previously reported that the average running speed in a marathon was significantly higher in elite Japanese endurance runners with the ACE DD + ID genotype than those with the ACE II genotype (45). In addition, we reported that the ACE I allele was over-represented in elite short-distance Asian swimmers (48). These findings suggest that the ACE I/D polymorphism exerts different effects among different human ethnic groups. Interestingly, the present study showed that men with the ACE DD + ID genotype had a significantly higher proportion of MHC-I than those with the ACE II genotype, which is consistent with the results of previous studies involving the Asian population. However, Zhang et al. (50) reported conflicting results—that the ACE I allele was associated with a high

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**Fig. 1.** Most fitting models of combined effects of ACTN3 R577X and ACE I/D on MHC-I (A), MHC-IIa (B), and MHC-IIx (C) in men. Data are expressed as means ± SD.
proportion of MHC-I in 41 young Japanese subjects. Whereas the men to women ratio in the study by Zhang et al. (50) was different among the II, ID, and DD genotype groups, they did not consider the sex differences of the association between the ACE I/D polymorphism and muscle fiber composition. In the present study, we found that the proportion of MHC-I was higher in women than in men, suggesting that the conflicting results between the previous study by Zhang et al. (50) and the present study may be caused by sex differences in the muscle fiber composition. However, further studies are necessary to confirm our finding of the association between the ACE I/D polymorphism and muscle fiber composition in the Asian population.

Although it has been reported that the HIF1A rs11549465 (2), VEGFR2 rs1870377 (3), and AGTR2 rs11091046 (25) polymorphisms are associated with muscle fiber composition, this was not observed in the present study. Previous studies included young athletes and/or physically active, healthy men. However, the present study included subjects with a sedentary lifestyle who were comparatively older than those included in previous studies. This difference in study subjects may produce inconsistent results. Furthermore, we did not observe significant associations among the five genetic polymorphisms that we examined and muscle fiber composition in women. Previous studies have reported that estrogen, a female sex hormone, is associated with skeletal muscle growth, regeneration, and compositions (31, 33, 46). Thus estrogen may decrease the effects of the examined genetic polymorphisms on a proportion of MHC isoforms in women.

The present study has several limitations. The first limitation is the evaluation of muscle fiber composition. We only measured the proportion of MHC isoforms MHC-I, MHC-IIa, and MHC-IIx as an index of skeletal muscle fiber composition and did not measure the number and cross-sectional area of each fiber. The second limitation is the number of subjects. The present study included 211 subjects, which is much larger than the number of subjects included in similar, previous studies (1–3, 25, 47, 50). However, classification of the subjects, according to sex, relatively decreased the sample size. The third limitation is the problem of a multiple comparison when we analyze the associations between the selected genetic polymorphisms and MHC isoforms independently (Table 4). When we corrected the multiple comparisons in the present study, the statistical significances disappeared, likely because of the lack of statistical power of the present study. To avoid false negatives, we have shown statistical significance without adjustments for multiple comparisons. However, there is consequently an inflated possibility of false positives in the present study. Therefore, further and larger studies are needed to overcome the above limitations.

In the present study, we investigated the effects of genetic polymorphisms in ACTN3, ACE, HIF1A, VEGFR2, and AGTR2 on the composition of human skeletal muscle fibers with respect to sex-based differences in the general Japanese population. Our results showed that men with the ACTN3 RR + RX genotype had a significantly higher proportion of MHC-IIx than those with the ACTN3 XX genotype and that men with the ACE ID + DD genotype had a significantly higher proportion of MHC-I than those with the ACE II genotype. Furthermore, results of multiple linear regression analysis showed that these effects remained significant even after an adjustment for living environmental factors, such as age and BMI. In contrast, no significant association was observed between muscle fiber composition and the examined genetic polymorphisms in women. Thus our results suggest that the ACE I/D and ACTN3 R577X polymorphisms affect the proportion of human skeletal muscle fibers MHC-I and MHC-IIx in men but not in women.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.F. conceived and designed research; H. Kumagai, T. Tobina, N.I-S., R.K., T. Tsuzuki, K.S., E.Y., H. Kumahara, M.A., Y.H., H. Kobayashi, A.K., H.N., H.T., and N.F. performed experiments; H. Kumagai, H.Z., and R.Y. analyzed data; H. Kumagai and N.F. interpreted results of experiments; H. Kumagai prepared figures; H. Kumagai and N.F. drafted manuscript; H. Kumagai, T. Tobina, N.I-S., R.K., H.Z., R.Y., H.N., and N.F. edited and revised manuscript; N.F. approved final version of manuscript.

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