ABSTRACT

Objective: Previous studies have shown controversial relationships between ACE and ACTN3 gene polymorphisms and sports performance. Thus, the aim of our study was to assess anaerobic and aerobic performance indicators of young female soccer players with different ACE/ACTN3 gene profiles. Methods: Twenty-seven female soccer players aged 16-18 underwent acceleration, speed, strength, anaerobic power and aerobic endurance tests and had their ACE and ACTN3 polymorphisms determined. Results: Based on genetic analysis, they were divided into the following groups: ACE II (n=2), ACE ID (n=11), ACE DD (n=14), ACTN3 XX (n=5), ACTN3 RR (n=7) and ACTN3 RX (n=15). ACE DD and ACE ID groups differed significantly in terms of results achieved on the 5 m sprint test (1.15±0.05 s vs 1.10±0.05 s, P=0.42). ACTN3 RR and RX achieved better results than the ACTN3 XX group in seven continuous vertical jumps (26.57±1.59 cm vs 25.77±2.51 cm vs 22.86±1.16 cm, respectively; P=0.007 for RR vs XX and P=0.021 for RX vs XX). Conclusion: High prevalence of ACE DD and ACTN3 RX genotypes in our subjects may suggest that faster and more powerful young females tend to perform better in soccer. Nevertheless, the absence of differences in most of the physical test results indicates that different genotypes are compatible with high-level soccer performance, meaning that it is the phenotype-genotype interaction that makes a successful female soccer player. Level of Evidence I, Prognostic studies — Investigating the effect of a patient characteristic on disease outcome.

Keywords: Genes; Sports performance; Soccer; Physical endurance.
INTRODUCTION
During the last two decades, there has been considerable interest in finding the genetic factors responsible for human sport capabilities. However, the findings on the relations between genetic polymorphisms and sports performance are frequently controversial.\(^1\) Race and sex seem to strongly affect the results, as well as the sporting modality. The most explored genes that are suggested to influence physical performance are the angiotensin converting enzyme (ACE) and \(\alpha\)-actinin-3 (ACTN3). After the discovery of ACE insertion/deletion (I/D) polymorphism,\(^2\) i.e. its three genotypes (II homozygote, ID heterozygote and DD homozygote), ACE gene became the first genetic element suggested to have substantial impact on human physical performance.\(^3\) Although there is some inconsistency in the literature, majority of studies have shown that the I allele is associated with improved performance in endurance sports, while the deleted form of the variant (D allele) is associated with enhanced performance in sports requiring sprinting or short bursts of power.\(^4\) The ACTN3 gene encodes for the synthesis of \(\alpha\)-actinin-3 in skeletal-muscle fibres, a sarcomeric protein necessary for producing explosive, powerful contractions.\(^5\) A genetic variation in the ACTN3 gene that results in the replacement of an arginine (R) with a stop codon (X) at amino acid 577 (R577X, rs1815739) can create different versions of the ACTN3 gene. Ma and colleagues\(^6\) analyzed 88 articles focused on ACTN3 and athletic performance and concluded that ACTN3 R allele is significantly associated with power performance, while ACTN3 XX might be postulated to contribute to endurance performance. Although a significant amount of papers on the relationship between ACE/ACTN3 genes and sports achievement was published during past 15 years, this topic is still considered unexplored.\(^7\) The lack of repeatability and variations in study design and the population studied prevent a final conclusion to be drawn from current literature data.\(^8\) Since ethnicity, gender, sport modality and other factors may influence the results, we conducted a study that explores the relationship between the abovementioned genes and athletic performance in a very specific group of subjects: young elite female football players. The aims of the study were to assess if certain ACE/ACTN3 genotype prevails in this cohort and if players with different ACE/ACTN3 genotypes achieve significantly different results in basic speed, power and endurance tests.

METHODS
The study was conducted among 27 female football (soccer) players pertaining to the Serbian national U18 team (16-18 years old). All participants and their parents gave a written informed consent (EFT) for data collection and participation in the study. The study was approved by the local Ethical committee (01-10988) and performed according to the principles of World Medical Association's Declaration of Helsinki. The first part of the study was directed towards the genetic analyses (ACE and ACTN3 genes), performed by trained personnel in laboratory at School of Medicine, University of Belgrade, Serbia. Using sterile swabs, buccal cells samples were collected from all the tested subjects. Genomic DNAs from buccal samples were extracted manually, using the commercial kit (PureLink genomic DNA, Invitrogen). ACE and ACTN3 genetic polymorphisms were determined by HRM-PCR, using an “in house” developed and validated method. After analysis of ACE polymorphism, subjects were divided into three groups: 1) ACE I group, 2) ACE ID group, 3) ACE II group. Furthermore, based on analysis of ACTN3 gene, subjects were divided into three groups: 1) ACTN3 XX group, 2) ACTN3 RX group, 3) ACTN3 RR group. Analysis of athletic capabilities of subjects was conducted a week before the start of the National league using six performance tests: 1) sprinting 5m (acceleration), 2) sprinting 20m (speed), 3) zig-zag test (agility), 4) squat jump (power), 5) seven continuous vertical jumps (anaerobic power), 6) Yo-Yo test (endurance). Subjects were familiarized with the testing protocol, and performed every test twice (better result was chosen for further analyses). All performance tests were conducted in Serbian Institute of Sport and Sports Medicine facilities, by Strength and Conditioning specialists.

Sprinting 20m with transitory time on 5m. Subject started running from the upper starting position, after auditory signal. Time needed to run 20m was used for estimation of speed, while time needed to pass the first 5m was used for estimation of acceleration ability of subjects.\(^9\) Time was measured using FITRO light gates (Fitronics.r.o., Slovakia) and results expressed in 1/100 seconds.

Zig-Zag test. Agility was estimated according to result achieved on Zig-zag agility test that consists of four 5-m sections set out at the angle of 100 degrees.\(^10\) Subject starts running from the upper starting position, after visual signal. Time was measured using FITRO light gates (Fitronics.r.o., Slovakia). Results are expressed in 1/100 seconds.

Squat jump. Explosive strength was estimated using squat jump, the test that measures concentric phase of contraction of body extensors.\(^11\) Starting position includes semisquat (90 degrees of flexion in knees) and arms on hips. After two seconds spent in this position, subjects jumps vertically and lands to similar semisquat. The height of jump was measured using FITRO jumper (Fitronics.r.o., Slovakia).

Seven continuous jump with bent legs. Anaerobic power of subjects was estimated using continuous jumping for 15 seconds.\(^12\) Subject places arms on hips and starts jumping after auditory signal. Contact phase with ground (landing phase) includes knee flexion of about 90 degrees. Jump height was measured using FITRO jumper (Fitronics.r.o., Slovakia), and measured with accuracy of 1mm.

Yo-Yo test. Aerobic endurance was estimated using Yo-Yo test level 1.\(^13\) This test involves continuous running between two lines 20m apart. The subjects start with their foot behind one of the lines, and begin running when instructed. They continue running between the two lines, turning when signaled by the recorded beeps. After each minute, the pace gets quicker. If the line is not reached in time the subject must run to the line turn and try to catch up with the pace within two more “beeps”. The test is stopped if the subject fails to catch up with the pace within the two ends. The athlete’s score is the total distance covered before they were unable to keep up with the recording.

Statistical analysis. The statistical analysis was performed with SPSS...
program 21.0 for Windows. Results are expressed as mean ± standard error of the mean (on Figures), or mean ± standard deviation (in brackets). The differences between three groups were assessed using Kruskal-Wallis test, followed by Mann Whitney test, if the difference between two groups was significant (probability set at 5% level).

RESULTS
The performance test boxplot results for subjects with different ACE- and ACTN3 genes version are presented in Figures 1 to 6 (median, the 1st and 3rd quartile, maximum and minimum values). ACE DD genotype was found in 14 subjects, ACE ID in 11 and ACE II in only two subjects. ACTN3 RR genotype was found in seven, ACTN3 XX in five and ACTN3 RX in 15 subjects.

Since ACE II genotype was found in only two subjects, this group was excluded from the statistical analysis, but their results are presented on the Figures for the descriptive purposes. ACE DD and ACE ID groups significantly differed only in results achieved on 5m sprint test (1.15±0.05 s vs 1.10±0.05 s, P=0.042). Difference between ACTN3 groups was found only in test of seven continuous jumps (P=0.022). ACTN3 RR and RX groups achieved significantly better result than ACTN3 XX group (26.57±1.59 cm vs 25.77±2.51 cm vs 22.86±1.16 cm, respectively; P=0.007 for RR vs XX and P=0.021 for RX vs XX).

DISCUSSION
The concept that genetics are strongly associated with human physical performance has been widely accepted in the last decades. Previous studies were mostly performed on athletes from pure strength/power oriented sports (swimmers, sprinters, etc.), or endurance-based sports (marathon runners, rowers, etc.), which is the most likely the best way to study the relationship between genes that are suggested to affect mechanisms responsible for anaerobic/aerobic capabilities. In our study, we explored the possibility that genetic polymorphism would be related to results in a number of performance tests usually used for testing athletes from team sports, more specifically football. In this context, we assessed in 27 young female elite football players, the relationship between two genes (ACE and ACTN3) and results obtained in six performance tests. Our results do not show significant relationship between ACE/ACTN3 polymorphism and performance in different tests of physical abilities of young female football players. The ACE gene was the first gene whose polymorphism was suggested to affect athletic performance, and the one mostly studied. ACE is a part of complicated cascade of molecules known as the renin-angiotensin system, thereby directly influencing blood pressure and fluid balance and indirectly influencing cardiovascular and musculoskeletal structure and function. A number of studies have shown that the ACE I/D polymorphism affects athletic performance and exercise duration in a variety of populations (athletes, adolescents, older people, pulmonary and cardiovascular patients, etc.). However, despite the numerous studies positively associating ACE gene polymorphisms with performance, other studies failed to find any association. With respect to the rapid increase in the number of original researches in this area, review articles and meta-analysis were recently published. A systematic review and meta-analysis by Ma and coworkers, in which 366 articles about the relationship between ACE gene and athletic performance were analyzed, concluded that there was no statistically significant association between ACE I allele and endurance sport events (but it was very close to be so), while...
ACE genotype (DD, II, ID) achieved on “Seven continuous jumping test” (Significantly different from ACTN3 RX, Significantly different from ACTN3 RR).

ACTN3 genotype (XX, RR, RX) and ACE genotype (DD, II, ID) achieved on “Yo-Yo” endurance test (estimated VO2max (mean±SD) based on ran distance: ACTN3 XX group 44.72±3.06, ACTN3 RR group: 44.35±2.84, ACTN3 RX group: 45.34±2.79 ml/kg, ACE DD group 43.60±3.11, ACE II group: 44.65±2.93, ACE ID group: 45.62±2.62 ml/kg).

Subject with different ACTN3 genotype (XX, RR, RX) and ACE genotype (DD, II, ID) achieved on “Seven continuous jumping test” (Figure 5).

Figure 5. Boxplot: results that subject with different ACTN3 genotype (XX, RR, RX) and ACE genotype (DD, II, ID) achieved on “Seven continuous jumping test” (Significantly different from ACTN3 RX, Significantly different from ACTN3 RR).

Figure 6. Boxplot: results that subject with different ACTN3 genotype (XX, RR, RX) and ACE genotype (DD, II, ID) achieved on “Yo-Yo” endurance test (estimated VO2max (mean±SD) based on ran distance: ACTN3 XX group 44.72±3.06, ACTN3 RR group: 44.35±2.84, ACTN3 RX group: 45.34±2.79 ml/kg, ACE DD group 43.60±3.11, ACE II group: 44.65±2.93, ACE ID group: 45.62±2.62 ml/kg).

a significant association between ACE II genotype and endurance group was observed. The results of our study show that our subjects with ACE DD genotype, which is suggested to be related to power and speed, achieved worse result that those with ACE ID genotype in test of acceleration (5m sprint). A similar study was performed by Micheli et al20 on young male football players, and they reported that the ID genotype of ACE was associated with the best performances in squat jump and countermovement jump. We did not compare the results that ACE II group achieved with other two groups due to small number of subjects with ACE II genotype (just two subjects). Such low frequency of ACE II genotype is in consent with some previous reports of ACE genotype distribution in Serbian population, such as around 16% in a study by Stankovic et al.20 That ACE II genotype is the least frequent genotype in population of football players was shown by many previous studies,11–12 proving the notion that although aerobic metabolism dominates the energy delivery during a football game, power and strength play a greater role in the determination of success in football.24

Unlike ACE, which is often connected with endurance sports, researchers generally concentrate on association between ACTN3 and power sports. Numerous studies have confirmed associations between the ACTN3 R577 allele and muscle power phenotypes in humans (i.e. sprint time, fiber type), suggesting that at least one copy of the ACTN3 R577 allele is beneficial for participation in sprint and power sports,25 while there is consistently strong association between RR genotype and elite power performance.12,26–29 This was confirmed by Alfred and colleagues27 who conducted a meta-analysis on the published association between ACTN3 and athletic status. That ACTN3 RR genotype determines anaerobic abilities of football players was shown by Pimenta et al,30 whose subjects with genotype ACTN3 RR were the fastest in short distances and presented higher jumping potential. Our subjects carried in most cases RX type of ACTN3 gene (15 subjects), followed by RR version (7 subjects) and XX version of the gene (5 subjects). Ulucan et al32 also reported the lowest frequency of ACTN3 XX genotype in Turkish football players, as well as Egorova et al33 in Russian football players, but contrary to our results, ACTN3 RR genotype was the most frequent genotype in many studies on male football players.22,23,34,35 The results of our study do not support the hypothesis on relationship between ACTN3 and anaerobic abilities of subjects, except in case of anaerobic power manifested through seven continuous vertical jumps. Subjects with ACTN3 XX genotype performed worse than both RR and RX groups, confirming that at least one R577 allele is enough to make difference in anaerobic disciplines.

CONCLUSIONS

Prevalence of ACE DD and ACTN3 RX genotypes in our subjects suggests that faster and more powerful girls distinguish in this sport. However, subjects with different ACE/ACTN3 genotype significantly differed in only one out of six performance tests, suggesting that assessing these specific genes polymorphisms in young elite female football players may not give important information regarding their physical abilities, and thus their use as one of factors for talent identification is questionable. Although genetics may predispose a subject for some sport, it is the interaction of the phenotype with genotype that makes the final result. Athletic performance is the result of many different factors, such as training characteristics, anthropometric and morphometric characteristics of the athlete, etc. These phenotypes are influenced by a variety of other processes and cellular pathways which are eventually influenced by a large number of individual and relevant genes.

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