The AGTR2 (RS11091046) Gene Polymorphism is Associated with Cycling Performance in Korean Cyclists

Seok-ki Min¹ PhD, Seung-taek Lim² PhD, Kwangkyu Lee³ PhD, Jieun Kim¹ MA, Eonho Kim¹ PhD, Eunbin Jee⁵ MA
¹Department of Sport Science, Korea Institute of Sport Science (KISS), Seoul; ²Olympic Studies Center, Kangwon National University, Chuncheon; ³Department of Sports Rehabilitation, Chungcheong University, Cheongju; ⁴Department of Physical Education, Dongguk University, Seoul, Korea; ⁵Graduate School of Health and Sport Science, Nippon Sport Science University, Tokyo, Japan

INTRODUCTION

Skeletal muscle is the largest organ in the human body. In the past decade, skeletal muscle has been also identified as a secretory organ. To understand how to communicate skeletal muscle and biochemical pathways in protein synthesis is getting important more and over not only from a pathological perspective but also in the field of athletic performance [1]. According to recent studies, training methods for competitive cyclists are changing to adding heavy strength training to endurance training [2,3]. Since, professional cyclists need to possess the ability to accomplish a comparatively high-power output of short duration during the steep climbing, the mass start, and the finish of the race [4].

To date, more than 200 health- and fitness-related phenotypes have been found [5]. As an example, angiotensin-converting enzyme (ACE) gene is most frequently researched in the field of sports genetics. ACE is a tonic regulator of circulatory homeostasis and also a family of the Renin-angiotensin system (RAS) which contains the angiotensinogen (AGT), Angiotensin II type 1 receptor (AGTR1) and Angiotensin II type 2 receptor (AGTR2) [6]. The AGTR2 Gene (A>C, rs11091046) of the X chromosome, located at Xq22-q23, has emerged recently as a crucial gene candidate that influences on athletic performance. The AGTR2, which is one of the important components of RAS, is known as a mediator of vasodilation and antiproliferative and apoptotic effects in vascular smooth muscle [7]. The presence of AGTR2 is in skeletal muscle fiber throughout the rat skeletal muscle microcirculation [8]. The evidence has demonstrated the possibility of a correlation between the expression...
of the AGTR2 gene and the function and metabolism of skeletal muscle.

There are few studies about the relationship between the AGTR2 rs11091046 (A > C) gene and athletic performance. At the first time, Mustafina et al. [9] have found AGTR2 gene as a novel genetic polymorphism, which might be a candidate gene in athletic performance in 2014. The C allele has been associated with slow-twitch muscle composition, maximal oxygen consumption, and athletic status in a cohort of Caucasian athletes. Furthermore, the frequency of the AGTR2 A allele was significantly higher in women power athletes in comparison to control subjects. More recently, another study has investigated the association between AGTR2 A allele and the power athletic performance in Brazil. In the top-level male and female power athletes, A allele or AA genotype was significantly higher than non-athletes or top-level endurance athletes. Additionally, male sprinters with the A allele were significantly faster than those with the C allele [10].

However, the opposite opinion of the above two studies through a case-control replication study has been argued. In the study, the author has replicated only a specific event cohort (track and field) and in two different ethnicities (Japanese and Caucasian). The frequency of athletes with the C allele was significantly higher than control subjects. In a Japanese population independently, the frequency of male athletes with C allele is significantly higher than the A allele, but not in Caucasian male athletes. In contrast to previous findings, the association between the C allele of the AGTR2 gene and sprint/power athlete status has been demonstrated.

Thus, it is necessary to investigate the association between AGTR2 (rs11091046 A > C) gene polymorphism and athletic performance by employing specific event and one ethnic group. The genotypic frequencies among athletes and non-athletes and the athletic performance by Wingate test, one-repetition maximum, isokinetic dynamometer were used to examine the preceding evidence. The aim of the study is to investigate the relationship between AGTR2 genotype and athletic performance of Korean national team cyclists.

METHODS

1. Participants

The study involved forty-four top-level Korean male competitive cyclists (track racing cyclists = 20, short-distance cyclists = 13, middle-distance cyclists = 10, long-distance cyclist = 1). All of the athletes were selected for the Korea national team in 2016 and 2017. The participants were informed about this research and genotyping process before they participated in this study. In addition, they signed in the Letter of Agreement and the pledge of ethics. All the experiments were approved by the Ethics committee of the Korea Institute of Sport Science.

2. DNA Extraction and Genotyping

Total DNA was isolated from buccal epithelium cells. Before the collection of buccal epithelial cells, all participants rinsed briefly their mouth. Using the sterile cotton Swab (4N6FLOQSwabs, COPAN flock technology, Italy), the participants were advised to rub their Buccal cells inside of their mouth for 15-20 seconds. Afterward, they were then instructed to deposit their swab in 1.5 mL E-Tube with 400 µL DNA Lysis solution until an available amount of Buccal cells were available collected. The Samples in the E-tube were deposited in heat box for 3 minutes at 95°C and 400 µL DNA stabilizing solution was added. Before the Genotyping, the samples were refrigerated at 4°C.

All genotyping experiments were performed in the biochemistry laboratory at the Korea Institute of Sports Science (Seoul, Korea). The AGTR2 (rs11091046) polymorphism was genotyped by using a pre-designed specific TaqMan SNP Genotyping Assays (Applied Biosystems, USA). The DNA samples containing Deionized distilled water, TaqMan GTXpress Master Mix, TaqMan genotyping assay were amplified on Thermal Cycle (Real-Time PCR 7,500, applied Biosystem, USA). To minimize the contamination errors, a negative control and positive controls were contained. The Analysis of genotyping was performed using 7500 Software Ver2.3 (applied Biosystem, USA).

3. Physical fitness tests

1) Wingate test

The Wingate test (WAnT) was completed on a Monark cycle ergometer (Ergomedic 894E, monark, Sweden) and used to measure the anaerobic mechanical power outputs; peak power (PP) and mean power (MP). All subjects (n = 44) performed a 5 minutes warm-up prior to completing the Wingate test. The subjects completed the 30 seconds “full-out” WAnT with the resistance on the flywheel wet to 7.5% of the body weight.

2) One-repetition maximum

For one-repetition maximum (1RM), squat and bench press by using the ACE-2,000 multi-function instrument (Ariel Dynamics Inc., USA) was performed once to measure the maximum weight one could lift.
3) Isokinetic muscle strength

Leg isokinetic muscle strength was evaluated by Cybex 770 (Human-norm, CSMI, USA) at the speed of 60°/sec. All subjects practiced flexion and extension motions three times and got a minute break before starting the main test. In the main test, the subjects tried three times with maximum strength.

4. Statistical analysis

Data were analyzed with SPSS/PC software ver.23.0 (IBM SPSS statistics, Armonk, NY, USA). The alleles (C allele or A allele; Since AGTR2 rs11091046 polymorphism is situated in the sexual X chromosome, the genotype of males contributes a single allele) frequency differences were tested by Pearson’s χ² test of independence. The t-test was performed to investigate the differences of sprint/power performance between C allele and A allele. The level of significance was established at p < .05.

RESULTS

Table 1 represents the frequencies of AGTR2 gene between C allele and A allele of participants. (C allele; 54.5% vs A allele 45.5%). The Hardy-Weinberg Equilibrium was omitted because the AGTR2 genotype of males contributes a single allele.

Table 2 shows the height, weight and BMI of the participants. The groups were separated in genotype to show the difference between C allele and A allele. We did not observe significant differences between C allele and A allele in physical characteristics.

The physical fitness tests (Isokinetic dynamometer and one-repetition maximum) are shown in Table 3. No significant differences were found between C allele and A allele in isokinetic muscle strength. In 1RM test, both results of squat and bench press show no significant difference between C allele and A allele.

Fig. 1. represents the association between the AGTR2 gene polymorphism and Wingate test. We found a significant difference between polymorphism and Wingate test in peak power and mean power. The peak power of C allele was significantly higher than A allele (C allele vs. A allele; 10.46 ± 0.82 W/kg vs. 9.92± 0.57 W/kg; p = .016). Furthermore, the mean power was higher in C allele than A allele (8.30± 0.48 W/kg in C allele and 7.95± 0.47 W/kg in A allele, p = .022).

DISCUSSION

The main finding of the present study was that the AGTR2 C allele have significantly higher peak power and mean power of Wingate test than A allele.
Mustafina et al. [9] have reported a positive association between C allele of AGTR2 gene and endurance athletic performance by comparing the frequency of allele and the percentage of muscle fiber type. However, the results of previous studies about AGTR2 gene and athletic performance show an obviously opposite opinion. The frequency of C allele is overrepresented in male strength athletes compared to controls. It has been considered the change of transcription factor-binding motifs in the rs11091046 locus of the AGTR2 gene for three different transcription factors using HaploReg. They have been involved in muscle development, neuronal differentiation, cell growth control and apoptosis. The AGTR2 gene might affect Type I muscle fiber composition, and endurance athletic performance.

In contrast, the C allele was significantly overrepresented in Japanese male field and track sprint/power athletes by a case-control replication study about AGTR2 gene and athletic performance of Asians and Caucasians. The study has indicated the positive association between C allele and sprint/power athletic performance. It has been suggested that the specific miRNA/mRNA, which is related in cardiomyocyte hypertrophy in human and muscle myosin, myofiber identity, and muscle performance in mice, binding affinity might be decreased by AGTR2 C allele. Therefore, it might increase the amount of AGTR2 production leading to increased muscle mass and type II muscle fiber type composition [11]. The AGTR2 gene is a mediator of vasodilatation, nitrogen oxide (NO) release and inhibition of proliferation and growth of the cell [7,12,13]. In the present study, we showed the results of the physical fitness test that the mean power and peak power increased in C allele. However, the Isokinetic dynamometer and 1RM showed no significance. The vasodilatation-related signaling is depending on the type of muscle contraction such as single brief contraction (such as vertical jump) and rhythmic activity (such as running and cycling). For example, for the rhythmic activity persist the slow onset vasodilatation until inhibition of nitric oxide synthesis [14]. Moreover, It is sufficiently reported that nitrogen species is important for skeletal muscle function and it is reported that the acute effect of NO can contribute to the development of muscle fatigue or alternatively help counter it [15]. Thus, the difference of vasodilatation and released nitrogen oxide depending on genotypes might the potential mechanism which explains the result of the study.

The function of genes in human athletic performance is complicated and multiple. Combinational and various approaches are needed to investigate the unequivocal association of complex interactions of biochemical functions. In addition, it must be considered that the functional mechanism of RAS is not completely established. The limitation of this study is the small sample size and the subdivided events. However, the subjects of the present study are all elite athletes who are participating in international events as the national team of Korea, not amateur athletes or semi-professionals.

In addition, further replication studies in various ethnicities with a bigger sample size and functional studies are necessary.

**CONCLUSION**

In summary, we investigated whether AGTR2 gene is related to athletic performance in Korean national team male cyclists. As main results, AGTR2 gene C allele have significantly higher peak power and mean power in the Wingate test than A allele. Thus, we concluded that the AGTR2 gene (rs11091046 A > C polymorphism) C allele is advantageous for the athletic performance of cyclists.
CONFLICT OF INTEREST

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization: SK Min; Data curation: SK Min, ST Lim, KK Lee; Formal analysis: SK Min, ST Lim, JE Kim; Funding acquisition: SK Min; Methodology: SK Min, ST Lim, KK Lee, EH Kim; Project administration: SK Min, EB Jee; Visualization: SK Min, JE Kim; Writing-original draft: SK Min, EB Jee; Writing-review & editing: SK Min.

ORCID

Seok-ki Min https://orcid.org/0000-0003-3134-9689
Seung-taek Lim https://orcid.org/0000-0002-0980-991X
Kwangkyu Lee https://orcid.org/0000-0003-1169-6368
Jieun Kim https://orcid.org/0000-0002-7873-6814
Eonho Kim https://orcid.org/0000-0003-1907-390X
Eunbin Jee https://orcid.org/0000-0003-3961-2058

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