THE ANGIOTENSIN CONVERTING ENZYME GENE I/D POLYMORPHISM IN POLISH ROWERS

Zbigniew Jastrzębski,[1] Agata Leońska-Duniec,[1, 2] Marek Kolbowicz,[2] Tomasz Tomiak[3]

[1] Faculty of Tourism and Recreation, Gdansk University of Physical Education and Sport, Gdańsk, Poland
[2] Faculty of Physical Culture and Health Promotion, University of Szczecin, Poland
[3] Faculty of Physical Education, Gdansk University of Physical Education and Sport, Gdańsk, Poland

Address for correspondence:
Zbigniew Jastrzębski
Gdansk University of Physical Education and Sport
Kazimierza Górskiego 1, 80-336 Gdańsk, Poland
tel. +48 (58) 55 47 323, fax +48 (58) 55 47 324
E-mail: zb.jastrzebski@op.pl

Abstract. Angiotensin converting enzyme gene (*ACE*) is the most frequently investigated genetic marker in the context of genetic conditioning of athletic predispositions. The product of the gene is a key component of the renin-angiotensin system (RAS) and the kallikrein-kinin system (KKS), mainly responsible for the regulation of blood pressure. The main aim of the study was to determine the possible interaction between the *ACE* I/D polymorphism and endurance athlete status in a group of Polish rowers in comparison with sedentary individuals. 121 male Polish rowers, members of academic sports clubs, and 115 unrelated volunteers, were recruited for the study. The PCR amplification of the insertion (I) or deletion (D) fragment of the *ACE* gene was performed. Compared with control group, the frequency of the I allele differ significantly from that found in rowers (57.4% vs. 44.3%; P = 0.013) and the ACE genotype frequency amongst the whole cohort of rowers (30.6% II, 53.7% ID, 15.7% DD) was also different from expected values (control group 19.1% II, 50.4% ID, 30.4% DD; P=0.017). Our investigation confirms a positive association of the I allele of the *ACE* gene with endurance athlete status in a group of Polish rowers.

Key words: sport genetics, ACE I/D polymorphism, athlete status, rowers

Introduction

Physical performance is a complex multifactorial phenotype, influenced by a combination of various genetic and environmental components. Within the group of genetic factors that are believed to play a role in individual's physical performance, there are gene variants that have a significant impact on human body composition and metabolism. The determination of these components is important in professional, academic as well as individual's sports (Leońska-Duniec 2013).

In 1998, for the first time Montgomery and co-workers described a positive association between endurance exercise performance and a genetic variation, the inversion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene (*ACE*). This paper determined the role of the gene for sport successes and was the

inspiration for several groups of scientists worldwide (Leońska-Duniec 2013). Nowadays, the *ACE* gene is the most frequently investigated genetic marker in the context of genetic conditioning of athletic predispositions. The polymorphism has been associated with improvements in performance and exercise duration in a variety of populations (Puthucheary et al. 2011).

The product of the *ACE* gene is one of the key components of the renin angiotensin system (RAS) which catalyses the production of angiotensin II (ANG II) from angiotensin I (ANG I), resultantly increasing blood pressure. Furthermore, the enzyme is the essential part of the kallikrein-kinin system (KKS) where it degrades kinins into inactive fragments, thus reducing blood pressure. Moreover, the *ACE* is expressed in skeletal muscles, where it influences its function and biomechanical properties (Gordon et al. 2001; Jones et al. 2002; Moreau et al. 2005; Wagner et al. 2006).

The human *ACE* gene is located on chromosome 17 in position 17q23.3 with polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair Alu repeat sequence in intron 16 (Rigat et al. 1992a; Rieder et al. 1999). In this case, three *ACE* genotypes include DD, II homozygotes, and ID heterozygotes (Villard and Soubrierr 1996).

Studies concerning the connection between the *ACE* genotype and athlete status have shown that the I allele is associated with lower ACE activity in blood and tissue, and improved performance in endurance sports as rowing (Jones et al. 2002; Collins et al. 2004). Additionally, it is responsible for an increased percentage of slow-twitch type I fibers in human skeletal muscles (Zhang et al. 2003). Meanwhile, the D allele is associated with greater circulation and the ACE activity. As a result, it causes an enhanced performance in sports requiring sprinting or short bursts of power (Myerson et al. 1999; Woods et al. 2001) and power-related phenotypes in non-athletic populations (Hopkinson et al. 2004; Yamin et al. 2007; Giaccaglia et al. 2008). On the other hand, there are also some studies which have found no evidence for such associations (Rankinen et al. 2000; Nazarov et al. 2001).

In the view of the mentioned facts, the main aim of the present study was to determine the possible interaction between the *ACE* I/D polymorphism and endurance athlete status in a group of Polish rowers, members of academic sports clubs, in comparison with sedentary individuals.

Materials and methods

Ethics Committee

The Pomeranian Medical University Ethics Committee approved the study and written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki (Kruk 2013).

Subjects

121 male Polish rowers, members of academic sports clubs were recruited for this study. Twenty-eight of them were national representatives with no less than ten years experience participating in sport. Forty-two of them were the Poland National Championship medalist. All of them were rowers with no less than six years experience participating in sport.

For controls, samples were prepared from 115 unrelated volunteers (male students of University of Szczecin, aged 19–23). The athletes and controls were all Caucasian to ensure no likely racial gene skew and to overcome any potential problems of population stratification.

Protocol

Genomic DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany), according to the producer protocol.

PCR amplification of the polymorphic region of the *ACE* gene containing either the insertion (I) or deletion (D) fragment was performed. One pair of primers (forward: CTG GAG ACC ACT CCC ATC CTT TCT and reverse: GAT GTG GCC ATC ACA TTC GTC AGA) was used to determine the ACE genotype, yielding amplification products of approximately 490 bp (for the I allele) and 190 bp (for the D allele), as it has been described earlier (Rigat et al. 1992b). PCR mixture and thermal-time profile were coequal as described by Cięszczyk et al. (2009). The amplified DNA fragments were visualized by using 1.5% agarose gels stained with ethidium bromide. The research was performed in the molecular laboratory of Gdansk University of Physical Education and Sport, Poland.

Statistical analysis

Genotype distribution and allele frequencies between the groups of athletes and controls were compared, and significance was assessed by $\chi 2$ test. P values of < 0.05 were considered statistically significant.

Results

The *ACE* genotype distributions amongst athletes and controls were in Hardy-Weinberg equilibrium, making selection bias less likely. The distributions of the *ACE* genotypes and alleles are presented in Table 1.

The genotype distribution amongst the whole cohort of rowers (30.6% II, 53.7% ID, 15.7% DD) was significantly different to that amongst controls (19.1% II, 50.4% ID, 30.4% DD; P = 0.017). The difference in the I allele frequency between athletes and controls also reach statistical significance (57.4% vs. 44.3%; P = 0.013).

Table 1. Frequency of the ACE insertion (I) and deletion (D) alleles and genotypes. Numbers are absolute values and percentage frequencies are in parentheses, n is the number of subjects studied

Group	ACE allele		P	ACE genotype			P
	I	D		II	ID	DD	
Rowers (n = 121)	139 (57,4%)	103 (42,6%)	0.013	37 (30,6%)	65 (53,7%)	19 (15,7%)	0.017
Controls (n = 115)	102 (44,3%)	128 (55,7%)		22 (19,2%)	58 (50,4%)	35 (30,4%)	

Discussion

In the present study we analyzed genotype distribution of the *ACE* I/D polymorphism and the I allele frequency among the Polish rowers, members of academic sports clubs. Our research shows a statistical significant association between an excess of the I allele and athletic endurance phenotype. The similar tendency was revealed in the genotype distribution as the rowers showed a higher frequency of the II genotype.

The obtained results are in accordance with previous research on endurance athletes including rowers representing the highest level in Poland, which demonstrated a greater frequency of the II genotype in the examined athletes compared to sedentary individuals (Cięszczyk et al. 2009). Cięszczyk et al. (2010) also showed a high occurrence of the II genotype in elite Polish and Lithuanian judo players. Additionally, our results are similar to research from around the world on professional endurance athletes such as Australian rowers (Gayagay et al.

1998), British runners (Myerson et al. 1999), Spanish cyclists and long-distance runners (Alvarez et al. 2000; Muniesa et al. 2010), British high-altitude mountaineers (Montgomery et al. 1998), as well as Slovenian marathon runners (Hruskovicova et al. 2006), which proved a higher frequency of the II genotype in the examined athletes compared to controls.

Investigators have shown that the I allele of the *ACE* gene is associated with improved physical fitness and this effect may influence general sport ability favoring endurance performance (Nazarov et al. 2001; Jones et al. 2002; Scanavini et al. 2002; Collins et al. 2004). Many studies have revealed that the I allele is related with increased muscle efficiency (Williams et al. 2000), higher anabolic activity in response to physical training (Montgomery et al. 1999), explained by an enhanced intake of oxygen by the muscles (MacArthur et al. 2005). It is also responsible for an increased percentage of slow-twitch type I fibers in human skeletal muscles (Zhang et al. 2003). The physiological explanation of such observations is that the I allele is associated with lower ACE activity in blood and tissue and, consequently, with significantly lower ANG II and higher bradykinin levels (Jones et al. 2002; Collins et al. 2004). It also works through improvements in substrate delivery, mainly due to increased skeletal muscle glucose uptake (Myerson et al. 1999; Woods et al. 2000) and adaptation of the enzymes responsible for glucose catabolism, the efficiency of skeletal muscle increases (Ueda et al. 1995) with subsequent conservation of energy stores like glycogen storage (Montgomery et al. 1999). These factors could lead to more efficient ventriculo-vascular coupling during exercise and also enhance endurance performance (Gayagay et al. 1998).

However, other results obtained by some authors are inconsistent with our findings and data presented above and show a lower frequency of the II genotype in endurance athletes. Muniesa and co-workers (2010) have obtained the lower percentage of the II genotype in world-class rowers. They have explained their results by mixed strength-endurance character of rowing, consequently this discipline can not be classified as regular endurance sport. According to the authors, the II genotype may give negative effect for more power oriented sports such as rowing. Other report suggests that the D allele may support endurance performance instead of sprint power activity in some ethnic groups (Amir et al. 2007). Increased production of the ANG II not only influences capillary density in skeletal muscle but is also a key factor in mediating vascular smooth muscle growth (Jones et al. 2003). Moreover, it regulates oxygen consumption and energy expenditure (Cassis et al. 2002), and is involved in the regulation of body fluid balance via higher aldosterone secretion which results in retention of sodium and water during extreme endurance exercises (Amir et al. 2007). Authors connect mentioned effects with better level of endurance performance which may promote athletic success in individuals with the DD genotype, whereas some studies have shown the D allele as a factor responsible for sprint power in working muscles (Amir et al. 2007). The possible positive effect of the D allele in power sports is supported by higher percentage of the D allele in sprinters (Myerson et al. 1999), swimmers (Woods et al. 2001), and non-training individuals (Charbonneau et al. 2008; Giaccaglia et al. 2008).

The problem of defining the connection between the *ACE* I/D polymorphism and physical performance is even more complex because some reports have found no association between higher I/D alleles frequency and athlete status (Taylor et al. 1999; Rankinen et al. 2000). These difficulties are connected with the interpretation of association studies e.g. different authors may categorize the same sport discipline as endurance or power orientated, or the definition of elite athlete phenotype is variable. It should be also emphasized that most of the studies have involved too small sample sizes and have not been replicated in independent studies. The problem in the research is the limitation of the number of genetic cohorts of elite athletes from a variety of countries, ethnic groups and sports disciplines with extensive physical performance phenotypes (Leońska-Duniec 2013).

In conclusion, our investigation have proved that the *ACE* I/D allele could be one of the factors influencing the endurance performance. The study demonstrates a positive association between Polish rowers, members of academic sports clubs, and the *ACE* I allele which is advantageous for the endurance-type athletes in studied population. The obtained results support the general statement that the I allele of the *ACE* gene improves performance in endurance sports as rowing.

Acknowledgments

Scientific work financed from the budget funds for science for 2011–2014.

References

Alvarez R., Terrados N., Ortolano R., Iglesias-Cubero G., Reguero J.R., Batalla A., Cortina A., Fernández-García B., Rodríguez C., Braga S., Alvarez V., Coto E. Genetic variation in the renin-angiotensin system and athletic performance. Eur. J. Appl. Physiol. 2000; 82 (1–2): 117–20.

Amir O., Amir R., Yamin Ch., Attias E., Eynon N., Sagiv M., Sagiv M., Meckel Y. The ACE deletion allele is associated with Israeli elite endurance athletes. Exp. Physiol. 2007; 92: 881–886.

Cassis L., Helton M., English V., Burke G. Angiotensin oxygen consumption. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2002; 282: 445–453.

Charbonneau D.E., Hanson E.D., Ludlow A.T., Delmonico M.J., Hurley B.F., Roth S.M. ACE genotype and the muscle hypertrophic and strength responses to strength training. Med. Sci. Sports Exerc. 2008; 40: 677–683.

Cięszczyk P., Krupecki K., Maciejewska A., Sawczuk M. The angiotensin converting enzyme gene I/D polymorphism in Polish rowers. Int. J. Sports Med. 2009; 30 (8): 624–627.

Cięszczyk P., Maciejewska A., Sawczuk M., Ficek K., Eider J., Jascaniene N. The angiotensin converting enzyme gene I/D polymorphism in ellite Polish and Lithuanian judo players. Biol. Sport 2010; 27: 119–122.

Collins M., Xenophonotos S.L., Cariolou M.A., Mokone G.G., Hudson D.E., Anastasiades L., Noakes T.D. The ACE gene endurance performance during the South African Ironman Triathlons. Med. Sci. Sports Exerc. 2004; 36: 1314–1320.

Gayagay G., Yu B., Hambly B., Boston T., Hahn A., Celermajer D.S., Trent R.J. Elite endurance athletes and the ACE I allele – the role of genes in athletic performance. Hum. Genet. 1998; 103 (1): 48–50.

Giaccaglia V., Nicklas B., Kritchevsky S., Mychalecky J., Messier S., Bleecker E., Pahor M. Interaction between angiotensin converting enzyme insertion/deletion genotype and exercise training on knee extensor strength in older individuals. Int. J. Sports Med. 2008; 29: 40–44.

Gordon S.E., Davis B.S., Carlson C.J., Booth F.W. ANG II is required for optimal overload-induced skeletal muscle hypertrophy. Am. J. Physiol. Endocrinol. Metab. 2001; 280: E150–E159.

Hopkinson N.S., Sharshar T., Ross E.T., Nickol A.H., Dayer M.J., Porcher R., Jonville S., Moxham J., Polkey M.I. Corticospinal control of respiratory muscles in chronic obstructive pulmonary disease. Respir. Physiol. Neurobiol. 2004; 41: 1–12.

Hruskovicová H., Dzurenková D., Selingerová M., Bohus B., Timkanicová B., Kovács L. The angiotensin converting enzyme I/D polymorphism in long distance runners. J. Sports Med. Phys. Fitness. 2006; 46 (3): 509–513.

Jones A., Montgomery H.E., Woods D.R. Human performance: a role for the ACE genotype? Exerc. Sport Sci. Rev. 2002; 30: 184–190.

Jones A., Woods D.R. Skeletal muscle RAS and exercise performance. Int. J. Biochem. Cell Biol. 2003; 35: 855–866.

Kruk J. Good scientific practice and ethical principles in scientific research and higher education. Cent. Eur. J. Sports Sci. Med. 2013; 1: 25–29.

Leońska-Duniec A. Genetic research in modern sport. Centr. Eur. J. Sport Sci. Med. 2013; 3: 19–26.

MacArthur D.G., North K.N. Genes and human elite athletic performance. Hum. Genet 2005; 116: 331–339.

Montgomery H.E., Marshall R., Hemingway H., Myerson S., Clarkson P., Dollery C., Hayward M., Holliman D.E., Jubb M., World M., Thomas E.L., Brynes A.E., Saeed N., Barnard M., Bell J.D., Prasad K., Rayson M., Talmud P.J., Humphries S.E. Human gene for physical performance. Nature. 1998; 393 (6682): 221–222.

Montgomery H., Clarkson P., Barnard M., Bell J., Brynes A., Dollery C., Hajnal J., Hemingway H., Mercer D., Jarman P., Marshall R., Prasad K., Rayson M., Saeed N., Talmud P., Thomas L., Jubb M., World M., Humphries S. Angiotensin-converting-enzyme gene insertion/deletion polymorphism and response to physical training. Lancet. 1999; 353: 541–545.

Moreau M.E., Garbacki N., Molinaro G., Brown N.J., Marceau F., Adam A. The kallikrein-kinin system: current and future pharmacological targets. J. Pharmacol. Sci. 2005; 99: 6–38.

Muniesa C.A., Gonzalez-Freire M., Santiago C., Lao J.I., Buxens A., Rubio J.C., Martin M.A., Arenas J., Gomez-Gallego F., Lucia A. World-class performance in lightweight rowing: Is it genetically influenced? A comparison with cyclist, runners and non-athletes. Br. J. Sports Med. 2010; 44 (12): 898–901.

Myerson S., Hemingway H., Budget R., Martin J., Humphries S., Montgomery H. Human angiotensin I-converting enzyme gene and endurance performance. J. Appl. Physiol. 1999; 87: 1313–1316.

Nazarov I.B., Woods D.R., Montgomery H.E., Shneider O., Kazakov V.I., Tomilin N.V., Rogozkin V.A. The angiotensin converting enzyme I/D polymorphism in Russian athletes. Eur. J. Hum. Genet. 2001; 9: 797–801.

Puthucheary Z., Skipworth J.R., Rawal J., Loosemore M., Van Someren K., Montgomery H.E. The ACE gene and human performance: 12 years on. Sports Med. 2011; 41 (6): 433–448.

Rankinen T., Wolfarth B., Simoneau J.A., Maier-Lenz D., Rauramaa R., Rivera M.A., Boulay M.R., Chagnon Y.C., Perusse L., Keul J., Bouchard C. No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. J Appl. Physiol. 2000; 88: 1571–1575.

Rieder M.J., Taylor S.L., Clark A.G., Nickerson D.A. Sequence variation in the human angiotensin converting enzyme. Nat. Genet. 1999; 22: 59–62.

Rigat B., Tiret L., Visvikis S., Breda C., Corvol P., Cambien F. Evidence, from combinet segregation and linkage analysis ACE levels. Am. J. Hum. Genet. 1992a; 51: 197–205.

Rigat B., Hubert C., Corvol P., Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 1992b; 20: 1433.

Scanavini D., Bernardi F., Castoldi E., Conconi F., Mazzoni G. Increased frequency of the homozygous II ACE genotype in Italian Olympic endurance athletes. Eur. J. Hum. Genet. 2002; 10: 576–577.

Taylor R.R., Mamotte C.D.S., Fallon K., van Bockxmeer F.M. Elite athletes and the gene for angiotesin-converting enzyme. J. Appl. Physiol. 1999; 87: 1035–1037.

Ueda S., Elliott H.L., Morton J.J., Connell J.M. Enhanced pressor response to angiotensin I in normotensive men with the deletion genotype (DD) for angiotensin-converting enzyme. Hypertension 1995; 25: 1266–1269.

Wagner H., Thaller S., Dahse R., Sust M. Biomechanical muscle properties and angiotensin-converting enzyme gene polymorphism: a model-based study. Eur. J. Appl. Physiol. 2006; 98: 507–515.

Williams A.G., Rayson M.P., Jubb M., World M., Woods D.R., Hayward M., Martin J., Humphries S.E., Montgomery H.E. The ACE gene and muscle performance. Nature. 2000; 403: 614–615.

Woods D.R., Humphries S.E., Montgomery H.E. The ACE I/D polymorphism and human physical performance. Trends Endocrinol. Metab. 2000; 11: 416–420.

Woods D., Hickman M., Jamshidi Y., Brull D., Vassiliou V., Jones A., Humphries S., Montgomery H. Elite swimmers and the D allele of the ACE I/D polymorphism. Hum. Genet. 2001; 108: 230–232.

Villard E., Soubrierr F. Molecular biology and genetics of the angiotensin I-converting enzyme: potential implications in cardiovascular diseases. Cardiovasc. Res. 1996; 32: 999–1007.

Yamin C., Amir O., Sagiv M., Attias E., Meckel Y., Eynon N., Sagiv M., Amir R.E. ACE ID genotype affects blood creatine kinase response to eccentric exercise. J Appl. Physiol. 2007; 103: 2057–2061.

Zhang B., Tanaka H., Shono N., Miura S., Kiyonaga A., Shindo M., Saku K. The I allele of the angiotensin-converting enzyme gene is associated with an increased percentage of slow-twitch type I fibers in human skeletal muscle. Clin. Genet. 2003; 63: 139–144.

Cite this article as: Jastrzębski Z., Leońska-Duniec A., Kolbowicz M., Tomiak T. The angiotensin converting enzyme gene I/D polymorphism in Polish rowers. Centr Eur J Sport Sci Med. 2014; 5 (1): 77–82.