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OPTIMISATION OF PCR CONDITIONS FOR MOLECULAR CHARACTERIZATION OF A GROUP OF 'SPORTS AND OBESITY GENES'

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(Short Communication)

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Abstract

Genetic variations of appropriate marker genes define an individual's predisposition to sport performance and body weight. The influence of many genes and their allelic polymorphism on sport performance and obesity has been identified in various studies. In this study we optimised the PCR condition for the amplification of six genes of which two are so called "sports genes", ACE and ACTN3, and four "obesity genes", FTO, PPARγ, FABP2, ADRB3, respectively. DNA was isolated from blood samples using Promega kit. PCR amplifications for all genes were performed in 25 μL final volume with different thermal cycling conditions. One polymorphism can be identified using end point PCR (ACE gene), while in other cases it is necessary to perform Restriction Fragment Length Polymorphism (RFLP) analysis using appropriate restriction endonucleases. The optimisation of PCR conditions is the first step followed by the molecular characterisation and the correlation analysis of those results with previously measured anthropometric parameters.

Keywords: *genetic factors, sport genes, obesity genes, sport performance, PCR condition.*

Introduction

Despite the environmental factors such as diet and physical activity, genetic factors play an important role in sport performance and obesity [1, 2]. A large number of genes that influence sport performance and the development of obesity have already been identified [3].

Among the large number of candidate genes, in this study we analyse six of them, two sports genes, ACE (angiotensin I-converting enzyme) and ACTN3 (alpha-actinin-3) and four obesity genes, FTO (fat mass and obesity), PPARγ (peroxisome proliferator-activated receptor - gamma), FABP2 (fatty acid binding protein) and ADRB3 (adrenoceptor beta 3).

The ACTN3 gene (chr 11) encodes the alpha actinin 3 protein primarily expressed in human skeletal muscle and plays role in the generation of contractions. A genetic variation in the ACTN3 gene results with the production of non-functional protein [4].

The ACE gene (chr 17) encodes the angiotensin-converting enzyme, which regulates the amount of salts in the human body, the balance of fluids and blood pressure [5]. The genetic variation of this gene influences sports endurance and the body's response to intense exercise.

One of the first identified candidate genes related to obesity is FTO (chr 16). The association between single nucleotide polymorphisms (SNPs) of the FTO gene with an increase in BMI (Body Mass Index) and risk of being overweight has been found in 2007 in many populations [6]. This gene encodes the alpha-ketoglutarate-dependent dioxygenase enzyme and is expressed in many human tissues predominantly in the hippocampus, cerebellum and hypothalamus [7]. All of this indicates that the FTO gene plays a role in controlling food intake and energy intake [8].

The PPARγ gene (chr 30) encodes a peroxisome proliferator-activated nuclear gamma receptor (PPARγ) that plays a key role in the gene expression in a large number of cells, including fat cells. Although there

have been controversial results from various studies, the primary effect of PPARγ is in overweight development [9].

The FABP2 gene (chr4) encodes a fatty acid-binding protein, an intracellular protein expressed only in the intestines. FABP2 plays a key role in the absorption and intracellular transport of long-chain fatty acids [5, 10].

The ADRB3 gene (chr9) encodes a protein belonging to the beta-adrenoceptor family. This receptor is located predominantly in adipose tissue and has an important role in regulating lipolysis and thermogenesis and causes a difference in energy consumption. Weight loss and obesity are associated with certain polymorphisms in three types of beta-adrenergic receptors, including ADRB3 [5].

To start unravelling the role and the impact of these genes on sport performance and obesity we optimized the PCR (polymerase chain reaction) condition for the amplification of these six genes.

Materials and Methods

DNA was isolated from blood samples using Promega kit, according to the protocol.

PCR amplifications for all genes were performed in 25 μL final volume.

ACTN3 amplification was performed with the following thermal cycling conditions: initial 5 minute denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 30 seconds, and a final elongation at 72°C for 10 minutes. Primers for ACTN3 locus: ACTN3-F 5′-CTGTTGCCTGTGGTAAGTGGG-3′ and ACTN3-R 5'-TGGTCACAGTATGCAGGAGGG-3.

PCR amplification for ACE was performed with the following thermal cycling conditions: initial 5 minute denaturation at 94°C, followed by 30 amplification cycles of denaturation at 94°C for 1 minute, annealing at 67°C for 1 minute, elongation at 72°C for 2 minute, and a final elongation at 72°C for 5 minutes. Primers for ACE locus: ACE-F 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and ACE-R 5′-GATGTGGCCATCACATTCGTCAGAT-3′.

FTO gene amplification was performed with the following thermal cycling conditions: initial 5 minute denaturation at 94°C, followed by 35 amplification cycles of denaturation at 94°C for 45 seconds, annealing at 51°C for 45 seconds, elongation at 72°C for 45 seconds, and a final elongation at 72°C for 10 minutes. Primers for FTO locus FTO-F 5′-AACTGGCTCTTGAATGAAATAGGATTCAGA-3' and FTO-R 5' AGAGTAACAGAGACTATCCAAGTGCAGTAC-3'

PCR amplifications for PPARγ and ADRB3 genes were performed with the following thermal cycling conditions: initial 10 minutes denaturation at 95°C, followed by 37 amplification cycles of denaturation at 95°C for 20 seconds, annealing at 58°C for 30 seconds, elongation at 72°C for 30 seconds, and a final elongation at 72°C for 7 minutes. Primers for PPARγ locus: PPARγ -F 5'-CCAATTCAAGCCCAGTCCTTTC - 3' and PPARγ-R 5'-CAGTGAAGGAATCGCTTTCCG -3'. Primers for ADRB3 locus: ADRB3 - F 5'-TGGGAGGCAACCTGCTGGTCAT-3' and ADRB3-R 5'-AGGAGTCCCATCACCAGGTC-3'

FABP2 amplification was performed with the following thermal cycling conditions: 35 amplification cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, elongation at 72°C for 1 minute, and incubation at 37°C for 3 hours.Primers for FABP2 locus: FABP2-F 5′-ACAGGTGTTAATATAGTGAAAAG -3′ and ACTN3-R 5'- TACCCTGAGTTCAG TTCCGTC-3'.

Results

As shown in Figure 1. the PCR conditions have been optimized, giving a positive signal for gene amplification. All genes gave one PCR amplification product, except ACE gene where two DNA fragments were found as polymorphism which should be verified as a next step, whith RFLP (Restriction Fragment Length Polymorphism).

Discussion and Conclusion

This work enables to continue with the research on a larger sample size. As a next step, the amplified gene fragments with polymorphism will be characterized using the RFLP technique. The results will be analysed for possible correlations with previously measured and appropriate anthropometric parameters.

The combinations of identified genotypes among the six genes and their interactions will be further tested in order to create haplotypes for this phenotypic manifestation. It could be used as a tool to predict the predisposition to athlete performance and gain weight.

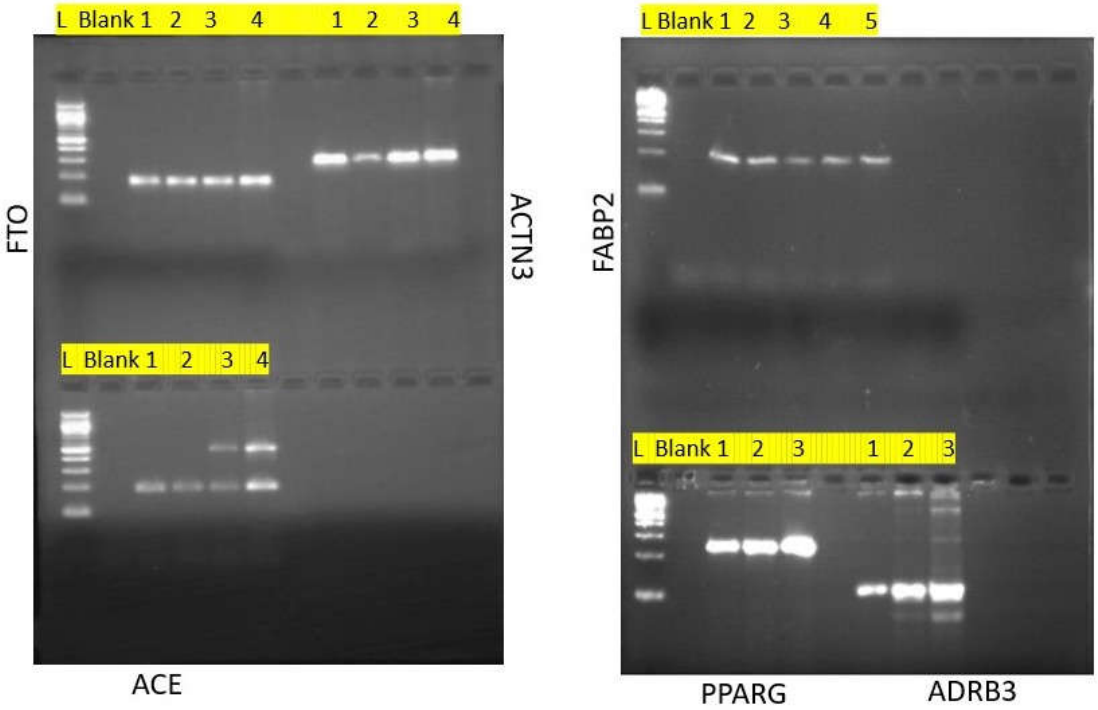

Fig. 1. Agarose Gel Electrophoresis (AGE) of amplicons from 6 analysed marker genes gained under optimised conditions

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