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Research Paper

AGTR2 gene polymorphism is associated with muscle fibre composition, athletic status and aerobic performance

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New Findings

- **What is the central question of this study?**
  Variations in genes are considered to be molecular determinants maintaining the expression of the slow or fast myosin heavy chains of adult skeletal muscle. The role of polymorphisms of candidate genes involved in skeletal muscle development, energy homeostasis and thyroid and calcium metabolism in the determination of muscle fibre type has not previously been reported.
- **What is the main finding and its importance?**
  We show that the *AGTR2* rs11091046 C allele is associated with an increased proportion of slow-twitch muscle fibres, endurance athlete status and aerobic performance. Such findings have important implications for our understanding of muscle function in both health and disease.

Muscle fibre type is a heritable trait and can partly predict athletic success. It has been proposed that polymorphisms of genes involved in the regulation of muscle fibre characteristics may predispose the muscle precursor cells of a given individual to be predominantly fast or slow. In the present study, we examined the association between 15 candidate gene polymorphisms and muscle fibre type composition of the vastus lateralis muscle in 55 physically active, healthy men. We found that rs11091046 C allele carriers of the angiotensin II type 2 receptor gene (*AGTR2*; involved in skeletal muscle development, metabolism and circulatory homeostasis) had a significantly higher percentage of slow-twitch fibres than A allele carriers [54.2 (11.1) *versus* 45.2 (10.2)%; $P = 0.003$]. These data indicate that 15.2% of the variation in muscle fibre composition of the vastus lateralis muscle can be explained by the *AGTR2* genotype.

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Next, we investigated the frequencies of the *AGTR2* alleles in 2178 Caucasian athletes and 1220 control subjects. The frequency of the *AGTR2* C allele was significantly higher in male and female endurance athletes compared with power athletes (males, 62.7 *versus* 51.7%, $P = 0.0038$; females, 56.6 *versus* 48.1%, $P = 0.0169$) and control subjects (males, 62.7 *versus* 51.0%, $P = 0.0006$; elite female athletes, 65.1 *versus* 55.2%, $P = 0.0488$). Furthermore, the frequency of the *AGTR2* A allele was significantly over-represented in female power athletes (51.9%) in comparison to control subjects (44.8%, $P = 0.0069$). We also found that relative maximal oxygen consumption was significantly greater in male endurance athletes with the *AGTR2* C allele compared with *AGTR2* A allele carriers [$n = 28$; 62.3 (4.4) *versus* 57.4 (6.0) ml min^{-1} kg^{-1}; $P = 0.0197$]. Taken together, these results demonstrate that the *AGTR2* gene C allele is associated with an increased proportion of slow-twitch muscle fibres, endurance athlete status and aerobic performance, while the A allele is associated with a higher percentage of fast-twitch fibres and power-oriented disciplines.

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Introduction

Individuals have different capacities to perform aerobic or anaerobic exercise, which can be explained partly by interindividual variability of muscle fibre composition. More specifically, the proportion of type I fibres in the vastus lateralis is typically ∼50%, but there is wide variation (range ∼5–90%; Simoneau & Bouchard 1989; Klitgaard *et al.* 1990; Staron *et al.* 2000). Endurance-oriented athletes are reported to have a remarkably high proportion of type I (slow-twitch, fatigue-resistant) fibres in their trained muscle groups (Ricoy *et al.* 1998; Zawadowska *et al.* 2004), whereas muscles of sprinters and weightlifters predominantly consist of type IIA (fast-twitch, fatigue-resistant) and IIX (fast-twitch, glycolytic) fibres (Andersen *et al.* 1994). Variability in the proportion of skeletal muscle fibres has also been found to contribute to susceptibility and aspects of several chronic diseases. Accordingly, a low percentage of type I muscle fibres was shown to be a risk factor for the development of obesity, metabolic syndrome and hypertension (Frisk-Holmberg *et al.* 1983; Lillioja *et al.* 1987; Tanner *et al.* 2002).

Simoneau & Bouchard (1995) concluded that the genetic component for the proportion of type I fibres in human muscles is of the order of 40–50%, indicating that muscle fibre type composition is determined by both the genotype and the environment. The genetic variance is that portion of interindividual phenotypic differences associated with sequence variations in the DNA. Genetic variance therefore includes the effects of single genes and gene–environment interaction, as well as gene–gene interaction.

Known determinants of fibre type in human skeletal muscle are innervation, intensity of different types of training, spaceflight and unloading, thyroid hormone levels and disease states (Baldwin & Haddad, 2001). Significant progress has been made during the last few years in the identification of the signalling pathways that control muscle fibre types. The function of specific genes has been defined by gain- and loss-of-function approaches using transgenic and knockout mouse models. These genes are involved in calcineurin/nuclear factor of activated T-cells (NFAT), peroxisome proliferator-activated receptor gamma, coactivator 1 (PGC-1)/peroxisome proliferator-activated receptor delta (PPARδ), calcium/calmodulin-dependent protein kinase and histone deacetylases, thyroid hormone and other pathways (Schiaffino & Reggiani, 2011). It can be suggested that DNA polymorphisms which influence gene expression of these signalling pathways predispose the muscle precursor cells of a given individual to be predominantly fast or slow. Consequently, gene variations could be considered as molecular determinants maintaining the expression of the slow or fast myosin heavy chain (MyHC) of adult skeletal muscle.

To date, few studies have sought to define the impact of gene polymorphisms on human muscle fibre composition. There have been reports that five polymorphisms of angiotensin-converting enzyme (*ACE*), alpha-actinin-3 (*ACTN3*), hypoxia inducible factor 1 alpha (*HIF1A*), peroxisome proliferator-activated receptor alpha (*PPARA*) and vascular endothelial growth factor receptor 2 (*VEGFR2*) genes (involved in the calcineurin/NFAT pathway, glucose and lipid metabolism, cytoskeletal function, hypoxia/angiogenesis and circulatory homeostasis) are associated with muscle fibre composition (Zhang *et al.* 2003; Ahmetov *et al.* 2006, 2008, 2009*a*,*b*, 2011; Akhmetov *et al.* 2006; Vincent *et al.*

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2007). Interestingly, most of these gene variants have also been associated with physical performance, athletic status or different metabolic and cardiovascular diseases, or even several of these, indicating that these phenotypes may share some common molecular mechanism of development (Ahmetov *et al.* 2012; Wang *et al.* 2013).

In order to extend the number of known molecular determinants for muscle fibre composition we have thus addressed this issue, in a study focused on 15 polymorphisms of genes involved in skeletal muscle growth and development, the thyroid hormone pathway, calcium and circulatory homeostasis, skeletal muscle metabolism, vascular tone and angiogenesis. The aim of the study was to examine the association between *ADRB2*, *AGT*, *AGTR1*, *AGTR2*, *AMPD1*, *BDKRB2*, *CALCR*, *IGF1*, *MYF6*, *NOS3*, *REN*, *TSHR*, *VDR* and *VEGFA* gene polymorphisms and muscle fibre composition of the vastus lateralis muscle, as well as the relationship between the most significant genetic marker, athletic status and aerobic performance.

Methods

Ethical approval

The study was approved by the Ethics Committee of St Petersburg University, the Physiological Section of the Russian National Committee for Biological Ethics and by the Pomeranian Medical University. Written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki. The experimental procedures were conducted in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the STrengthening the REporting of Genetic Association studies (STREGA) Statement.

Study participants

The study involved 2178 Caucasian athletes (1780 Russians and 398 Poles; 1473 males and 705 females) stratified into four groups according to type, intensity and duration of exercise (Akhmetov *et al.* 2007). The first group (‘group I’ or ‘endurance group’; n = 487) included long- and middle-distance athletes. The second group (‘group II’ or ‘mixed group’; n = 907) comprised athletes whose sports use mixed anaerobic and aerobic energy production. The third group (‘group III’ or ‘power group’; n = 615) included sprinters and explosive power athletes with predominantly anaerobic energy production. The fourth group (‘group IV’ or ‘strength group’; n = 169) included strength athletes. There were 679 athletes classified as ‘elite’ (prize winners of major international competitions). There were 1050 athletes classified as ‘subelite’ (participants in international competitions). The other athletes (n = 449) were classified as ‘non-elite’ athletes, being regional competitors with at least 4 years of experience participating in their sports.

Additionally, 55 physically active healthy men and 40 elite endurance athletes (23 rowers, 11 kayakers and six all-round speed skaters) participated in the study of muscle fibre proportion and aerobic performance (all Russians; for details see Table 1). It has been estimated that the sample size required in these studies to obtain a statistical power of 80% was sufficient.

Control subjects were 1220 (631 male and 589 female) healthy, unrelated citizens of Russia (n = 751) and Poland (n = 469) without any competitive sport experience. The athletes and control subjects were all Caucasians of Eastern European descent.

Genotyping

Russian samples. Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells (scrape) or leucocytes (venous blood). DNA from mouth cells was extracted using a DNK-sorb-A sorbent kit according to the manufacturer’s instructions (Central Research Institute of Epidemiology, Moscow, Russia). Four millilitres of venous blood was collected in tubes containing EDTA (Vacuette EDTA tubes; Greiner Bio-One, Kremsmünster, Austria). Blood samples were transported to the laboratory at 4°C, and DNA was extracted on the same day. The DNA extraction and purification were performed using a commercial kit according to the manufacturer’s instructions (Technoclon, Moscow, Russia) and included chemical lysis, selective DNA binding on silica spin columns and ethanol washing. The quality of the extracted DNA was assessed by agarose gel electrophoresis at this step.

Genotyping for the adrenoceptor beta 2 (*ADRB2*) G16R (rs1042713) and Q27E (rs1042714), angiotensinogen (*AGT*) M235T (rs699), angiotensin II type 1 receptor (*AGTR1*) A1166C (rs5186), angiotensin II type 2 receptor (*AGTR2*) C3123A (rs11091046), bradykinin receptor B2 (*BDKRB2*) T58C (rs1799722) and renin (*REN*) G83A (rs2368564) gene polymorphisms of DNA samples obtained from the muscle biopsy group was performed using multiplex PCR with subsequent hybridization on the biochip. Mutations were discriminated by analysing fluorescence intensities from separate units on the biochip, as previously described (Glotov *et al.* 2007). Genotyping for the adenosine monophosphate deaminase 1 (*AMPD1*) Q12X (rs17602729), calcitonin receptor (*CALCR*) L447P (rs1801197), insulin-like growth factor 1 (*IGF1*) CA-repeat (rs10665874), myogenic factor 6 (*MYF6*) C964T (rs3121), nitric oxide synthase 3 (*NOS3*) 27 bp repeat, thyroid stimulating hormone receptor

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(*TSHR*) D727E (rs1991517), vitamin D receptor (*VDR*) TaqI (rs731236) and vascular endothelial growth factor A (*VEGFA*) −2578 A/C (rs699947) gene polymorphisms was performed by PCR and restriction enzyme digestion (where appropriate), as previously described (Chistiakov *et al.* 2002; Oganov *et al.* 2008; Ahmetov *et al.* 2009*b*; Fedotovskaya *et al.* 2013; Zheng *et al.* 2014).

Genotyping for the *AGTR2* C3123A (rs11091046) variant of DNA samples obtained from 1149 Russian athletes and control subjects was performed by PCR and restriction enzyme digestion. The PCR primers were forward GGATTCAGATTTCTCTTTGAA and reverse GATTAAATCATACTCCTATGC, generating a fragment of 323 bp. The PCR products were digested with *Alu*I (SibEnzyme, Novosibirsk, Russia) for 12 h at 37°C and were separated by 8% polyacrylamide gel electrophoresis, stained with ethidium bromide, and visualized in ultraviolet light. All analysis was done blind to genotype. Additionally, HumanOmni1-Quad BeadChips (Illumina Inc., San Diego, CA, USA) were used for genotyping of the *AGTR2* rs11091046 C/A polymorphism in 1382 subjects. The assay required 200 ng of DNA sample as input with a concentration of at least 50 ng μl^{-1}. Exact concentrations of DNA in each sample were measured using a Qubit Fluorometer (Invitrogen, Grand Island, NE, USA). All further procedures were performed according to the instructions of Infinium HD Assay.

Polish samples. Genomic DNA was isolated from buccal epithelium using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Hamburg, Germany) according to the manufacturer's instructions. All samples were genotyped for the *AGTR2* gene rs11091046 polymorphism in duplicates using allelic discrimination assays with TaqMan® probes (Applied Biosystems, Carlsbad, CA, USA) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). To discriminate *AGTR2* alleles, TaqMan® Pre-Designed SNP Genotyping Assay was used (assay ID: C_1841568_10), including appropriate primers and fluorescently labelled (FAM and VIC) MGB™ probes to detect the alleles. Genotypes were assigned using all of the data from the study simultaneously.

Immunohistochemistry

Samples of vastus lateralis muscle of 55 physically active healthy men were obtained with the Bergström needle biopsy procedure under local anaesthesia with 1% lidocaine solution. Prior to analysis, samples were frozen in liquid nitrogen and stored at −80°C. Serial sections (10 μm thick) were prepared using a cryostat and microtome at −20°C, then sections were mounted on slides. The immunoperoxidase technique was employed for immunohistochemical identification of myosin isoforms. Antibodies against the slow (MHCs) and fast myosin isoforms (MHCf) were used [clones NCL-MHCf (a+b) and NCL-MHCs; Novocastra Laboratories, Newcastle, UK]. Sections incubated without primary antibodies were used to detect non-specific staining. The antigen–antibody marking was intensified with the Vectastain ABC kit (Vector Labs Inc., Burlingame, CA, USA) to visualize the diaminobenzidine peroxidase reaction. Fibre distribution was expressed as the ratio of the number of fibres of each type in a section to the total number of fibres. All fibres (200–300) were measured in each section using an image analysis system (QUANTIMET-500; Leica, Cambridge Ltd, Cambridge, UK) and a colour digital video camera (JVC TK-1280E, Tokyo, Japan; image resolution 720 × 512 pixels with 8 bits per pixel). Sections used for analysis were all prepared and stained together with reagents from Sigma (St Louis, MO, USA).

Measurement of maximal oxygen consumption

Maximal oxygen consumption rate ($\dot{V}_{O_2 \max}$) in rowers was determined using an incremental test to exhaustion on a PM 3 rowing ergometer (Concept II, Morrisville, VT, USA). The initial workload was 150 W. The duration of exercise at each workload was 3 min, with a 30 s rest period between increments of 50 W. The oxygen consumption was determined breath by breath using a MetaMax 3B gas analysis system (Cortex, Leipzig, Germany). Using the MetaMax system, O_2 and CO_2 contents were measured using an electrochemical cell and non-dispersive infrared sensor, respectively, and air flow was measured using a turbine transducer (Triple V). Two-point gas calibrations (first gas, 15% O_2–5% CO_2; second gas, ambient air) were performed daily. A one-point gas calibration with ambient air was performed before each test as well as a flow transducer calibration using a 3 l syringe (Hans Rudolph, Kansas City, KS, USA). The criteria used to confirm a maximal test were a decrease in power of >30 W from the target power despite strong verbal encouragement and a respiratory exchange ratio >1.1 before cessation of exercise. The $\dot{V}_{O_2 \max}$ was recorded as the highest mean value observed over a 30 s period.

The $\dot{V}_{O_2 \max}$ in kayakers was determined using an incremental test to exhaustion on a kayaking ergometer (Efremov, Moscow, Russia). The initial workload was 8 kg for men and 5 kg for women. The duration of exercise at each workload was 2 min, with a 30 s rest period between increments of 1 kg. The $\dot{V}_{O_2 \max}$ was determined breath by breath using a MetaLyzer II gas analysis system (Cortex). The $\dot{V}_{O_2 \max}$ was recorded as the highest mean value observed over a 30 s period.

The $\dot{V}_{O_2 \max}$ in speed skaters was determined using a ramp test to exhaustion on an electromagnetic cycle

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Table 1. Characteristics of muscle biopsy and aerobic performance groups

		Endurance athletes	
Characteristics	Physically active men	Men	Women
n	55	28	12
Age (years)	21.9 (2.6)	24.5 (3.9)	22.3 (3.1)
Body mass (kg)	73.0 (5.4)	91.1 (6.1)	72.1 (7.7)*
Height (cm)	179.4 (7.4)	191.3 (6.3)	176.8 (6.2)*
Body mass index (kg m^{-2})	22.8 (2.7)	24.9 (1.6)	23.1 (2.2)*
$\dot{V}_{O_2 max}$ (ml min^{-1} kg^{-1})	—	60.5 (5.4)	55.4 (6.7)*
Slow-twitch fibres (%)	50.3 (11.5)	—	—
Fast-twitch fibres (%)	52.8 (11.8)	—	—
CSA of slow-twitch fibres (μm^2)	5240 (1090)	—	—
CSA of fast-twitch fibres (μm^2)	5747 (1275)	—	—

Values are means (SD). Abbreviations: CSA, cross-sectional area; and $\dot{V}_{O_2 max}$, maximal oxygen consumption. *$P \leq 0.05$, significantly different between female and male athletes.

ergometer (Ergoselect 200K; Ergoline, Bitz, Germany). The initial workload was 60 W, the increment was 15 W min^{-1}, and the target cadence was 60–70 r.p.m. The $\dot{V}_{O_2 max}$ was determined breath by breath using a MetaMax 3B gas analysis system (Cortex). The criteria used to confirm a maximal test were a decrease in cadence to <50 r.p.m. despite strong verbal encouragement and a respiratory exchange ratio >1.1 before cessation of exercise. The $\dot{V}_{O_2 max}$ was recorded as the highest mean value observed over a 30 s period.

Statistical analysis

Genotype distribution and allele frequencies between athletes and control subjects were compared using χ^2 tests. Spearman's (non-parametric) correlations were used to assess the relationships between the physiological phenotypes (muscle fibre characteristics, aerobic performance) and the *AGTR2* genotypes (dummy coded as 1, 2 and 3 for CC, AC and AA, respectively). The squared correlation coefficient (r^2) was used as a measure of explained variance. Differences in phenotypes between groups were analysed using Student's unpaired *t* tests. All data are presented as mean values (SD). Values of $P < 0.05$ were considered statistically significant. Bonferroni's correction for multiple testing was performed by multiplying the *P* value by the number of tests where appropriate. Statistical analyses were conducted using GraphPad InStat software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Gene polymorphisms and muscle fibre composition

Muscle fibre type distribution was measured in biopsy samples obtained from the vastus lateralis muscle of physically active healthy men. On average, the relative fractions of slow-twitch (type I) and fast-twitch (type II) fibres were 50.3 (11.5) and 52.8 (11.8)%, respectively. Of the 15 candidate genetic markers located in *ADRB2*, *AGT*, *AGTR1*, *AGTR2*, *AMPD1*, *BDKRB2*, *CALCR*, *IGF1*, *MYF6*, *NOS3*, *REN*, *TSHR*, *VDR* and *VEGFA* genes, only *AGTR2* rs11091046 A/C polymorphism was shown to be associated with muscle fibre composition [for slow-twitch fibres, C genotype 54.2 (11.1)%, A genotype 45.2 (10.2)%, $P = 0.003$; and for fast-twitch fibres, C genotype 48.9 (12.4)%, A genotype 57.7 (8.9)%, $P = 0.003$], indicating that *AGTR2* C allele carriers exhibit a higher proportion of slow-twitch fibres (Table 2). This association remained statistically significant after Bonferroni correction for multiple testing ($P_{corrected} = 0.045$). The *AGTR2* genotype explained 15.2% of the variation in muscle fibre composition of the vastus lateralis muscle. We therefore felt justified in retaining only the *AGTR2* gene polymorphism for further analyses.

Case–control study

Given that the *AGTR2* gene is located on the X chromosome (men can have only one of either the C or the A allele, while women may have zero, one or two copies of either allele), all analyses were done separately for men and women. The frequencies of the *AGTR2* genotypes and alleles did not differ between Russian and Polish control subjects or athletes (data not shown), supporting our previous observations that Russian and Polish populations have similarities in their genetic profile (Maciejewska *et al.* 2012; Eynon *et al.* 2013; Sawczuk *et al.* 2013; Zarebska *et al.* 2014). Therefore, for the main analyses we used the combined data (i.e. combined groups of Caucasians, independent of precise ethnicity).

The frequency of the *AGTR2* C allele was significantly higher in male and female endurance athletes compared

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Table 2. Muscle fibre type composition of vastus lateralis of physically active healthy men (*n* = 55) by 15 candidate genotypes

Gene/variation	Genotype	*n*	Slow-twitch fibres [%; mean (SD)]	*P* Value	Fast-twitch fibres [%; mean (SD)]	*P* Value
ADRB2 G16R	RR	34	51.0 (11.3)	0.8417	52.4 (11.7)	0.8543
	RG	5	48.8 (12.8)		51.0 (14.1)	
	GG	16	49.1 (12.3)		54.0 (11.8)	
ADRB2 Q27E	QQ	48	50.4 (11.7)	0.9164	52.3 (11.3)	0.7217
	QE	2	52.0 (15.6)		58.0 (19.9)	
	EE	5	48.4 (11.2)		55.2 (15.5)	
AGT M235T	MM	17	51.6 (11.8)	0.7706	50.8 (11.0)	0.607
	MT	23	50.4 (11.5)		52.8 (11.9)	
	TT	15	48.6 (11.8)		55.0 (12.6)	
AGTR1 A1166C	AA	35	51.0 (12.6)	0.5446	53.2 (12.4)	0.7078
	AC	19	48.6 (9.6)		52.4 (10.8)	
	CC	1	56.0		45.0	
AGTR2 C3123A	C	31	54.2 (11.1)	0.003*	48.9 (12.4)	0.003*
	A	24	45.2 (10.2)		57.7 (8.9)	
AMPD1 Q12X	QQ	24	52.1 (12.2)	0.6865	49.9 (11.1)	0.4158
	QX	15	49.8 (11.5)		55.2 (12.2)	
	XX	3	46.2 (18.4)		52.3 (19.4)	
BDKRB2 T58C	TT	10	49.7 (13.5)	0.986	55.8 (14.8)	0.6835
	TC	25	50.3 (11.9)		52.2 (11.1)	
	CC	20	50.5 (10.7)		52 (11.2)	
CALCR L447P	PP	5	50.1 (9.2)	0.9083	53.6 (12.5)	0.913
	PL	20	49.1 (11.7)		53.5 (12.4)	
	LL	29	50.6 (12.0)		52.1 (11.8)	
IGF1 (*CA rep*)	130/130	23	47.8 (12.7)	0.482	55.6 (13.5)	0.3008
	130/M	26	51.4 (10.5)		51.1 (10.3)	
	MM	5	52.7 (10.5)		48.7 (10.5)	
MYF6 C964T	CC	8	49.3 (15.4)	0.7812	52.9 (13.7)	0.9033
	CT	31	49.8 (11.3)		53.4 (11.2)	
	TT	15	52.2 (10.7)		51.7 (12.9)	
NOS3 27 bp rep	5/5	30	47.4 (11.6)	0.1306	55.1 (11.4)	0.3236
	5/4	20	54.4 (11.5)		49.8 (12.7)	
	4/4	4	49.5 (6.9)		51.8 (9.0)	
REN G83A	GG	31	48.8 (12.7)	0.5718	54.3 (12.4)	0.4958
	GA	16	52.5 (9.3)		51.5 (11.4)	
	AA	8	51.4 (11.1)		49.3 (9.9)	
TSHR D727E	DD	46	50.0 (11.6)	0.74	52.4 (12.1)	0.4648
	DE	7	48.5 (11.8)		56.0 (11.0)	
	EE	0	—		—	
VDR TaqI	TT	26	50.4 (10.5)	0.9722	52.4 (10.9)	0.9346
	Tt	23	50.4 (13.8)		53.6 (13.8)	
	tt	4	48.9 (6.7)		53.0 (8.3)	
VEGFA −2578 A/C	AA	11	45.4 (14.9)	0.0705	57.5 (12.1)	0.021
	AC	30	53.6 (9.4)		48.9 (9.8)	
	CC	13	47.2 (11.6)		58.4 (13.5)	

*$P < 0.0033$, statistically significant differences between men with different genotypes (after Bonferroni's correction for multiple testing).

with power athletes (males, 62.7 *versus* 51.7%, $P = 0.0038$; and females, 56.6 *versus* 48.1%, $P = 0.0169$) and control subjects (males, 62.7 *versus* 51.0%, $P = 0.0006$; Tables 3 and 4). Furthermore, the frequency of the *AGTR2* A allele was significantly over-represented in female power athletes (51.9%) in comparison to control subjects (44.8%, $P = 0.0069$; Table 4). Additionally, the C allele was found to be more frequent in male strength athletes compared with male control subjects (65.8 *versus* 51.0%, $P = 0.0037$), but this association was not replicated in female cohorts. Compared with A allele carriers, the odds ratio of being an endurance athlete in male C variant carriers was 1.612

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Table 3. *AGTR2* genotype distribution in male athletes and control subjects

Group	*n*	*AGTR2* genotypes (%) A	C	*P* Value
Endurance	327	37.3	62.7	0.0006*
Mixed	678	45.6	54.4	0.2190
Power	354	48.3	51.7	0.8413
Strength	114	34.2	65.8	0.0037*
All male athletes	1473	43.5	56.5	0.0213
Male control subjects	631	49.0	51.0	—

*$P \leq 0.01$, statistically significant differences between groups of male athletes and control subjects (after Bonferroni's correction for multiple testing).

Table 4. *AGTR2* genotype distribution in female athletes and control subjects

Group	*n*	*AGTR2* genotypes (%) AA	AC	CC	C allele (%)	*P* Value
Endurance	160	22.5	41.9	35.6	56.6	0.6586
Mixed	229	25.8	42.8	31.4	52.8	0.3935
Power	261	26.4	51.0	22.6	48.1	0.0069*
Strength	55	18.2	54.5	27.3	54.5	0.8985
All female athletes	705	24.7	46.5	28.8	52.1	0.1129
Female control subjects	589	23.8	42.1	34.1	55.2	—

*$P \leq 0.01$, statistically significant differences between groups of female athletes and control subjects (after Bonferroni's correction for multiple testing).

(95% confidence interval 1.227–2.119, $P = 0.0006$). In contrast, the odds ratio for a power female athlete to carry the A allele (AC+AA) compared with female control subjects was 1.774 (95% confidence interval 1.267–2.483, $P = 0.0007$).

Additional analyses showed only one association between the *AGTR2* polymorphism and the competitive standard achieved by the athletes. Accordingly, we found that the frequency of the *AGTR2* C allele was significantly higher in elite female endurance athletes (65.1%) compared with control subjects (55.2%, $P = 0.0488$) or subelite female endurance athletes (52.4%, $P = 0.0391$).

AGTR2 genotype and aerobic performance

Relative $\dot{V}_{O_2\,max}$ was significantly greater [62.3 (4.4) *versus* 57.4 (6.0) ml min^{-1} kg^{-1}; $P = 0.0197$] in male endurance athletes with the *AGTR2* C allele ($n = 18$) compared with the *AGTR2* A allele carriers ($n = 10$). The *AGTR2* genotype explained 19.2% of the variation in $\dot{V}_{O_2\,max}$ of male endurance athletes.

Discussion

This is the first study to demonstrate that variation in the *AGTR2* gene is associated with muscle fibre composition, athletic status and aerobic performance. Specifically, the *AGTR2* rs11091046 C allele seems to be associated with an increased proportion of slow-twitch muscle fibres, endurance athlete status and aerobic performance, and the A allele with a higher percentage of fast-twitch fibres and power-oriented disciplines.

The angiotensin II type 2 receptor is a component of the renin–angiotensin system, which is considered to be one of the regulators of skeletal muscle growth and differentiation (Zhang *et al.* 2003; Johnston *et al.* 2011; Ahmetov *et al.* 2012). The renin–angiotensin system is linked with calcineurin, mitogen-activated protein kinase, Akt, NO and insulin signalling pathways, as well as with thyroid, carbohydrate and calcium metabolism and mitochondrial biogenesis, which may influence skeletal muscle characteristics (Wei *et al.* 2000; Mitsuishi *et al.* 2009; Yang *et al.* 2010; Chai *et al.* 2011; Carrillo-Sepúlveda *et al.* 2013).

The angiotensin II type 2 receptor mediates the effects of angiotensin II on cellular differentiation and growth, and has a metabolic role that is in contrast to the vascular role of the angiotensin II type 1 receptor (AGTR1; Matsubara, 1998; Seip *et al.* 2008; Chai *et al.* 2011). The *AGTR2* gene is expressed abundantly in the fetus, but in the adult it is limited to a few tissues, such as brain, ovary and adrenal gland (Matsubara, 1998). Furthermore, AGTR2 is upregulated in certain pathological conditions, such as hypertension, vascular injury, inflammation and hyperthyroidism (Akishita *et al.* 2000; Carneiro-Ramos *et al.* 2010; Carrillo-Sepúlveda *et al.* 2013). Qi *et al.* (2005), using radioligand binding, demonstrated that human skeletal muscle myoblasts possess both AGTR1 and AGTR2. Results obtained by Johnston *et al.* (2011) confirmed a regulatory role of a local angiotensin signalling system in proliferating and differentiated muscle stem cells. In proliferating cells, AGTR1 appeared to be predominately localized to cytoskeletal, filamentous proteins, while AGTR2 demonstrated a more diffuse expression pattern (Johnston *et al.* 2011).

Over the past decade, several polymorphisms in the *AGTR2* gene have been identified. The C3123A (also known as rs11091046 or C4599A) polymorphism of the *AGTR2* gene located within the 3′-untranslated region of exon 3 has been reported to be associated with several cardiovascular phenotypes. The A variant of the *AGTR2* gene was shown to be a risk allele for acute myocardial infarction (Aoki *et al.* 2001), hypertrophic

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cardiomyopathy (Deinum *et al.* 2001) and hypertension (Jin *et al.* 2003). Therefore, one might suggest that the positive relationship between hypertension and a higher proportion of fast-twitch fibres (Frisk-Holmberg *et al.* 1983) may be explained partly by the association of the *AGTR2* A allele with both an increased percentage of fast-twitch muscle fibres and the risk of hypertension.

The use of the HaploReg (version 2, http://www.broadinstitute.org/mammals/haploreg/haploreg.php) online program predicted the change of transcription factor-binding motifs in the rs11091046 locus of the *AGTR2* gene for HOXA9, HOXD8 and MEF2A transcription factors. Both HOXA9 and HOXD8 regulate morphogenesis and differentiation (de Wilde *et al.* 2010), while MEF2A is involved in several cellular processes, including muscle development (it promotes the transformation of type II fast glycolytic fibres into type I slow oxidative fibres), neuronal differentiation, cell growth control and apoptosis (Wu *et al.* 2001; Theobald & DiMario, 2011). In contrast, given that the *AGTR2* rs11091046 polymorphism is located in the untranslated region of the X chromosome, the discovered associations might be explained by linkage disequilibrium in a functional variant of the same or a different gene. For instance, given that *AGTR2* rs1403543 (in strong linkage disequilibrium with rs11091046) is associated with *AGTR2* expression *in vitro* (Warnecke *et al.* 2005), one would expect such an association for *AGTR2* rs11091046 polymorphism. Thus, the mechanisms through which such altered *AGTR2* activity influences muscle fibre composition, $\dot{V}_{O_2\,max}$ and athletic performance remain speculative, and further *in vitro* and *in vivo* studies of gene function are advocated.

In fact, the interrelationship between the *AGTR2* genotype and muscle fibre composition is not surprising, given that genetic polymorphism within another renin–angiotensin system component, the angiotensin I converting enzyme gene (*ACE*; it encodes an enzyme involved in catalysing the conversion of angiotensin I into the physiologically active peptide angiotensin II), has already been linked with muscle fibre type. Zhang *et al.* (2003) tested the hypothesis that the *ACE* insertion/deletion (I/D) polymorphism may influence muscle characteristics in humans, which could in part explain the association of gene variation with endurance performance. Indeed, they revealed that the greater the I allele frequencies, the higher the percentage of type I skeletal muscle fibres; and the greater the D allele frequency, the higher the percentage of type II fibres in the vastus lateralis muscle of 41 Japanese healthy subjects. Furthermore, in a hypertensive and insulin-resistant animal model (fructose-fed rats), the angiotensin-converting enzyme inhibitor temocapril was shown to produce recovery of the composition ratio of type I fibres of soleus muscle to the same as the control muscle and improved insulin sensitivity (Higashiura *et al.* 2000).

Data from many studies indicate that muscle fibre type proportion influences physical performance capability and competition specialty (Ricoy *et al.* 1998; Andersen *et al.* 1994; Zawadowska *et al.* 2004; Ahmetov *et al.* 2011). For instance, within skeletal muscle itself, fibre type proportion is a useful marker of skeletal muscle functional properties, and a high proportion of slow-twitch fibres is related to high mitochondrial volume, high oxidative capacity and high fatigue resistance. As successful endurance athletes have a relative preponderance of slow-twitch rather than fast-twitch fibres in the trained musculature (and sprinters an excess of fast-twitch fibres), part of the allelic association with performance phenotypes might have been mediated though genotype-associated alterations in fibre type proportion. Our data suggest that 15.2% of the variation in muscle fibre composition of the vastus lateralis muscle can be explained by the *AGTR2* genotype. Nevertheless, given the substantial heritable component of muscle fibre composition (∼45%), there are clearly more (and probably many more) genetic variants associated with muscle fibre composition that need to be identified and the findings replicated. Future research that embraces the advancing technology available in genomics, such as gene chips with wide genomic coverage or next-generation sequencing, will be needed to determine a more comprehensive list of the important polymorphisms involved in the regulation of muscle fibre composition. Genes encoding transcription factors, such as myf5, myoD, MRF4 and myogenin, could be targets for deep sequencing in this context.

Although more replication studies are needed, our data suggest that the *AGTR2* C allele is likely to be advantageous for performance in endurance sports in relationship to men and women, and the *AGTR2* A allele for power disciplines in relationship to women. Given that power and endurance are located at opposite extremes of the muscle performance continuum, we also compared allelic frequencies between endurance and power athletes, and found significant differences in both male and female athletes. The prevalence of the *AGTR2* C allele in male strength athletes (in comparison with power athletes and control subjects) can be explained by the fact that the proportion of type IIA fibres (fast and oxidative) rather than type IIX fibres (fast and glycolytic; dominant type in sprinters) have been shown to be greater in weightlifters (Fry *et al.* 2003), but further replication studies are warranted.

Importantly, we demonstrated that the *AGTR2* C allele was positively associated with three phenotypes (percentage of slow-twitch muscle fibres, endurance athlete status and $\dot{V}_{O_2\,max}$) and the A allele with two (percentage of fast-twitch muscle fibres and power athlete

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status), which are highly correlated with each other (Bergh *et al.* 1978). Thus, a consistent picture is presented, where the associations of the *AGTR2* C allele with greater maximal oxygen uptake and an increased proportion of type I skeletal muscle fibres explains, at least in part, the association of the *AGTR2* rs11091046 polymorphism with elite athletic status.

In conclusion, we have demonstrated that the *AGTR2* rs11091046 C allele is associated with an increased proportion of slow-twitch muscle fibres, endurance athlete status and aerobic performance, and the A allele with a higher percentage of fast-twitch fibres and power athlete status. Such findings have important implications for our understanding of muscle function in both health and disease.

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Additional Information

Competing interests

None declared.

Author contributions

Conception and design of the experiments: I.I.A., O.L.V. and V.M.G. Collection, analysis and interpretation of data: L.J.M., V.A.N., P.C., D.V.P., E.V.L., E.S.K., O.N.F., A.M.D., I.V.A., A.S.G., D.G.A., M.M.M., E.S.E., A.M.-K., A.K.L., E.V.G., R.E.N., Z.J., N.A.K., E.A.O., A.V.P., M.S., E.B.A., A.A.D., P.Ž. and I.I.A. Drafting the article or revising it critically for important intellectual content: I.I.A., L.J.M., D.V.P., P.Ž., O.L.V. and V.M.G. All authors approved the final version of the manuscript. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

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