Distribution of Angiotensin-1 Converting Enzyme Insertion/Deletion and α-Actinin-3 Codon 577 Polymorphisms in Turkish Male Soccer Players

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ABSTRACT: Angiotensin-1 converting enzyme (ACE) gene and α-actinin-3 (ACTN3) gene polymorphisms are considered to be the most important candidate genes for genetic predisposition to human athletic performance. In the present study, we aimed to analyze the distribution of *ACE* and *ACTN3* polymorphisms for the first time in male Turkish soccer players. In this prospective study, our cohort consisted of 25 professional players, all with Turkish ancestry. Polymerase chain reaction (PCR)-restriction length polymorphism was used for the characterization of the genotype of *ACTN3* and single PCR for *ACE*. For *ACE* genotype, 16%, 44%, and 40% of the players had insertion/insertion (II), insertion/deletion (ID), and deletion/deletion (DD) genotypes, respectively, whereas 20% had XX, 36% had RX, and 44% had RR genotypes for *ACTN3*. When we examined the allelic percentages, for *ACE*, D allele was recorded as 62 and I as 38, and for *ACTN3*, R allele was 62 and X was 38. Our results were in agreement with the previous reports, indicating the presence of *ACTN3* D and *ACE* X allele in soccer players. We suggest that *ACE* and *ACTN3* genotypes are important biomarkers for genetic counseling for the individuals who are prone to be successful soccer players.

KEYWORDS: *ACE*, *ACTN3*, soccer, polymorphism, sport genetics

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Introduction

Human performance is affected by both the genetic makeup of the individual and the environmental factors. Current research and interest in sports genetics focus on genetic variants that may make a significant contribution to the individual's performance. It is known that personal traits such as endurance, strength, power, muscular coordination, and psychological willingness and motivation, all have a genetic background.[1] The most frequently investigated genetic polymorphisms in terms of athletic performance or predisposition to athletic capacity are angiotensin-1 converting enzyme (ACE) gene and α-actinin-3 (ACTN3) gene.[2] Variants in these genes have been reported to be associated with elite athletic performance and with quantitative physical performance traits in the general population.[3–5]

ACTN3 was the first structural gene specific to skeletal muscle that has been associated with athletic performance.[6] The actinins, major and important Z line functional and structural proteins in sarcomers, are members of the actin-binding protein family.[7] *ACTN3* codes for ACTN3 in humans, and its expression is restricted to fast, type 2 fibers.[8] North et al[9] reported that a deficiency of α-actinin protein because of a premature stop codon in *ACTN3* alters the designation for the amino acid arginine to a stop codon at position 577 (R577X; dbSNP rs1815739) in exon 16 and may contribute to the possibility of differential fitness. The *ACTN3* 577R allele and 577RR genotype are associated with top-level, power-orientated athletic performance in a wide array of ethnic groups.[10] The *ACTN3* R577X polymorphism has been reported to be associated with elite athletic status,[6] endurance athletes,[11] and many other groups.[12–16]

ACE is located on chromosome 17q23 and comprises 26 exons and 25 introns. It contains a polymorphism due to an insertion (I) or a deletion (D) of a 287 bp Alu sequence in intron 16, resulting in the three genotypes of insertion/insertion (II), insertion/deletion (ID), and deletion/deletion (DD).[17] The I/D polymorphism is associated with circulating and tissue ACE levels. Individuals homozygous for the D allele had higher tissue and plasma ACE concentrations than heterozygotes and II homozygotes.[18] Many of the case–control studies reported that success in speed-strength disciplines, such as short-distance running, long jump, high jump, and short-distance swimmers, is associated with *ACE* DD genotype.[19,20] On the other hand, individuals with the II genotype

have a lower ACE serum concentration and have more success in endurance-related disciplines such as medium- and long-distance running, race walking, and rowing.[21,22] Two different studies involving Spanish and Lithuanian football players showed that players had a significantly higher percentage of the *ACE* ID genotype when compared to the nonathletic population.[23,24]

Previous studies, including related genetic polymorphisms and football players, are very limited, especially in Turkish subjects. The aim of this study was to determine the genotype and allele distribution of *ACTN3* and *ACE* in Turkish male football players and assess the impact on predisposition to football.

Materials and Methods

Study subjects. All players enrolled for the study had Turkish ancestry and were members of a Turkish football team. The study was conducted in accordance with the principles of the Declaration of Helsinki II. Üsküdar University Ethical Committee approved the study protocol, and written informed consent was obtained from each player once prospective participants understood and accepted the aim and protocol of the study.

Genotyping. DNA isolation was carried out by using High Pure Polymerase Chain Reaction Template Preparation Kit (Roche Diagnostics) using peripheral blood. The region of interest, *ACTN3*, was amplified using the following primers: forward 5′-CTG TTG CCT GTG GTA AGT GGG-3′ and reverse 5′-TGG TCA CAG TAT GCA GGA GGG-3′, as described previously.[5] Polymerase chain reaction (PCR) was performed by initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing and extension at 72°C for one minute, and a final extension for seven minutes at 72°C. Genotyping of the *ACTN3* R577X was maintained by restriction length polymorphism method. The 290-bp amplicons were digested by DdeI (New England Biolabs) as recommended by the manufacturer. Digested fragments were separated on 10.0% polyacrylamide gel electrophoresis and visualized under UV light by ethidium bromide staining. The wild-type allele, 577R, showed fragments of 205 and 85 bp, whereas the variant allele, 577X, showed fragments of 108, 97, and 85 bp (Fig. 1).

For the *ACE* genotype, conventional PCR amplifications were carried out. Primers 5′-CTGGAGACCAC TCCCATCCTTTCT-3′and5′-GATGTGGCCATCACAT TCGTCAGAT-3′ were used for the amplification. The final volume of the PCR mixture was 50 µL and contained 50–100 ng genomic DNA, 1 mM of each primer, 50 mM KCl, 1 mM deoxynucleotide triphosphate (dNTP), 1.5 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.0, and 1 U Taq DNA polymerase. An initial denaturation at 94°C for five minutes was followed by annealing at 58°C for one minute and extension at 72°C for two minutes. Amplification was finalized with 30 cycles: denaturation at 94°C for one minute, annealing at 58°C for one minute, and extension at 72°C for two minutes, followed by a final elongation at 94°C for one minute, annealing at 58°C for one minute, and extension at 72°C for seven minutes. Amplicons were separated by electrophoresis on a 2% agarose gel and visualized under UV light after ethidium bromide staining. Electrophoresis gave rise to three possible patterns: a 490-bp band (II genotype), a 190-bp band (DD genotype), or both 490- and 190-bp bands (I/D genotype) (Fig. 2). Amplicons had 190 bp in the presence of the D allele and 490-bp fragment in the presence of the I allele.

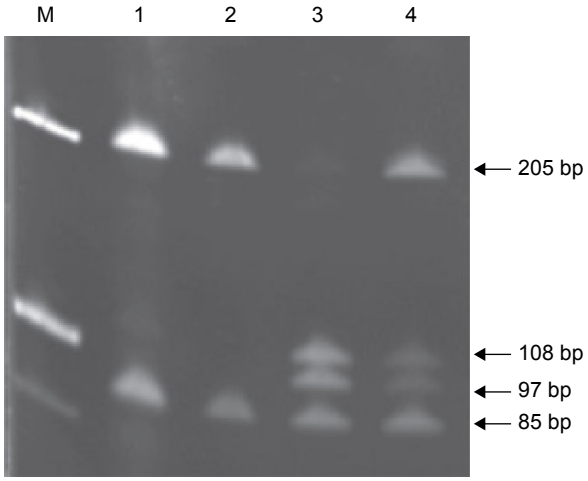

Figure 1. Polyacrylamide gel electrophoresis images of *ACTN3* polymorphisms (M: 100 bp molecular marker, Lanes 1 and 2: RR genotype, Lane 3: XX genotype, Lane 4: RX genotype).

Results

The percentage of the *ACE* genotype in the examined players was 16, 44, and 40 for II, ID, and DD genotypes, respectively. For the *ACTN3* genotype, the respective frequencies were 20, 36, and 44 for XX, RX, and RR. An allelic count gave rise to 19 (38%) I and 31 (62%) D alleles for *ACE* and 31 (62%) R and 19 (38%) X alleles for *ACTN3*. Table 1 lists the genotype and allelic frequencies of *ACE* and *ACTN3*. According to the genotypes, nine different combinations were found, five of the players had DD + RR, the same number of the players had ID + RR, four players had ID + RX, three players had DD + RX, two players had DD + XX, the same

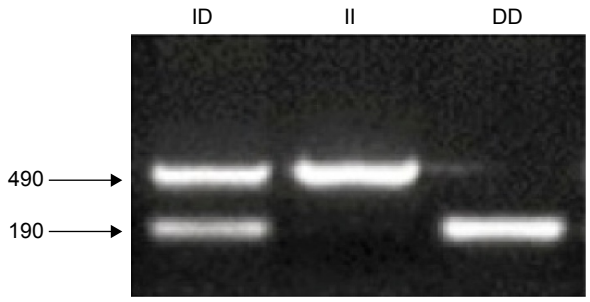

Figure 2. Agarose gel images of *ACE* polymorphisms.

Table 1. Genotypic and allelic distribution of the *ACE* and *ACTN3* in the examined players.

		GENOTYPE			ALLELE FREQUENCY	
		II	ID	DD	I	D
ACE (n = 25)	Number	4	11	10	19	31
	Percentage (%)	16	44	40	38	62
		XX	RX	RR	X	R
ACTN3 (n = 25)	Number	5	9	11	19	31
	Percentage (%)	20	36	44	38	62

number of the players had ID + XX, two players had II + RX, one player had II + XX, and one player had II + RR (data not shown in table).

Discussion

Recent studies have indicated that several genes are involved in determining the performance of the players, both physiologically and psychologically.[25] Genetic models could be developed and used to find the optimal genetic endowment of a player to help scientists establish which genetic polymorphisms are advantageous for proper performance in different sports types. Therefore, the creation of genomic databases will be very useful for sport scientists. In this study, we solely examined 25 senior football players in terms of *ACE* and *ACTN3* polymorphisms and aimed to associate these polymorphisms with predisposition to football.

In our study cohort, the RR genotype for *ACTN3* genotype was higher than XX and RX. The R allele, considered to be the wild-type allele of the gene and associated with the rapid contraction of the sarcomers, was also high when compared to X allele. Egorova et al[26] reported similar results in Russian football players; they examined 240 football players, and 46.25% had the RR genotype. Santiago et al[16] analyzed 60 Brazilian football players, and in their cohort, 48.3% of the players had RR genotype. Pimenta et al[27] aimed to compare acute inflammatory responses, muscle damage, and hormonal variations with eccentric training in soccer athletes and examined 37 professional soccer players of which 40.5% were RR. To the best of our knowledge, this is the first report in Turkish male professional soccer players, and our results were in agreement with previous studies, indicating R allele and RR genotype superiority for soccer players.

In our cohort, 44% of the players had ID genotype, 40% of the players had DD genotypes for *ACE,* and 84% of the players had at least one D allele. The allelic count revealed D allele as 62% in players. D allele is responsible for high ACE concentration and is associated with success in speed-strength disciplines.[17] Gineviciene et al[24] examined 199 Lithuanian football players and reported similar results to ours. They showed that ID was the highest genotype (46.7%), and together with DD genotype, the percentage of the players having at least one D allele was 76.3%. Juffer et al[23] analyzed 54 male professional soccer players, and ID was the most detected genotype. Unlike our results, Egorova et al[26] examined 213 Russian football players and found the frequency of ID and DD genotypes as 28.6% and 50.7%, respectively, giving both genotypes an overall frequency of 79.3%, which is similar to our results.

The cumulative effect of genotypes in human metabolism had always received great attention. In an attempt to find the optimal genotypes, several researchers have examined candidate genes separately, in different populations, to create a pool of genomic data. Genotype combinations are very useful for identifying a certain metabolism, or a part of a metabolism, if they are part of the same reaction. For example, *ACTN3* and *ACE* have crucial roles for their metabolisms, but they do not have roles in the same biologic pathway. But it is important to understand performance-enhancing polymorphisms to create an optimal genomic score for being an elite athlete. In our cohort, five of the players had the DD + RR genotype combinations and the same number had ID + RR genotypes. The D and R alleles were found together in these genotypes, the most important similarity between these genotypes. On the other hand, one player had the II + XX genotype, thought to be associated with endurance capacity.

The number of the subjects examined in the current study is the main limitation. We hope this preliminary study will guide new studies, with extended numbers of players in different kinds of sports to evaluate the effect of the combination of these polymorphisms more accurately.

Conclusion

In this study, we examined the distribution of *ACE* and *ACTN3* polymorphisms in male Turkish professional soccer players for the first time. *ACTN3* RR genotype and R allele and *ACE* ID genotype and D allele dominated our cohort. These polymorphisms are well known and have been examined in different populations. Therefore, we did not compare the genomic results with sedentary people; rather, we examined the combined effect of these polymorphisms. According to our previous and current results, we hypothesize that elite soccer players tend to have a power/strength-oriented genotype. These polymorphisms, alone or in combination with the additional polymorphisms, should be taken into account when deciding a genomic score profile for success in sports.

Author Contributions

Conceived and designed the experiments: KU. Analyzed the data: CS, TB. Wrote the first draft of the manuscript: KU. Contributed to the writing of the manuscript: CS. Agree with manuscript results and conclusions: KU, CS, TB. Jointly developed the structure and arguments for the paper: KU, CS. Made critical revisions and approved final version: All authors. All authors reviewed and approved of the final manuscript.

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