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Research Article

T786C Polymorphisms of Endothelial Nitric Oxide Synthase 3 (eNOS3) in basketball players

Sarab Hussain Khaleel

Department of Anatomy, College of Medicine, University of Al-Qadisiyah, Iraq

ABSTRACT

Genetic factor plays an important role in athletes performance. This study was amid to explain individual variability in eNOS3 – T786C polymorphism that acts a vital role athletes performance. The endothelial nitric oxide synthase three gene variant has been studied. The T786C polymorphism of eNOs3 gene studied in power and endurance sport. The genotypic and allelic frequencies of the eNOS3 T786c polymorphism in basketball players compare with control group. DNA extracts from 35 man player basketball and 40 healthy men as a control. The T786C polymorphism was determined genotype by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The frequency of the T allele was significant for basketball player compared to the control group (60% vs 40.5%, P= 0.334). While genotypes disruption there is no statically significant among basketball player (40% TT, 40% TC, 20% CC) compared with control (30% TT, 25% TC, 18% CC), (p=0.365, 0.167, 0.069 respectively). It was concluded that T allele has a significant relationship with basketball players, and it has a beneficial effect on performance parameters, but it needed further study.

Keywords: Endothelial Nitric Oxide synthase; T786C polymorphism; PCR.

INTRODUCTION

Genetic variants are showing a number of the differences between athletic individual and non-athletic individual, and between individuals with some sports to occupy a number of physiological factors. Recently many studies showed the α-actinin-3 (ACTN3) gene have dominance R allele in R5`77X polymorphism, and another gene the angiotensin-converting enzyme (ACE) have D allele was the best allele in endurance and power athletes respectively (Abdulla, 2017; Juffer et al., 2009; Santiago et al., 2008).

Elite of basketball players demonstrate both endurance and power in phenotypic effect for related traits. So, muscles need greater than before for oxygen and metabolic molecules for exercise (Wolfarth et al., 2008) and the main factor that influences endurance performance that capacity of bloodstream in muscles and changed manage of whole and local muscle bloodstream (Cięszczyk et al., 2010). A molecule of Nitric oxide NO is a vasodilator that acting to provide blood to the tissues, as well as working muscles (Wilkerson et al., 2004).

* Corresponding Author
Email: sarab.khaleel@qu.edu.iq
Contact: +96- 47707207768
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NO is concerned with heart-protection (Otani, 2009), respiration of heart (Loke et al., 1999a; Loke et al., 1999b), and" in insulin-independent glucose uptake into the working muscle fibres" (McConnell et al., 2006. Also, NO organises Kinetic of O_2 when the muscle was working (Wilkerson et al., 2004). Endothelial cells in circulatory system synthesis Nitric oxide from rich L-arginine via an enzyme called endothelial nitric oxide synthetase, that powerful for vasodilator (Shankarishan et al., 2011). NO acts a very vital outcome on modifiable the sympathetic and parasympathetic system, circulatory system and the immune organization. (Safarinejad et al., 2013; Liu et al., 2014; Polat et al., 2014).

The gene of nitric oxide synthase3 (*NOS3*), situated on human chromosome 7q35–36, encodes endothelial nitric oxide synthase3 (eNOS), which convert L-arginine to L-citrulline and nitric oxide (NO). Moreover, it contains 26 exons (Kara et al., 2006).

In the long arm of chromosome 7q35-36 situated gene eNOS3. That encodes eNOS enzyme which converts L-arginine to L-citrulline and nitric oxide molecule. Moreover, it contains 26 exons (Kara et al., 2006). Some of studies for NOS3 polymorphisms show different variations in different locations on region of eNOS3 gene include promoter -786 T/C (rs2070744) variable, Glu298Asp (E298D or G894T or rs1799983) in exon 7, 27 bp repeats in intron 4 (4B/4A) and microsatellite (CA)n repeats in intron 13 (Ahmetov and Fedotovskaya,2012). The T-786C variant (rs2070744), a transition mutation convert thymine to cytosine, is present in the 5' flanking region of eNOS3 gene and decrease the activity of the

promoter for eNOS, follow-on decrease of endothelial NO production (Nakayama et al., 1999). The C allele reduced eNOS transcription, so production of Nitric oxide molecules was decreased (Dengel et al., 2007). It has been confirmed that a sharp session of doing exercises enhances the manufacture of NO, and thus contribute to expanding the vessel in skeletal muscle (Higashibata et al., 2012; Silva et al., 2011). The recent study of T786C polymorphism of eNOS3 gene in basketball player demonstrated individual variability in eNOS3 – T786C polymorphism that acts a vital role athletes performance. This promoted us that the genotypes of this polymorphism are linked with the grade of being a best basketball group and control group in this study.

MATERIALS & METHODS

Samples collections

The study population consisted of 35 basketball players aged between (16-19) and 42 unrelated controls aged between (16-20) who had no competitive sports experience. Subjects had similar ethnic backgrounds, and they were all from the same geographic area.

DNA Extraction

Deoxyribonucleic acid was extracted from freezing blood samples through use "(Genomic DNA Mini Kit, Geneaid, USA)". According to" company instructions" by using frozen Blood extraction Protocol method with Proteinase K. Then the extracted gDNA was check by" Nanodrop spectrophotometer", and store at -20C at refrigerator awaiting used in PCR amplification.

PCR amplification"

PCR assay was carried out for detection eNOS3 gene by using specific primer forward primer (5'-TGGAGAG-TGCTGGTGTACC CCA-3) and reverse primer (5'-GCCTCCACCCCCACCCTGTC-3) that use to amplify (180 bp) PCR product. The primers were provided by (Bioneer company . Korea). PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the reaction of PCR was prepared according to kit instructions in 20µl final volume by "added 5µl of purified genomic DNA and 1.5µl of 10 pmole of forward primer and 1.5µl of 10 pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Techne TC-3000. USA) by set up the following thermocycler conditions as Touchdown protocol; initial denaturation temperature of 95°C for 5 min; followed by 15 cycles at denaturation 95°C for 30 s, annealing 69.6°C decrease 0.5°C per cycle for 30 s, and extension 72°C for 20 s and then another 20 cycles denaturation 95°C for 30 s, annealing 62.6°C for 30s, and extension 72°C for 20 s and final extension at 72°C for 5 min. The PCR products were examined by electrophoresis in a 2% agarose gel, stained with ethidium bromide, and visualised under UV illumination.

RFLP-PCR

RFLP-PCR were using for detection T-786C promoter polymorphism of $eNOS_3$ and genotypes of T-786C polymorphism "(rs2070744) located in the 5' flanking region of $eNOS_3$. The amplified products of 180 bp were digested with Msp I (New England Biolabs, US) at 37°C/ 1 hours. The producing fragments of 140 and 40 bp for the T allele, and fragments of 90, 50 and 40 bp for the C allele. The fragments were separated by 2% agarose gel electrophoresis containing ethidium bromide and visualized under UV Transilluminator".

Any differences in allele and genotype frequency were analysed using P values. Odd ratio (OR) with 95% confidence intervals (95%CI) were calculated. All calculations were performed using the Statistica Hardy-Weinberg equilibrium, adjusted P values, which were analyzed by using Michael H. Court's (2005-2008) online calculator (Polat et al., 2015). P values <0.05 were considered statically significant.

RESULTS

Genotypes and allele frequencies for eNOS T-786C polymorphisms in player and controls were listed in Table 1. The genotype frequencies of the T786C polymorphisms be to evaluate, no deviation from Hardy-Weinberg equilibrium observed for both basketball players and controls.

The T-786C polymorphism of the eNOS gene, in the basketball players group was 14players (40%) represented in TT genotype, 14 basketball players (40%) represented in TC genotype, and (15%) were 7 basketball players CC genotype; in the control group, it was 12 healthy individuals (30%) represented in TT genotype, 10 individuals (25%) represented in TC genotype, and 18 individuals (45%) represented in CC genotype. No significant differences will be observed between both groups for T-786C genotypes while alleles frequencies show a statistically significant difference the T allele frequency was 0.60 for basketball players group and 0.425 for the control group and C allele frequency was 0.40 for basketball players group and 0.575 for the control group in the present study. The difference of eNOS C786T polymorphism T allele occurrence be statistically significant when compared to the control group (Table 1), for T allele (OR: 2.029, CI: 1.0573-3.8954, p=0.0334), T allele represent high frequency in the player group (60%) and (42.5%) in the control group. While C allele frequency was also statistically significant when compared to the control group (OR: 0.492, CI: 0.2567-0.9458, p=0.0334). The C allele is the low frequency in basketball players group (40%) versus in control group was (57.5 %).

Genotype distributions in Hardy-Weinberg equilibrium (HWE) were a calculator in control ($\chi2=4.009$, P=0.o45) and basketball players $\chi2=18.4$, P=0.000017).

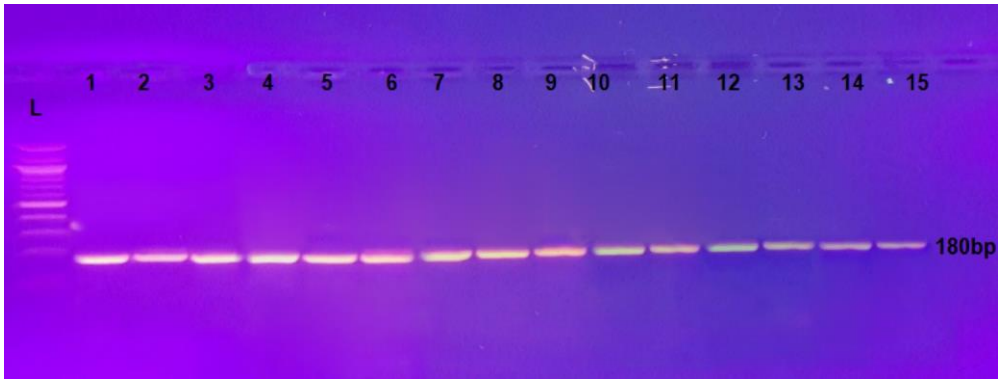

Figure 1: PCR amplification of the T786C eNOS3 polymorphism, PCR product of the T786C eNOS3 polymorphism has been the size (180 bp)

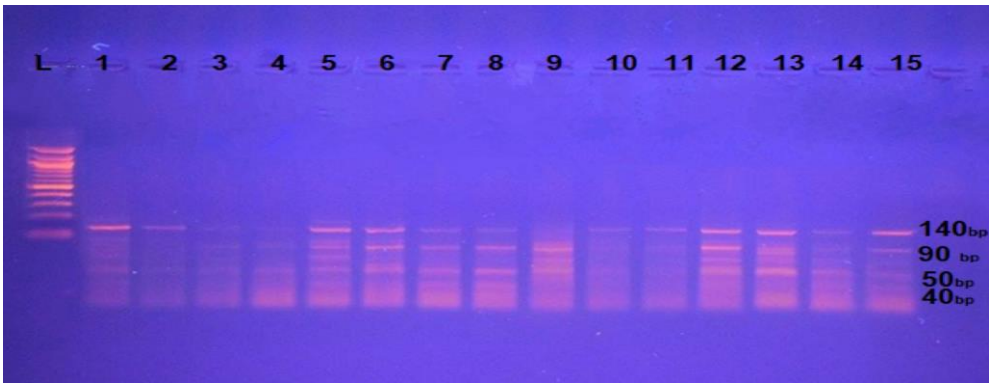

Figure 2: Enzymatic digestion of T786C polymorphism of eNOS gene

TT is represented in 140 bp and 40bp in lanes 1,2,3,4,10,11& 12, The product PCR digested by the mspI enzyme. Which digests the 180-bp fragment into140, 90,50 and 40-bp fragments (heterozygous genotype TC which has 140, 90, 50&40 bp fragments in lanes 5,6,7,8,12,13&15) (homozygous mutated genotype CC is represented in 90, 50&40 bp fragments for lanes 9 only) (By using a ladder)" this results in basketball players.

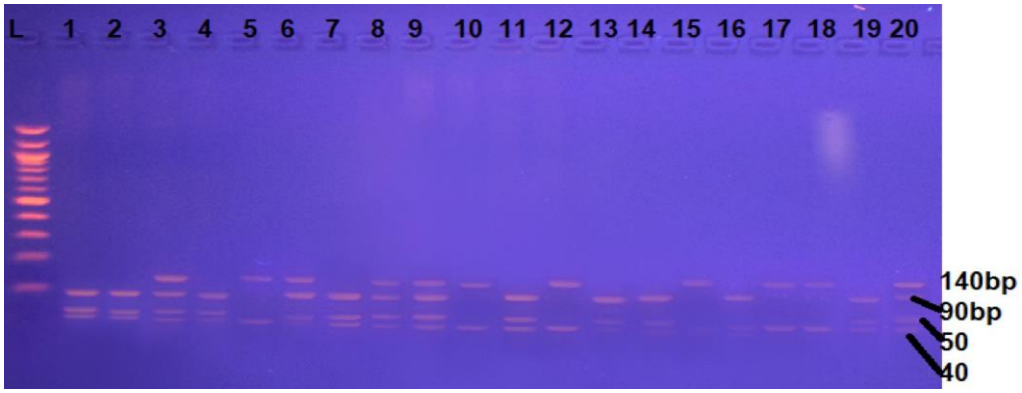

Figure 3: Enzymatic digestion of T786C polymorphism of eNOS gene

Wild-type TT is represented in 140 bp and 40bp in lanes 5,10,12,15,17 &18, the product PCR digested by the mspI enzyme. Which digests the 180-bp fragment into140, 90,50 and 40-bp fragments (heterozygous genotype TC which has 140, 90, 50&40 bp fragments lanes 3,6,8,9 & 20) (homozygous mutated genotype CC is represented in 90, 50&40 bp fragments lanes 1,2,4,7,11,13,14,16&19) (By using ladder) this results in control group.

Table 1: Distribution of Genotype and Allele Frequencies of eNOS T-786C Polymorphisms in Basketball players and Control group

Gene/Genotypes	Players (n=35) n%	Control (n=40) n/%	P value	Odds ratio	95%(CI)
T-786C- TT	14(40)	12(30)	0.3652	1.55	0.5978 to 4.0480
TC	14(40)	10(25)	0.1677	2	0.7471 to 1.0765
CC	7(20)	18(45)	0.069	0.388	0.1405 to 3.8954
Alleles T	42(60)	34(42.5)	0.0334	2.029	1.0573 to 3.8954
C	28(40)	46(57.5)	0.0334	0.492	0.2567 to 0.9458

DISCUSSION

In the current study. We have found relationships between" T786C polymorphism and basketball players. In our finding, the T786 allele was high-frequency n basketball player compared to the control group. The eNOS3 gene was one of the contestant genes explanation human being variation in the control group and do exercises ability associated with phenotypes because it encodes the eNOS3 enzyme (Bray et al., 2009). Which responsible for making of nitric oxide molecule (NO). This molecule has a vital role within the regulation of the circulatory system in human (Eider et al., 2014). By dilated vascular and permits blood supply to work muscle (Heydemann et al., 2009). So (NO) was supply the working muscle in oxygen and glucose (Wilkerson et al., 2004). Also, it can have a favourable effect in reacting to aerobic do exercises in the good physical community.

In subsequent studies provide inconsistent results on the relationship between the T786C eNs3 polymorphisms and athletes status or sporty act (Wolfarth et al., 2008; Cieszczyk et al., 2012; Buxens et al., 2011). The study among 71 endurance-oriented Ukrainian athletes (30 under 'water fin swimmers, 41 rowers") and 147 controls group. It shows significant differences in the frequency of the eNOs3 rs2070744 T allele "(75.4% vs. 65.0%; P = 0.029)" (Drozdovska et al., 2009). Gómez-Gallego et al., (2009a) have been established the T allele enrich in power athletes compare to control and endurance athletes Gómez-Gallego et al., (2009b) while Gómez - Gallego et al., (2009b) conferred there were no any differences in the occurrence of the eNOS3 rs2070744 T allele among 100" Spanish first-class endurance athletes and 100 control (Gómez-Gallego et al., 2009a) Also other study showed eNOS T-786C genotypes no significant differences among athletes and control groups (p>0.05) (Cenikli1 et al., 2016). However, the C"allele within 786T/C polymorphism was overrepresented within the elite soccer player (Andrew et al., 2012). Data et al., found the C allele association with endurance exercise (Data et al., 2003).

We found in current study no significant differences in frequencies of the genotypes of NOS3 T786C polymorphisms, while alleles frequencies were significant. The T allele was overrepresented in player basketball .while the C allele showed low frequency in player basketball compared with control. Suggested the small size of the sample due to this result. Because of that not easy to overcome since it is imperative in these studies to consist of only select few athletes as experiments. The importance of a particular sport-related heritable sign is based on a number of criteria, like the sort of the polymorphism (intronic, missense, nonsense etc.), in a given people that frequently, some of cross-sectional and case-control research, total number of study athletes, etc. (Ahmetov et al., 2012).

CONCLUSION

In conclusion, the T allele frequency was significantly in basketball players compared with control. Moreover, it has shown with the intention of T allele is linked with muscle athlete status. Additional, research are looked-for these genotypes on other power and endurance athletes.

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