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Speed and power-related gene polymorphisms associated with playing position in elite soccer players

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ABSTRACT: Heritability studies on sport-related traits accepted that endurance, speed, power, and strength abilities include an active genetic predisposition to elite soccer participation. This study evaluates the influence of selected genetic variants on performance in speed, power, and strength laboratory tests on a group of elite soccer players, including their playing position. A ninety-nine male elite soccer players were compared to controls ($n = 107$) and tested for quadriceps and hamstrings isokinetic strength at speed 60°/s, 180°/s, and 300°/s, jump performance, and genotypes of *ACTN3* (R577X, rs1815739), *ACE* (I/D, rs1799752), *NOS3* (Glu298Asp, rs1799983), *AMPD1* (34C/T, rs17602729), *UCP2* (Ala55Val, rs660339), *BDKRB2* (+9/-9, rs5810761) and *IL1RN* (VNTR 86-bp). The *ACTN3* XX homozygotes in defenders had lower quadriceps and hamstring isokinetic strength in all tested speeds than *ACTN3* RX and RR genotypes ($p < 0.05$). The *ACTN3* RR homozygotes in defenders had higher quadriceps strength in all tested velocities than the RX heterozygotes ($p < 0.05$). We also found other associations between playing-position in soccer and increased strength of lower limbs for *AMPD1* CC and *NOS3* Glu/Glu genotypes, and *IL1RN*2* allele carriers. Total genetic score regression explained 26% of the variance in jump performance and isokinetic strength. The *ACTN3* R allele, *NOS3* Glu/Glu genotypes, and *IL1RN*2* allele pre-disposed the attackers and defenders playing position in elite soccer, where those positions have higher strength and power measures than midfielders. Midfielders have lower strength and power conditions than other playing positions without relation to strength and power genes.

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INTRODUCTION

Elite soccer is highly competitive, and only a minority of players can participate in the world's best soccer leagues. This fact also relates to the high complexity of soccer requirements, including technical, tactical, psychological, and physiological domains. The main physiological factors are the combination of endurance and speed [1–4], like repeated short sprints [5, 6]; moreover, current soccer increases strength and power requirements. Based on estimations of heritability studies on sport-related traits, it is generally accepted that endurance, speed-power, and strength abilities include a genetic determination [7–10], which might be explained at least partly through the genetics of muscle fibers specificity [11, 12].

Among various physical constraints for soccer players, the knee extensors and flexors' concentric and eccentric strength correlate with soccer sprints [13–15] and deceleration ability [16] and differs by soccer-playing positions [17, 18]. Therefore, the knee flexors and extensors' force at different speeds can generally explain the necessary force-velocity predisposition of elite players [19], which are possible predictors of soccer players' agility and jump performance [20, 21]. Concerning the force-velocity profile, the soccer performance is specific by high speed running and sprinting [1, 22], acceleration and deceleration [23], isokinetic strength [18], and vertical jump performance [24, 25]. Since there is a clear cue between playing position and conditioning specificity, it is still unknown whether the force-velocity specificity might be genetically determined for playing position in the elite soccer level. On the other hand, some genetic markers have been associated with soccer attackers' metabolic traits [26].

Many candidate gene studies have investigated the influence of several genetic polymorphisms on athletes' speed, power, and strength performance during the past few years [7, 27]. In those studies, positive associations of "speed, power, and strength" genotypes have been found in groups of soccer players for the *ACTN3* gene [28–32]

or the *PPARA* gene [32–34]. Recently, the metanalyses of Weyerstrass et al. (2018) identified nine genetic polymorphisms for power phenotype: *ACE* (rs4363, rs1799752), *ACTN3* (rs1815739), *AGT* (rs699), *IL6-174* (rs1800795), *MnSOD* (rs1799725), *NOS3* (rs1799983, rs2070744) and *SOD2* (rs4880) [35], whereas some of them overlap with findings of current review from 2020 [36].

Since there are known genetic determinants for soccer conditioning, there is a lack in understanding genetic determinants for elite soccer level and playing position. Therefore, this study evaluates the influence of genetic variants on performance in speed, power, and strength laboratory tests on a group of elite soccer players concerning their playing position (attacker, defender, midfielder, and goalkeeper). We target the seven gene polymorphisms previously associated with speed, power, and strength *ACTN3* (R577X, rs1815739), *ACE* (I/D, rs1799752), *NOS3* (Glu298Asp, rs1799983), *AMPD1* (34C/T, rs17602729), *UCP2* (Ala55Val, rs660339), *BDKRB2* (+9/-9, rs5810761) and *IL1RN* (VNTR 86-bp).

MATERIALS AND METHODS

We performed a cross-sectional study with genotyping, vertical jumps, and isokinetic measurement of knee flexion and extension at three different angular speeds. Before the measurement, there was no training session or other demanding physical activity. The genotype samples were gathered before the participant general warm-up, which included 5 to 10 minutes of aerobic exercise on a treadmill/bike ergometer up to 140 beats per minute, followed by 5 to 10 minutes of individual static stretching, and 5 to 10 minutes of dynamic stretching presented by hops and dynamic lunges. After the warming-up, all players underwent vertical jump testing, followed by testing on an isokinetic dynamometer with 3 minutes rest interval between individual attempts.

Subjects

A ninety-nine Caucasian male soccer players (25.4 ± 4.51 y, 181.4 ± 6.11 cm, 77.4 ± 7.22 kg, Supplementary material Table S1) were recruited from five professional Czech soccer teams participating in the first (88 soccer players) and second national (11 soccer players) soccer league. Fifteen players reached the level of playing in the national team. For later frequency comparison of genotypes and alleles, we used 107 Czech healthy controls (from age 18 to 65) from whole-genome sequencing Czech national project Enigma, CZ.01.1.02/0.0/0.0/16_084/0010360. We calculated most represent allele frequency from targeted gene sites, which were used as a reference in our study. We could not analyze some of the hotspots due to the sequencing kit's limitations, uncovered regions, and low mapping quality. Some of the genes cannot be studied due to the repetitive areas, which cause a wildly inaccurate variant calling process. All subjects signed informed consent at the beginning of the study participation, approved by the Ethics Committee of Faculty of Physical Education, Charles University (No. 145/2016, issued on October 21, 2016).

Vertical jump testing

The measurement was performed using the force plates (Kistler 8611, Switzerland) with a sampling frequency of 1000 Hz for three types of standardized vertical jumps, countermovement jump with the support of upper limbs swing, countermovement jump without hands on the waist, and a squat jump. Each jump test was repeated three to five times based on the subject's choice, with at least 10 seconds between each jump and the jump type. The data were processed by BioWare software (Kistler Holding AG, Winterthur, Switzerland) and further calculation of the maximum height of the jump, the maximum force, the maximum force per kg of bodyweight [N/kg], and the achieved impulse of force per kg of the subject's body weight [N·s/kg].

Isokinetic strength

Knee extensors and flexors strength was measured at three different angular velocities of 60°/s, 180°/s, and 300°/s using isokinetic dynamometer Cybex Human Norm (Cybex Norm®, Humac, CA, USA) and manufactural software (HUMAC2015®, version 15.000.0044). Subjects performed two repetitions for each angular velocity with 30 s rest between the velocities. During the testing, subjects were verbally encouraged and had full visual contact with the screen showing the current performance level. The extracted data included peak force [Nm] and peak force per kg [Nm/kg] at the concentric phases.

Genotyping

Molecular genetic analysis was performed in the Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital in Prague. We performed genotyping with DNA samples obtained from epithelial mouth cells collected by a trained individual by cheek brushes (microRheologics, Italy) against the inside of each subject's cheek for approximately 15 s. Subjects were asked not to consume any food or drink in the 30 minutes before sample collection [37]. Cheek brushes were air-dried for at least 8 hours and later stored at -80°C until the DNA extraction, which was performed for a maximum of 2 weeks after the collection. The head of every cheek brush was cut and insert into a screw 2 ml cap tube before the extraction. The head of every cheek brush was cut and insert into a screw 2 ml cap tube before the extraction. DNA was extracted using the isolation kit QIAamp DNA Mini Kit (QIAgen, Germany) according to the manufacture's instructions, with minor adjustments. Extracted samples were stored at -80°C. The DNA samples were quantified using a Polymerase chain reaction method (PCR) based on previous studies [38, 39]; the procedure details are in Supplementary material Tables S2 and S3. The gradient thermocycler Labcycler (SensoQuest, Germany) was used for PCR reaction. The digested product was visualized by 3% agarose gel electrophoresis in the horizontal electrophoresis device HU10 (SCIE-PLA, England) and identified by ethidium bromide staining [40]. Software visualization was performed by the UV light device G: BOX Chemi HR16 (Syngene, England).

The combined influence of the studied polymorphisms

The combined influence of the studied polymorphisms for each soccer player was done using the total genetic score (TGS) by Williams and Folland algorithm [41], where the raw score was transformed to a scale of 0–100: $TGS = (100/14) \times (GS_{ACE} + GS_{ACTN3} + GS_{BDKRB2} + GS_{NOS3} + GS_{AMPD1} + GS_{UCP2} + GS_{IL1RN})$. In this calculation, 14 results from multiplying 7 (the number of studied polymorphisms) by 2 (the score is given to the optimal explosive-leg-strength genotype, where the score given to the optimal strength and power genotype is described in Table 1. The TGS was also calculated for genotypes with phenotype significant results TGS_{sig}, where $TGS_{sig} = (100/2 \times n) \times$ (GS 1st gene + GS 2nd gene + GS n gene). In this calculation n is the number of genes.

TABLE 1. Total genetic score counts for each examined genotype.

Genotype	Total genetic score count
ACE (I/D)	0 = II, 1 = ID, 2 = DD
ACTN3 (R577X)	0 = XX, 1 = RX, 2 = RR
BDKRB2 (9/+9)	0 = +9 + 9, 1 = +9 – 9, 2 = –9 – 9
NOS3 (Glu298Asp)	0 = Asp/Asp, 1 = Glu/Asp, 2 = Glu/Glu
AMPD1 (Gln12X)	0 = TT, 1 = CT, 2 = CC
UCP2 (Ala55Val)	0 = CC, 1 = CT, 2 = TT
IL1RN (VNTR 86-bp)	0 = 1/1 or 1/3, 1 = 1/2 or 2/3, 2 = 2/2

Statistical Analyses

NCSS statistical software (NCSS, USA) was used to calculate Hardy-Weinberg equilibrium and chi-square analysis for testing the allele frequencies determined by gene counting. We used Chi-square analysis to compare genotype distribution, allele frequencies between the group of soccer players and healthy, and frequencies concerning the playing position inside the soccer players group, where p values of < 0.05 were considered statistically significant. The regression analyses, correlation, and group comparison have been performed in STATISTICA software (13.5. TIBCO software, Palo Alto, CA, USA), with a statistical significance level set up for 0.05. The Kolmogorov Smirnov test has calculated the data normality.

One way ANOVA was used to compare performance differences by genotype groups of soccer players (isokinetic/jump performance × genotype), and the two way ANOVA has been used to compare performance differences between genotypes in each playing position (isokinetic/jump performance × genotype × playing position) considering effect size by partial eta square (μ^2) and differences by Unequal HSD post hoc test. μ^2 was considered 0.02–0.12, 0.13–0.25, and > 0.26 as weak, moderate, and strong associations, respectively [42]. The two way ANOVA has been performed only in the sub-groups with n above 6.

A Spearman correlation coefficient and multiple linear regression model with a step-down (backward) procedure were used to explore the predictive role of the TGS variable with correlated muscle-strength and jump performance phenotypes. The TGS was calculated for all analyzed genotypes and for genotypes with phenotype significant results – TGS_{sig}.

RESULTS

Physiological studies

The data from subgroups did not show disruption of normality. One way ANOVA showed differences in m. quadriceps strength among positions at 60°/s ($F_{3, 181} = 7.3$, $p < 0.001$, $\mu^2 = 0.08$), 180°/s ($F_{3, 181} = 6.7$, $p < 0.001$, $\mu^2 = 0.04$) and 300°/s ($F_{3, 170} = 8.6$, $p < 0.001$, $\mu^2 = 0.10$) in whole group, where midfield players resulted in lower quadriceps strength than other playing positions (Figure 1). The hamstring strength resulted in difference at 60°/s ($F_{3, 181} = 6{,}4$, $p < 0.001$, $\mu^2 = 0.04$), 180°/s ($F_{3, 181} = 5.9$, $p = 0.0081$ $\mu^2 = 0.06$) and 300°/s ($F_{3, 170} = 6.5$, $p < 0.001$, $\mu^2 = 0.07$) in whole group, where midfield players resulted in lower hamstring strength than other playing positions (Supplementary material Figure S1).

Case-control genetic studies

All genotype data did not disrupt the Hardy-Weinberg equilibrium, and only *NOS3* Glu298Asp differ in the allelic frequency in defenders, where defenders have higher Glu allele frequency than controls (Table 2). The *ACE* ID, *BDKRB2* +9/-9, and *IL1RN* VNTR polymorphisms did not have reference values in our control group because genotyping was not available in these polymorphisms; therefore, this comparison was not possible (Table 2). We identified no subjects in the following subgroups: *ACTN* XX in attackers, *NOS3* Asp/Asp in attackers and goalkeepers, *AMPD1* TT in all subjects, and *IL1RN*2*/*IL1RN*2* in goalkeepers (Table 2). Chi-square analysis of genotype and allele distribution is in Table 2.

Genotype-phenotype studies

The genotype differences were found in *ACTN3* gene between quadriceps strength at 60°/s ($F_{2, 156} = 4.8$, $p = 0.009$, $\mu^2 = 0.09$), 180°/s ($F_{2, 156} = 3.7$, $p = 0.026$, $\mu^2 = 0.16$) and 300°/s ($F_{2, 146} = 7.04$, $p = 0.001$, $\mu^2 = 0.08$) in whole group, where XX genotypes resulted in lower quadriceps strength than RX heterozygotes and RR homozygotes whereas RR homozygotes has higher values than other genotypes (Figure 2). The *ACTN3* genotypes differ also among hamstring strength at 60°/s ($F_{2, 156} = 3.2$, $p = 0.042$, $\mu^2 = 0.04$) and 300°/s ($F_{2, 147} = 4.1$, $p < 0.017$, $\mu^2 = 0.05$) in whole group, where XX genotype resulted in lower quadriceps strength than RX heterozygotes and RR homozygotes and RR homozygotes (Figure 1).

TABLE 2. Allele and genotype frequencies Chi-square comparison for the soccer players and the controls.

Genotype / allele / comparison type			Defenders	Attackers	Goalkeepers	Midfielders	All	Controls
			n 31	15	14	39	99	107
	Allele n (%)	R	35 (56.5)	22 (73.3)	14 (50.0)	47 (60.2)	118 (59.6)	128 (59.8)
		X	27 (43.5)	8 (26.7)	14 (50.0)	31 (39.7)	80 (40.4)	86 (40.2)
	p	Compared to controls	*0.636*	*0.154*	*0.321*	*0.945*	*0.964*	-
ACTN3	*p*	Compared to all	*0.661*	*0.150*	*0.335*	*0.920*	-	-
R577X	Genotype n (%)	RR	10 (32.3)	7 (46.7)	5 (35.7)	12 (30.8)	34 (34.3)	36 (33.6)
		RX	15 (48.4)	8 (53.3)	4 (28.6)	23 (59.0)	50 (50.5)	56 (52.3)
		XX	6 (19.4)	0 (0)	5 (35.7)	4 (10.3)	15 (15.2)	15 (14.0)
	p	Compared to controls	*0.765*	*0.254*	*0.840*	*0.735*	*0.958*	-
	p	Compared to all	*0.857*	*0.241*	*0.124*	*0.615*	-	-
	Allele n (%)	I	35 (56.5)	17 (56.7)	13 (46.4)	39 (50.0)	104 (52.5)	
		D	27 (43.5)	13 (43.3)	15 (53.6)	39 (50.0)	94 (47.5)	
	p	Compared to controls						
ACE	*p*	Compared to all	*0.589*	*0.672*	*0.546*	*0.705*	-	
I/D	Genotype n (%)	II	8 (25.8)	4 (26.7)	2 (14.3)	10 (25.6)	24 (24.2)	
		ID	19 (61.3)	9 (60.0)	9 (64.3)	19 (48.7)	56 (56.6)	
		DD	4 (12.9)	2 (13.3)	3 (21.4)	10 (25.6)	19 (19.2)	
	p	Compared to controls						
	p	Compared to all	*0.725*	*0.860*	*0.709*	*0.640*	-	
	Allele n (%)	Glu	51 (82.3)	24 (80.0)	22 (78.6)	54 (69.2)	151 (76.3)	146 (68.2)
		Asp	11 (17.7)	6 (20.0)	6 (21.4)	24 (30.8)	47 (23.7)	68 (31.8)
	p	Compared to controls	***0.031****	*0.189*	*0.264*	*0.870*	*0.069*	-
	p	Compared to all	*0.322*	*0.652*	*0.787*	*0.229*	-	-
NOS3	Genotype n (%)	Glu/Glu	21 (67.7)	9 (60.0)	8 (57.1)	18 (46.2)	56 (56.6)	48 (44.9)
Glu298Asp		Glu/Asp	9 (29.0)	6 (40.0)	6 (42.9)	18 (46.2)	39 (39.4)	50 (46.7)
		Asp/Asp	1 (3.2)	0 (0)	0 (0)	3 (7.7)	4 (4.0)	9 (8.4)
	p	Compared to controls	*0.075*	*0.361*	*0.445*	*0.985*	*0.166*	-
	p	Compared to all	*0.542*	*0.728*	*0.740*	*0.447*	-	-
	Allele n (%)	C	52 (83.9)	26 (86.7)	25 (89.3)	69 (88.5)	172 (86.9)	179 (83.6)
		T	10 (16.1)	4 (13.3)	3 (10.7)	9 (11.5)	26 (13.1)	35 (16.4)
	p	Compared to controls	*0.966*	*0.672*	*0.440*	*0.309*	*0.357*	-
AMPD1	*p*	Compared to all	*0.551*	*0.976*	*0.720*	*0.720*	-	-
34C/T	Genotype n (%)	C/C	21 (67.7)	11 (73.3)	11 (78.6)	30 (76.9)	73 (73.7)	76 (71.0)
		C/T	10 (32.3)	4 (26.7)	3 (21.4)	9 (23.1)	26 (26.3)	27 (25.2)
		T/T	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.0)	4 (3.7)
	p	Compared to controls	*0.441*	*0.748*	*0.707*	*0.439*	*0.152*	-
	p	Compared to all	*0.515*	*0.974*	*0,698*	*0.699*	-	-
	Allele n (%)	Ala	38 (61.3)	20 (66.7)	13 (46.4)	41 (52.6)	112 (56.6)	126 (58.9)
		Val	24 (38.7)	10 (33.3)	15 (53.6)	37 (47.4)	86 (43.4)	88 (41.1)
	p	Compared to controls	*0.733*	*0.415*	*0.210*	*0.335*	*0.635*	-
UCP2	*p*	Compared to all	*0.511*	*0.296*	*0.313*	*0.547*	-	-
Vla55Val	Genotype n (%)	Ala/Ala	10 (32.3)	6 (40.0)	4 (28.6)	9 (23.1)	29 (23.3)	37 (34.6)
		Ala/Val	18 (58.1)	8 (53.3)	5 (35.7)	23 (59.0)	54 (54.5)	52 (48.6)
		Val/Val	3 (9.7)	1 (6.7)	5 (35.7)	7 (17.9)	16 (16.2)	18 (16.8)
	p	Compared to controls	*0.530*	*0.594*	*0.235*	*0.402*	*0.665*	-
	p	Compared to all	*0.670*	*0.528*	*0.187*	*0.762*	-	-
	Allele n (%)	+9	32 (51.6)	15 (50.0)	11 (39.3)	36 (46.2)	94 (47.5)	
		-9	30 (48.4)	15 (50.0)	17 (60.7)	42 (53.8)	104 (52.5)	
	p	Compared to controls						
BDKRB2	*p*	Compared to all	*0.569*	*0.796*	*0.416*	*0.843*	-	
+9/-9	Genotype n (%)	+9/+9	9 (29.0)	3 (20.0)	1 (7.1)	10 (25.6)	23 (23.2)	
		+9/-9	14 (45.2)	9 (60.0)	9 (64.3)	16 (41.0)	48 (48.5)	
		-9/-9	8 (25.8)	3 (20.0)	4 (28.6)	13 (33.3)	28 (28.3)	
	p	Compared to controls						
	p	Compared to all	*0.807*	*0.693*	*0.350*	*0.725*	-	
	Allele (%)	*IL1RN*1*		39 (62.9)	21 (70,0)	23 (82.1)	56 (71.8)	
		*IL1RN*2*		23 (37.1)	9 (30,0)	5 (17.9)	22 (28.2)	
	p	Compared to controls						
IL-1RN	*p*	Compared to all		*0.280*	*0.982*	*0.189*	*0.794*	
(VNTR	Genotype (%)	*IL1RN*1/ IL1RN*1*		11 (35.5)	8 (53,3)	9 (64.3)	21 (53.8)	
86-bp)		*IL1RN*1/ IL1RN*2*		17 (54.8)	5 (33.3)	5 (35.7)	14 (35.9)	
		*IL1RN*2/ IL1RN*2*		3 (9.7)	2 (13.3)	0 (0)	4 (10.3)	
	p	Compared to controls						
	p	Compared to all		*0.374*	*0.782*	*0.267*	*0.836*	

p = the "p" values of the Chi-square test, * statistically significant difference according to the Chi-square test.

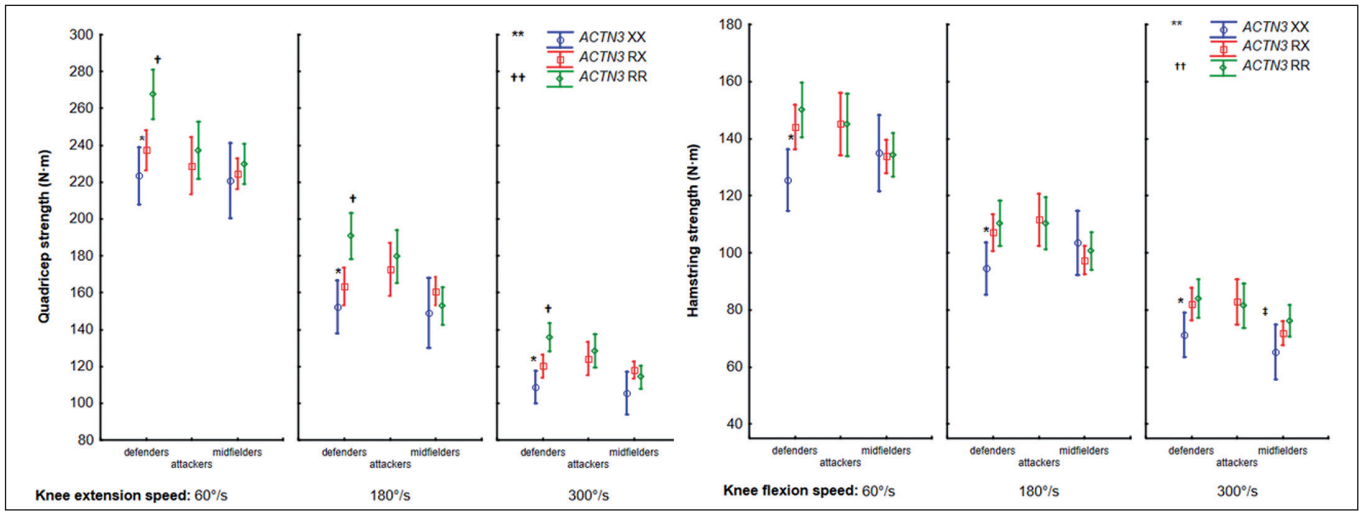

FIG. 1. Quadriceps and hamstring strength for *ACTN3* R577X genotypes and speeds of contraction in soccer player positions. †Significantly higher than other genotype groups at defined playing position and speed of contraction. †† significantly higher than other genotypes groups at all speeds of contraction regardless of playing-position. *Significantly lower than other genotype groups at defined playing position and speed of contraction. ** Significantly lower than other genotypes groups at all speeds of contraction regardless of playing position. ‡ Significantly lower than other genotype groups in midfielders at 300° speed of contraction. Significance is according to ANOVA and HSD test.

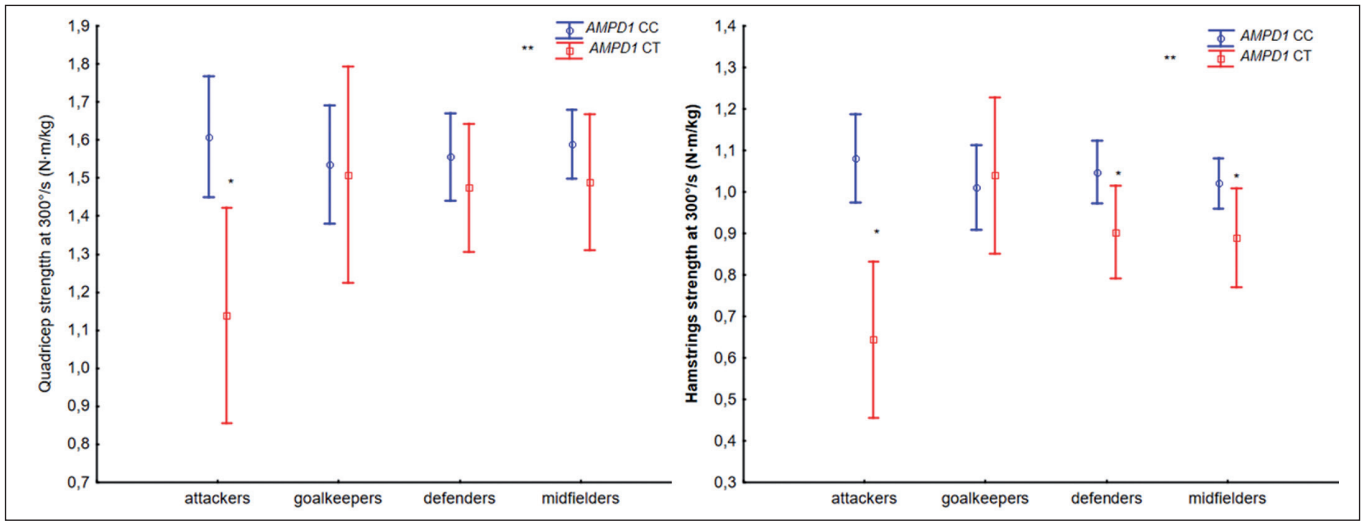

FIG. 2. Quadriceps and hamstring strength for *AMPD1* C34T genotypes at high contraction speed. *Significantly lower than other genotypes in the playing position. ** Significantly lower than different genotypes regardless of playing position. Significance is according to ANOVA and HSD test.

Further differences were found for *ACTN3* genotypes and playing position interaction for quadriceps strength at speed of 60°/s ($F_{3, 151} = 3.2$, $p = 0.025$, $\mu^2 = 0.06$), speed of 180°/s ($F_{3, 151} = 5.05$, $p = 0.002$, $\mu^2 = 0.09$) and 300°/s ($F_{3, 141} = 3.2$, $p = 0.024$, $\mu^2 = 0.06$), where post hoc test showed that RR genotype in defenders position had higher quadriceps strength than other genotype groups in each tested speed and XX genotype in defenders had lower quadriceps strength than other genotype groups in each tested speed (Figure 1). No difference among genotypes has been observed in midfielders and for the attackers.

The differences in *ACTN3* genotypes and playing position interaction were found for hamstring strength at speed of 60°/s ($F_{3, 151} = 2.4$, $p = 0.05$, $\mu^2 = 0.05$), speed of 180°/s ($F_{3, 151} = 2.9$, $p = 0.034$, $\mu^2 = 0.06$) and 300°/s ($F_{3, 142} = 8.2$, $p = 0.047$, $\mu^2 = 0.06$), showing that XX genotype in defenders had lower hamstring strength than other genotype groups in each tested speed and XX genotype

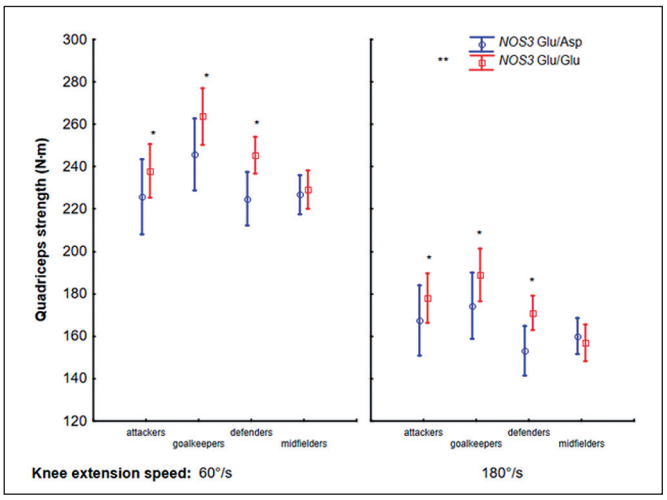

FIG. 3. Quadriceps strength for *NOS3* Glu298Asp genotypes and speeds of contraction in soccer player positions.
*Significantly higher than other genotypes in the playing position according to ANOVA and HSD test.

had lower hamstring strength than other genotype groups in midfielders at 300°/s speed (Figure 1).

The ANOVA showed differences in *AMPD1* genotypes for hamstrings ($F_{1, 176} = 13.9$, $p < 0.001$, $\mu^2 = 0.073$) and quadriceps ($F_{1, 176} = 4.99$, $p = 0.027$, $\mu^2 = 0.027$) at 300°/s in whole cohort, where CT genotype resulted in lower relative strength that CC genotype. The differences in *AMPD1* by playing positions were found for hamstrings relative strength ($F_{3, 170} = 3.2$, $p = 0.025$, $\mu^2 = 0.0754$) and quadriceps relative strength ($F_{3, 170} = 6.14$, $p = 0.014$, $\mu^2 = 0.034$) at 300°/s, where CT heterozygotes showed lower relative hamstring strength in attackers, defenders and midfielders and lower relative quadriceps strength in attackers than CC homozygotes (Figure 2).

The differences in *NOS3* genotypes for quadriceps absolute strength were found at speeds 60°/s ($F_{1, 175} = 8.85$, $p = 0.003$, $\mu^2 = 0.048$) and 180°/s ($F_{1, 175} = 4.93$, $p < 0.027$, $\mu^2 = 0.027$) in whole cohort, where Glu/Glu homozygotes showed higher strength than Glu/Asp heterozygotes (Figure 3). The differences in *NOS3* genotypes by playing positions were found for quadriceps strength at speeds 60°/s ($F_{1, 169} = 8.32$, $p = 0.004$, $\mu^2 = 0.046$), 180°/s ($F_{1, 169} = 5.26$, $p = 0.023$, $\mu^2 = 0.030$), where Glu/Glu homozygotes showed higher strength in attackers, defenders and goalkeepers than Glu/Asp heterozygotes (Figure 3).

The differences in *IL1RN* genotypes and playing position were found for hamstrings absolute strength at speeds 60°/s ($F_{1, 167} = 6.9$, $p = 0.009$, $\mu^2 = 0.040$), 180°/s ($F_{1, 167} = 7.06$, $p < 0.009$, $\mu^2 = 0.041$) and 300°/s ($F_{1, 167} = 4.83$, $p < 0.029$, $\mu^2 = 0.029$), where *IL1RN*2* allele carriers (*IL1RN*1/IL1RN*2+IL1RN*2/IL1RN*2*) resulted in higher strength than IL1RN*1/IL1RN*1 homozygotes in attackers and goalkeepers (Figure 4). The differences in *IL1RN* genotypes in context of playing position have been found for quadriceps strength at 300°/s ($F_{1, 158} = 4.8$, $p = 0.029$, $\mu^2 = 0.029$), where *IL1RN*2* allele carriers resulted in higher strength than *IL1RN*1/IL1RN*1* in attackers and defenders (Figure 4).

We found no differences between tested phenotype traits in our soccer players concerning their playing position and genotypes for *ACE* (I/D, rs1799752), *UCP2* (Ala55Val, rs660339), *BDKRB2* (+9/-9, rs5810761).

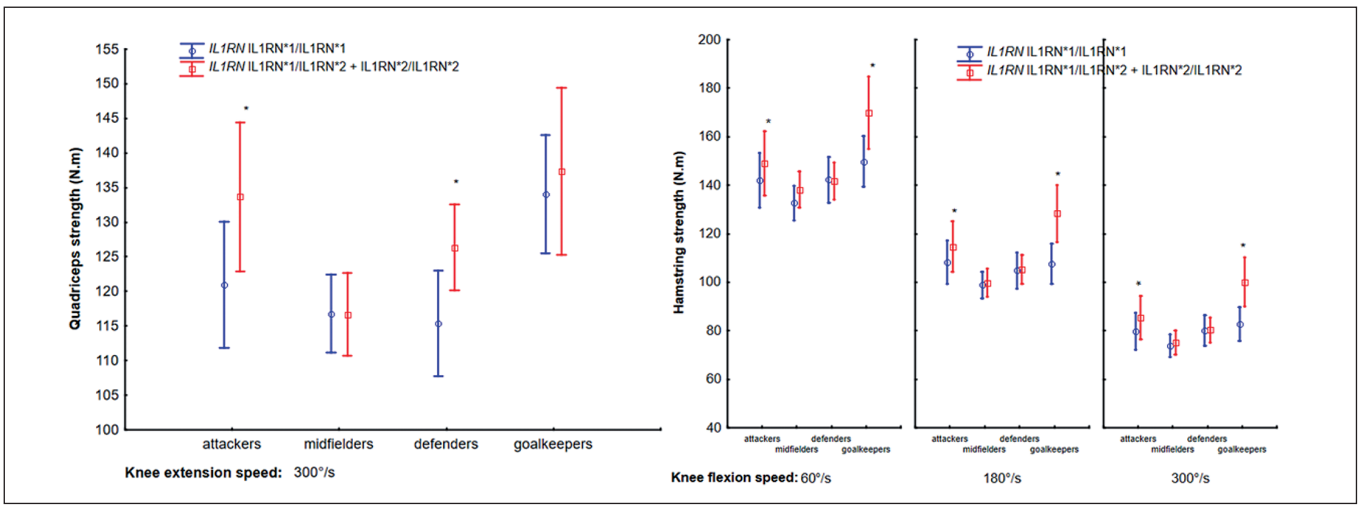

FIG. 4. Quadriceps and hamstring strength for *IL1RN* genotypes and speeds of contraction in soccer player positions.
*Significantly higher than other genotypes in the playing position according to ANOVA and HSD test.

TABLE 3. The multiple backward regression for correlated phenotypes in all analyzed genes (TGS) and four significant genotypes (TGS_{sig}).

Phenotypes		*b*	SE	t	p
Jump height: countermovement jump, squat jump **Relative strength:** quadriceps 60°/s and 300°/s, hamstring strength at 300°/s	Whole model	2.25	0.31	10.02	0.005
	ACE (I/D)	0,15	0.14	2.21	0.030
	BDKRB2 (9/+9)	0,22	0.18	2.54	0.013
	NOS3 (Glu298Asp)	0,38	0.14	4.20	0.001
	IIL1RN (VNTR86-bp)	0,24	0.16	2.61	0.011
	AMPD1 (C34T)	0,18	0.13	1.60	0.112
	UCP2 (Ala55Val)	0,19	0.16	2.32	0.023
	ACTN3 (R577X)	0,12	0.15	1.62	0.109
	TGS *	-0,02	0.28	-2.07	0.042
Jump height: countermovement jump, squat jump **Relative strength:** quadriceps 60°/s and 300°/s, hamstring strength at 300°/s	Whole model	2,50	0,20	12,68	< 0,001
	NOS3 (Glu298Asp)	0,32	0,10	3,37	< 0,001
	IIL1RN (VNTR86-bp)	0,16	0,09	1,76	0,083
	AMPD1 (C34T)	0,14	0,11	1,27	0,209
	ACTN3 (R577X)	0,09	0,08	1,09	0,277
	TGS_{sig} **	-0,01	0,00	-1,17	0,246

SE = standard error, * explained phenotype variance $R^2 = 0.26$, $p = 0.005$, ** explained phenotype variance $R^2 = 0.19$, $p = 0.009$.

Polygenic study

The TGSsig included four genotypes (*IL1RN, AMPD1* C34T*, ACTN3* R577X*,* and *NOS3* Glu298Asp) based on ANOVA results. Those TGS_{sig} genotypes correlated with jump height in countermovement jump, squat jump, relative quadriceps strength at 60°/s, 300°/s, and hamstring strength at 300°/s by r = 0.19, 0.20, 0.25, 0.20, and 0.19; respectively. Further linear-regression model of TGS including countermovement jump height, squat jump height, relative quadriceps strength at 60°/s, 300°/s, and hamstring strength at 300°/s can explain 19% of this phenotype variance ($R^2 = 0.19$, $p = 0.009$, Table 3).

The TGS of all seven analyzed genotypes correlated with jump height in countermovement jump, squat jump, relative quadriceps strength at 60°/s, 300°/s, and hamstring strength at 300°/s by r = 0.30, 0.24, 0.24, 0.27, and 0.30; respectively. Further linear-regression model of TGS including countermovement jump height, squat jump height, relative quadriceps strength at 60°/s, 300°/s, and hamstring strength at 300°/s can explain 26% of this phenotype variance ($R^2 = 0.26$, $p = 0.005$, Table 3).

DISCUSSION

Soccer belongs to the sport where speed is one of the main factors defining the difference between an excellent and an average athlete. Under this assumption, monitoring the lower extremities' maximum strength on an isokinetic dynamometer is a non-invasive and indirect way to determine a predictor of speed capability [43, 44]. Our findings confirm the genetic connection to power-speed performance in soccer players' positions in four out of seven previously reported gene polymorphisms [45], where *ACTN3* had the most evident influence, including the phenotypes of both hamstring and quadriceps strength at all three speeds of contractions. Moreover, our results confirm the previous finding that TGS is associated with professional soccer players [32] and can predict the jump performance [46], where we are adding the TGS link to the muscle strength at different speeds of contractions (regardless of players´ position). An interesting result was that our TGS score of seven selected genotypes had more robust model prediction than TGS_{sig}, including four genotypes. This confirms the major idea of TGS calculation that optimal genotypes profile for certain sport-related phenotypes requires multiple polymorphisms combinations, which are-related phenotypes that require multiple polymorphisms combinations related to different phenotype traits [46].

According to previous studies [7], our results confirm that the *ACTN3* RR genotype is associated with speed, power, and strength in elite athletes. With other conditioning assumptions, this genotype can be one of the player's premises [47], especially the in defenders. Conversely, the *ACTN3* XX genotype seems to be related to a less pronounced phenotype in speed, power, and strength predispositions, which we observed in midfielders (Figures 1 and 2). Moreover, the *ACTN3* XX genotype is possibly disadvantageous for attackers who did not contain any elite athlete in our cohort. This result corresponds

to the link between the *ACTN3* gene and muscle fiber type II, where the mutated X allele, especially of XX genotype, is less frequent in sprint and power athletes [48–50]. The physiological effect of the *ACTN3* X allele leading to missing protein on the structural properties of the sarcomere is described elsewhere [48, 51]. *ACTN3* gene coding alpha-actinin-3 protein belongs to groups of α-actinin isoforms, which are one of the main components of Z-line in muscle fiber [52], these form dimers that cross-link actin filaments. Expression of the *ACTN3* gene is restricted only to type 2 fibers; thus, muscle fibers containing α-actinin-3 can achieve higher absorption and transfer of force potential in Z-lines during rapid contractions [53]. The main difference in playing position is the distance cover requirements in the soccer match, where midfielders track greater distance than attackers and defenders [54, 55]. Specifically, the central midfielders spend less time in maximum sprints [54]. On the other hand, there is also a record that midfielders have the highest number of acceleration and deceleration activities in the most physically demanding period (10 min) over a game [56].

Our results also show a connection between *IL1RN*2* allele carriers and the speed, power, and strength hamstrings in attackers and goalkeepers and quadriceps strength in attackers and defenders; these carriers achieve increased strength levels. Thus *IL1RN*2* allele carriers probably relate more to the speed predispositions, which is typical for attackers sprinting or fast goalkeeper reactions. In the past, the VNTR polymorphism in the *IL1RN* gene was associated with athlete status; the *IL1RN*2* allele frequency was increased in professional athletes compared to amateur athletes with training times less than 10 hours a week [57]. Cauci et al. (2010) [57] also suggest that the *IL1RN*2* alleles may favor adaptation to high-intensity exercise. Unfortunately, a comparison of our cohort soccer players' allele/genotype frequencies with controls was not possible due to the unavailability of genotyping for this polymorphism in the control group.

Our study also indicates a connection between *NOS3* Glu298Asp polymorphism and the measured parameters of lower limb strength/power. *NOS3* Glu/Glu homozygotes showed a higher level of strength in the attackers, defenders, and goalkeepers than Glu/Asp heterozygotes. This polymorphism's possible effect on sports performance is related to the differential expression of endothelial NO synthase and the production of NO [58]. Our findings are consistent with [59] higher frequencies of the Glu298 allele in speed-strength-trained athletes than controls [59].

Finally, *AMPD1* CT heterozygotes resulted in lower relative strength than CC homozygotes, while rare TT homozygotes were completely missing in our cohort. Nucleotide change C to T at position 34 in exon 2 (34C/T) leads to a nonsense codon mutation (Gln12X) prematurely terminating translation associated with AMP deaminase enzyme deficiency. Several studies consistently showed lower T allele frequencies in athletes compared to controls [60, 61].

No relationship has been found between tested strength/power parameters and genotypes for *ACE* (I/D, rs1799752), *UCP2* (Ala-55Val, rs660339), *BDKRB2* (+9/-9, rs5810761); therefore, we suggest no significant contribution of these genetic variants on power and strength of lower limbs in our group soccer players. These findings are not exceptional as there is an inconsistency between genetic influence and speed/power performance in the literature for all mentioned polymorphisms [62–64].

Many studies evaluate the effects of genetic variants on elite (or sub-elite) soccer status [28, 65] or different traits that might be advantageous for soccer performance, including speed and power [66], endurance [67], or injury prevention [68]. For a comprehensive overview, current systematic reviews of McAuley et al. (2020) [7] or Sarmento et al. (2020) [69] suggest several gene variants, which can be beneficial for soccer and specifically for playing position in soccer as our findings exhibit. Nevertheless, sports scientists should keep in mind several things when interpreting the presented results, especially the noncoding biological variability, which continues to be uncovered in the human genome (e.g., epigenetic modifications, microRNAs, etc.). These other types of variability may contribute significantly to differences in athletic performance [70].

CONCLUSIONS

The strength and power measures are higher in elite soccer attackers and defenders, where some genetic markers can support these findings. Specifically, the *ACTN3* RR and *NOS3* Glu/Glu homozygotes and *IL1RN*2* allele carriers have higher strength and power, and thus they seem to be pre-disposed to those attacker or defender playing positions. The midfielders have lower strength and power conditions than other players without relation to "strength and power genes." The total genetic score regression explained 26% of the jump performance variance and isokinetic strength regardless of playing position.

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Authors' contributions

All authors met the authorship criteria for this journal and each author made a significant contribution to the final version of this paper.

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SUPPLEMENTARY MATERIAL

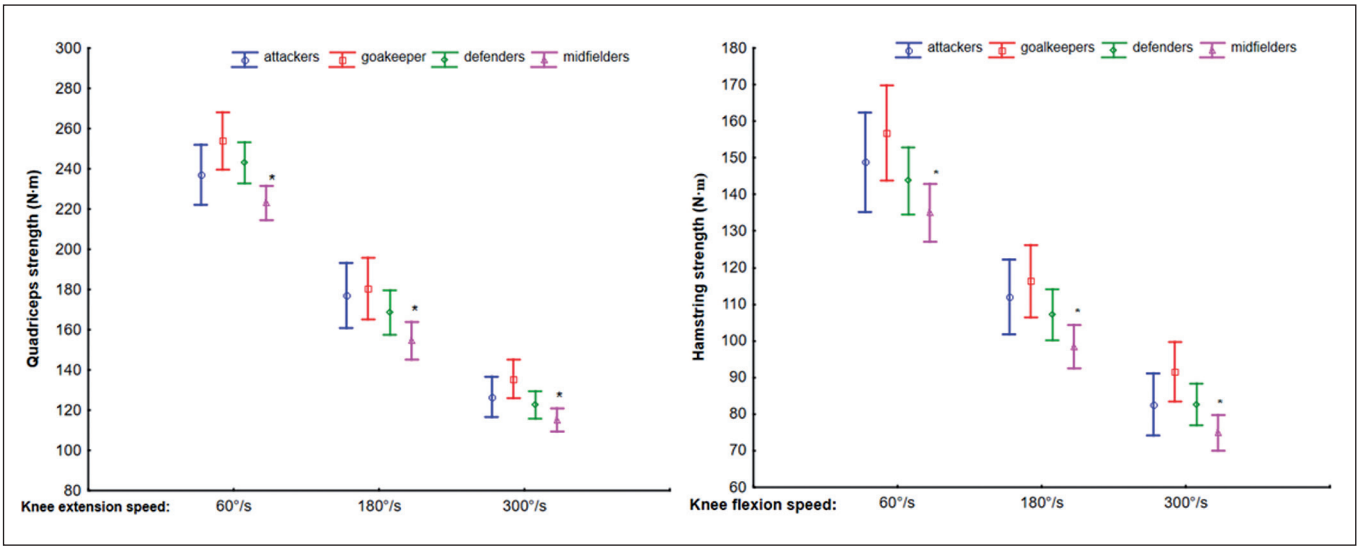

FIG. S1. Quadriceps and hamstring strength at different speeds in the soccer player group.
*Significantly lower than other playing position groups at a defined speed of contraction according to ANOVA and HSD test.

TABLE S1. The characteristic of the subjects

All soccer players:	**99**
– Goalkeepers	14
– Defenders	31
– Midfielders	39
– Attackers	15
Age (y)	25.4 ± 4.51
Height (cm)	181.4 ± 6.11
Weight (kg)	77.4 ± 7.22

TABLE S2. Quantity of the components used for the PCR

Genotype	DNA (μl)	DNA polymerase (μl)	Attackers primer/reverse primer (μl)	Buffer (μl)	dNTP (μl)	M_gCl_2 (μl)	Distilled H_2O (μl)	Betain (μl)
ACE (I/D)	2	Phusion 0,2	1	5xGC Buffer 4	4	Buffer included	3,8	4
ACTN3 (R577X)	2	Phusion 0,2	1	5xGC Buffer 4	4	Buffer included	3,8	4
BDKRB2 (9/+9)	2	Phusion 0,2	1	5xGC Buffer 4	4	Buffer included	3,8	4
NOS3 (Glu298Asp)	2	Phusion 0,2	1	5xGC Buffer 4	4	Buffer included	3,8	4
AMPD1 (Gln12X)	2	Taq(5U/μl) 0,9	0,8	10xTaq Buffer with KCl 2,0	1,5	25mM $M_gCl_2$1,6	6,4	4
UCP2 (Ala55Val)	2	Taq(5U/μl) 0,4	F 0,8 R 3,2	10xTaq Buffer with KCl 2,0	6	25mM $M_gCl_2$1,6	/	4
IL1RN (VNTR 86-bp)	2	Phusion 0,2	1	5xGC Buffer 4	4	Buffer included	3,8	4

TABLE S3. Forward/reverse primers and PCR conditions for the examined genotypes

Genotype	Forward primer (5'–3')	Reverse primer (5'–3')	PCR reaction conditions		
			Denaturation	Annealing and cycles	Final extension
ACE (I/D)	CTGGAGAGCCACTCCCATCCTTTCT	GACGTGGCCATCACATTCGTCAGAT	98°C 30 s 98°C 10 s	63°C 30 s – 35 cycles 72°C 30 s	72°C 5 min
ACTN3 (R577X)	CTGTTGCCTGTGGTAAGTGGG	TGGTCACAGTATGCAGGAGGG	94°C 30 s	70°C 1 min – 35 cycles	72°C 10 min
BDKRB2 (9/+9)	TCCAGCTCTGGCTTCTGG	AGTCGCTCCCTGGTACTGC	98°C 30 s 98°C 10 s	68°C 30 s – 35 cycles 72°C 30 s	72°C 5 min
NOS3 (Glu298Asp)	CATGAGGCTCAGCCCCAGAAC	AGTCAATCCCTTTGGTGCTCAC	98°C 30 s 98°C 10 s	62°C 30 s – 35 cycles 72°C 10 s	72°C 5 min
AMPD1 (Gln12X)	CTTCATACAGCTGAAGAGACA	GAATCCAGAAAAGCCATGAGC	95°C 30 s	56,4°C 1 min 72°C 30 s – 45 cycles	72°C 5 min
UCP2 (Ala55Val)	TGGGAGTCTTGATGGTGTCTAC	CACCGCGGTACTGGGCGCTG	95°C 30 s	61,2°C 50 s 72°C 30 s – 46 cycles	72°C 5 min
IL1RN (VNTR 86-bp)	CTCAGCAACACTCCTAT	TCCTGGTCTGCAGGTAA	98°C 30 s	57°C 30 s 72°C 30 s – 35 cycles	72°C 5 min