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The Relationship between Genetic Variability and Seasonal Changes in Vertical Jump Performance in Amateur Soccer Players

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Abstract: This study aimed to investigate the longitudinal evolution of vertical jump height in soccer players and its association with their genotypic profiles. The genotypes of 37 soccer players were characterized and the countermovement jump (CMJ) height, concentric mean power, force, and jump strategy were assessed at two time points: at the onset of the regular season (Pre) and at the conclusion of the first half (Mid). *AMPD1* (rs17602729), *ACE* (rs4646994), *ACTN3* (rs1815739), *CKM* (rs8111989), and *MLCK* (rs2849757 and rs2700352) polymorphisms were genotyped. No significant differences were found between Pre and Mid in concentric power (p = 0.068; d = 0.08) or force (p = 0.258; d = 0.04), while jump height displayed trivial increases (p = 0.046; d = 0.15). Individual analysis revealed that 38% of participants increased the vertical jump height and mean concentric power over the smallest worthwhile change (SWC), while 27% experienced an increased mean concentric force. The positive responders were characterized by a decreased frequency of the AA genotype and an increased frequency of the CA genotype for the c.37885C>A polymorphism of *MLCK* (p = 0.035), as well as a decreased frequency of the TT genotype of *ACTN3* (p = 0.042) and the CC genotype of *AMPD1* (p = 0.022). Our findings suggest that genetic analysis could explain some variability in neuromuscular adaptations during the in-season.

Keywords: football; DNA; ACTN3; polymorphism; genotype; CMJ

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1. Introduction

High-intensity actions characterized by requiring high-force production in short periods of time are the determinants of final performance in football. Notably, linear sprinting and vertical jumping account for 61% of the physical actions preceding a goal-scoring opportunity [1]. In this context, the incorporation of diverse neuromuscular performance assessments should be conducted in a manner that both informs and aligns with the specific actions of the sport. This will contribute to providing relevant information about neuromuscular performance and athlete adaptations during the competitive period. The countermovement jump (CMJ) emerges as a widely embraced tool in team sports within this framework [2]. Moreover, jump height, kinetics and kinematics of the CMJ are frequently linked to pivotal actions in team sports, including muscle strength, maximal sprint durations, and change of direction times [2]. Indeed, several studies have reported changes in the various metrics of the CMJ, whether in discrete variables or through the analysis of force, power, velocity, and displacement–time curves, suggesting that this test is a sensitive tool for identifying longitudinal and cross-sectional adaptations [3,4].

While interventions incorporating strength and/or power training have exhibited increases in the kinetics, kinematics, and outcomes of the CMJ [5], the scientific literature presents a less definitive stance on the adaptations in the CMJ over the course of a season (or multiple seasons) in team sports [3,4,6]. This ambiguity may arise due to the concurrent training of various physical capacities and the regular weekly competition schedule

inherent to team sports [3,4,6]. However, when individual analysis was carried out, 60% of the players have shown a meaningful change in jump height at certain points in the season. Several factors may influence these variable responses among individuals and between studies: the type of equipment used to measure jumping, sporting background, or genetic profile [7], among others. This latter aspect (i.e., genetic characteristics) have been recognized as a key factor in sporting success [7]. However, until recently, analyzing these traits in athletes was considered impractical due to high costs. Nevertheless, advancements in technology have made genetic testing more affordable, opening up possibilities for growth in this area and the potential to influence the daily practices of coaches, fitness trainers, and athletes.

The relationship between genetics and aspects related to strength and power in sports constitutes an area of great interest in sports research [8]. In this context, an athlete's ability to develop muscle strength and power is significantly influenced by their genetic makeup [9]. Research has demonstrated substantial genetic diversity in response to strength and power training, as well as in the predisposition to specific types of muscle fibers [10,11]. It is relevant to note that specific genetic variants play a crucial role in determining the response to training and neuromuscular capacity, especially in genes such as Angiotensin Converting Enzyme (*ACE*) and Alpha-actinin 3 (*ACTN3*). For example, the DD genotype of the I/D polymorphism (rs4646994) in the *ACE* gene and the CC genotype of the c.1729C>T polymorphism (rs1815739) in the *ACTN3* gene indicate a predisposition to power and strength [12]. Specifically, the T allele of the c.34C>T polymorphism (rs17602729) in the Adenosine Monophosphate Deaminase 1 (*AMPD1*) gene, along with genes related to muscle damage such as the c.*800A>G polymorphism (rs8111989) in Creatin Kinase Muscle-specific (*CKM*) and two polymorphisms [c.49C>T (rs2700352) c.37885C>A (rs28497577)] in Myosin-like chain kinase 1 (*MLCK*), have been associated with professional football players [13].

Despite genetics and the neuromuscular state of the lower limbs being determining factors in physical performance in team sports, it has not yet been identified whether there are different training adaptations throughout a season in male and female soccer players. Due to the relationship and internal logic between the neuromuscular state of soccer players and physical performance during a soccer match and different measurements of performance [2,14], it is essential to understand whether variations exist throughout the season in jump performance based on the different genetic profiles of the players. Therefore, the objective of this study is to describe the evolution of the CMJ performance during the competitive period in amateur soccer players and to compare the response to training according to genotype distribution.

2. Materials and Methods

2.1. Design

This longitudinal, prospective observational study was designed to monitor the CMJ performance according to genotypic distribution in the *ACE*, *ACTN3*, *AMPD1*, *CKM,* and *MLCK* polymorphisms of 37 players belonging to three different teams within the same Spanish football club. Throughout the 2021–2022 season, participants competed in the Spanish fourth division, the second U18 category, and the women's third division of football.

2.2. Participants

Initially, 60 participants volunteered to take part in the study, all of whom underwent genetic evaluation. However, due to long-term injuries (n = 10), absences during one of the two CMJ evaluations (n = 9), and transfers to other teams during the month of December (n = 4), only 23 men and 14 women were ultimately included in the study (n = 37). All the football players were of Spanish Caucasian descent (Caucasian descent for the population of $\geq$3 generations). Descriptive characteristics of men and women players are displayed in Table 1.

Table 1. Descriptive characteristics of men and women players.

	Men (n = 23)		Women (n = 14)	
	Mean	**SD**	**Mean**	**SD**
Weight (Kg)	76.07	6.42	58.95	5.8
Height (m)	1.82	0.25	1.64	0.14
BMI (kg/m^2)	22.9	2.18	21.82	1.97
Hours of competition (h)	18.32	10.06	18.73	10.24
Hours of training (h)	370.49	43.77	183.73	56.83

BMI, body mass index.

To be included in the research, participants had to meet the following inclusion criteria: (i) be amateur players aged >18 and <40; (ii) have attended more than 80% of the training sessions with their team; and (iii) engage in at least 1 h of physical exercise more than 3 times per week. Athletes who did not complete one evaluation due to injury were excluded from the study according to the criteria set by the team's medical staff and following the consensus statement on injuries in football. All players agreed to participate in the study by giving their written informed consent. The study protocol was approved by the research ethics committee of the Francisco de Vitoria University (UFV 32/2020) and the confidentiality of the participants was ensured, complying with the Declaration of Helsinki 1964 (latest update 2013).

2.3. Vertical Jump Testing

Measurements were taken at two different time points during the in-season: at the end of the preseason (first week of September) and mid-season, after concluding the first round (second week of January). In both assessments, a standardized warm-up, led by each team's physical coach, included running activities, dynamic stretching, core, and lower extremity activation, as well as a submaximal approach to the jump test. Both measurements were conducted in weeks with the same competitive density (1 match) for all three teams, and all assessments were performed on Wednesdays, three days after the last competition (Sunday). To assess the jumping performance, each participant executed three CMJ during pre- and mid-season testing sessions using the mean value for statistical analysis. The jumps were conducted on a Force-Decks FD4000 Dual Force platform (ForceDecks, London, UK) with a sampling rate of 1000 Hz. Athletes performed three maximal trials with a 1 min rest between each, involving a self-selected depth in the countermovement to maintain consistent jumping coordination [15]. The prescribed movement consisted of a downward motion followed by a swift and full extension of the lower limbs. Athletes were instructed to jump as high as possible and land in proximity to the take-off point. Center-of-mass (COM) velocity was calculated by dividing the vertical force (subtracting body weight) by body mass and integrating the product using the trapezoid rule. Instantaneous power was determined by multiplying the vertical force by the COM velocity. COM displacement was obtained through double integration of the vertical force data. A CMJ was considered successful if it performed with the arms akimbo, and participants maintained complete stillness for at least one second during the weighing phase [16]. The initiation of the movement was identified when a drop of 20 N from baseline force (recorded during the weighing phase) was observed. The concentric phase started when velocity became positive and ended at take-off. In accordance with the conceptual framework developed by Bishop et al. [14], we selected the following variables from the CMJ due to their appropriate reliability and their reflection of different aspects of vertical jump performance (i.e., outcome, kinetics, kinematics, and jump strategy) [15]. The variables we considered to quantify the CMJ are jump height (the highest displacement of the center of mass calculated from vertical velocity at take-off), concentric mean power (mean value of instant power during the concentric phase), concentric mean force (mean value of instant force during the concentric phase), player weight, BMI, and countermovement depth (the difference in the

displacement of the center of mass between the weighing phase and the lowest point of the center of mass from the onset of the movement to takeoff).

2.4. DNA Sample Collecting and Genotype

The samples were collected during the 2020/2021 season in the month of January with SARSTED swabs by buccal smear and kept refrigerated until genotyping.

Deoxyribonucleic acid (DNA) extraction from the swabs was carried out in VIVOLabs laboratory (Madrid, Spain) by automatic extraction in QIACube equipment (QIAGEN, Venlo, The Netherlands), yielding a DNA concentration of 25–40 ng/mL, which was kept in a solution in a volume of 100 µL at −20 °C until genotyping.

ACE I/D (rs4646994), *ACTN3* c.1729C>T (rs1815739), *AMPD1* c.34C>T (rs17602729), *CKM* c.*800A>G (rs8111989), and *MLCK* c.49C>T (rs2700352) and c.37885C>A (rs28497577) polymorphisms were genotyped using single-nucleotide primer extension (SNPE) with the SNaPshot Multiplex Kit (Thermo Fisher Scientific, Waltham, MA, USA), with the analysis of the reaction result by capillary electrophoresis fragments, in an ABI3500 unit (Applied Biosystems, Foster City, CA, USA) with bioinformatic analysis performed by GeneMapper 5.0 software. The genomic location of each polymorphism is present in Table 2.

Table 2. Genomic location and minor allele frequency (MAF) for selected genes in muscle injuries.

Symbol	Gene	dbSNP	Genomic Location	MAF Football Players	MAF (IBS) *	HWE	FIS
ACE	Angiotensin-converting enzyme	rs4646994	17q23.3	21.6% (I)	36.7% (I) **	$p = 0.089$	0.53
ACTN3	alpha-actinin-3	rs1815739	11q13.2	52.1% (T)	43.9% (T)	$p = 0.163$	−0.31
AMPD1	Adenosine monophosphate deaminase 1	rs17602729	1p13.2	16.2% (T)	14.0% (T)	$p = 0.844$	−0.08
CKM	Muscle-specific creatine kinase	rs8111989	19q13.32	24.3% (G)	26.6% (G)	$p = 0.895$	−0.06
MLCK	Myosin light chain kinase	rs28497577	3q21.1	5.4% (A)	10.3% (A)	$p = 0.348$	0.23
MLCK	Myosin light chain kinase	rs2700352	3q21.1	16.2% (T)	20.1% (T)	$p = 0.521$	0.19
Overall *SNPs*						$p = 0.476$	0.11

IBS, Iberian population in Spain *; ** FIS, inbreeding coefficient; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

2.5. Statistical Analysis

IBM SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA) was employed for conducting statistical tests and GraphPad prism v.9 (San Diego, CA, USA) was used for graphing. Data from preseason testing exhibited a normal distribution by using Shapiro–Wilk normality test. Participants exhibiting positive, negative, or no adaptations to training were identified based on the absolute change compared to the smallest worthwhile change (SWC = 0.2 · between-subject SD). A repeated-measures within-factors MANOVA test was carried out using Evaluation and Sex as predictive factors and BMI as covariate. A paired *t*-test was employed to detect the differences between pre- and mid-season testing. The magnitudes of estimated effects (Cohen's d) were calculated between the assessments and categorized as follows: ≤0.2 trivial, ≥0.2–0.6 small, ≥0.6–1.2 moderate, ≥1.2–2.0 large, and ≥2 very large [16]. The significance level was set at $p < 0.05$. The disequilibria of SNPs were estimated using the Hardy–Weinberg equilibrium (HWE) followed by the approach proposed by Weir and Cockerham [17]. The probability of having an optimal genotype for these polymorphisms was calculated using the χ^2 test with a fixed α error of 0.05. To determine what genotype was associated with an unexpected distribution, the standardized residuals were calculated based on the difference between the observed and the expected values. Briefly, within each variable, a genotype was considered to have a statistically different distribution from the expected value when its distribution was greater than or less than the critical Z-score value (i.e., 1.96). The genotypic frequencies of the polymorphisms were compared between the responders' and non-responders', using a χ^2 test. Post hoc power analysis was carried out using free software (G*Power v3.1). With a sample size of 37 participants, assuming an alpha error of 0.05 and an effect size of 0.38 based on the mean and standard deviation of both assessments of the CMJ height and Pearson correlation between measurements of 0.92, the statistical power achieved was 0.982.

3. Results

3.1. Overall CMJ Performance

A main effect of Sex was observed for all CMJ height (F = 74.076; $p < 0.001$; $\eta p^2 = 0.514$) concentric mean force (F = 199.959; $p < 0.001$; $\eta p^2 = 0.714$), and concentric mean power (F = 212.703; $p < 0.001$; $\eta p^2 = 0.752$) without differences between sex in CM depth (F = 2.470; $p = 0.121$; $\eta p^2 = 0.034$). No main effect of evaluation or interaction between Evaluation x Sex were observed ($p > 0.05$).

Men jump higher ($p < 0.001$), producing more concentric force ($p < 0.001$) and power compared ($p < 0.001$) to women, without differences in jump strategy.

Collectively, the jump height demonstrated a trivial non-significant increase between pre- and mid-season assessments (F = −0.884; $p = 0.350$; Δ% [95%CI] = 3.2 [0.2–6.7]; d [95%CI] = 0.15 [−0.01–0.30]). The remaining variables analyzed exhibited p-values greater than the predetermined significance level ($p > 0.05$), with effect sizes (d) below 0.2, indicating trivial magnitudes of change. According to the responder analysis based on the smallest worthwhile change (SWC), approximately 38% (14/37) of the study participants increased both jump height and concentric power above the minimum threshold to consider positive adaptations. Approximately 27% (10/37) of the participants also increased their mean concentric force (SWC = 51.64 N) (Figure 1). The jumping strategy remained consistent across measurements, as evidenced by the absence of differences in the countermovement depth (F = −0.564; $p = 0.455$; Δ% [95%CI] = −1.1 [−11.6 to 10.6]; d [95%CI] = −0.03 [−0.32 to 0.26]). There were also no differences in the weight of the subjects between the pre- and mid-assessment (F = 0.010; $p = 0.922$; Δ% [95%CI] = −0.1 [−0.9–0.7]; ES [95%CI] = −0.01 [−0.06–0.04]).

Similarly, the differences between the evaluations based on sex indicated that neither men nor women exhibited a generalized increase in any of the jump performance metrics (jump height: men: $p = 0.960$; women: $p = 0.824$; Concentric mean force: men: $p = 0.451$; women: $p = 0.930$; concentric mean power: men: $p = 0.422$; women: $p = 0.820$) (Figure 2).

3.2. DNA-Based Analysis of CMJ Performance

The polymorphisms analyzed met the HWE (all $p > 0.05$; Table 2).

3.2.1. Jump Height

Genotype distribution of muscle performance genes in the jump height responder's football players group, when compared with the non-responder's players, was no different in muscle performance polymorphisms between responder and non-responder players (Table 3).

3.2.2. Concentric Mean Power

The genotype distribution of muscle performance genes in the concentric mean power responder's football players group, when compared with the non-responder's players, was statistically significant for the *MLCK* c.37885C>A polymorphism ($p = 0.035$), showing a higher frequency in the "heterozygous" genotype in responder players (CA 21.4%) than the non-responder players (CA 4.3%), since there was a higher frequency of the "worse" genotype in the non-responder players (AA 91.3%) than in the responder players (AA 78.6%). No differences were shown in the other muscle performance polymorphisms between the responder and non-responder players (Table 4).

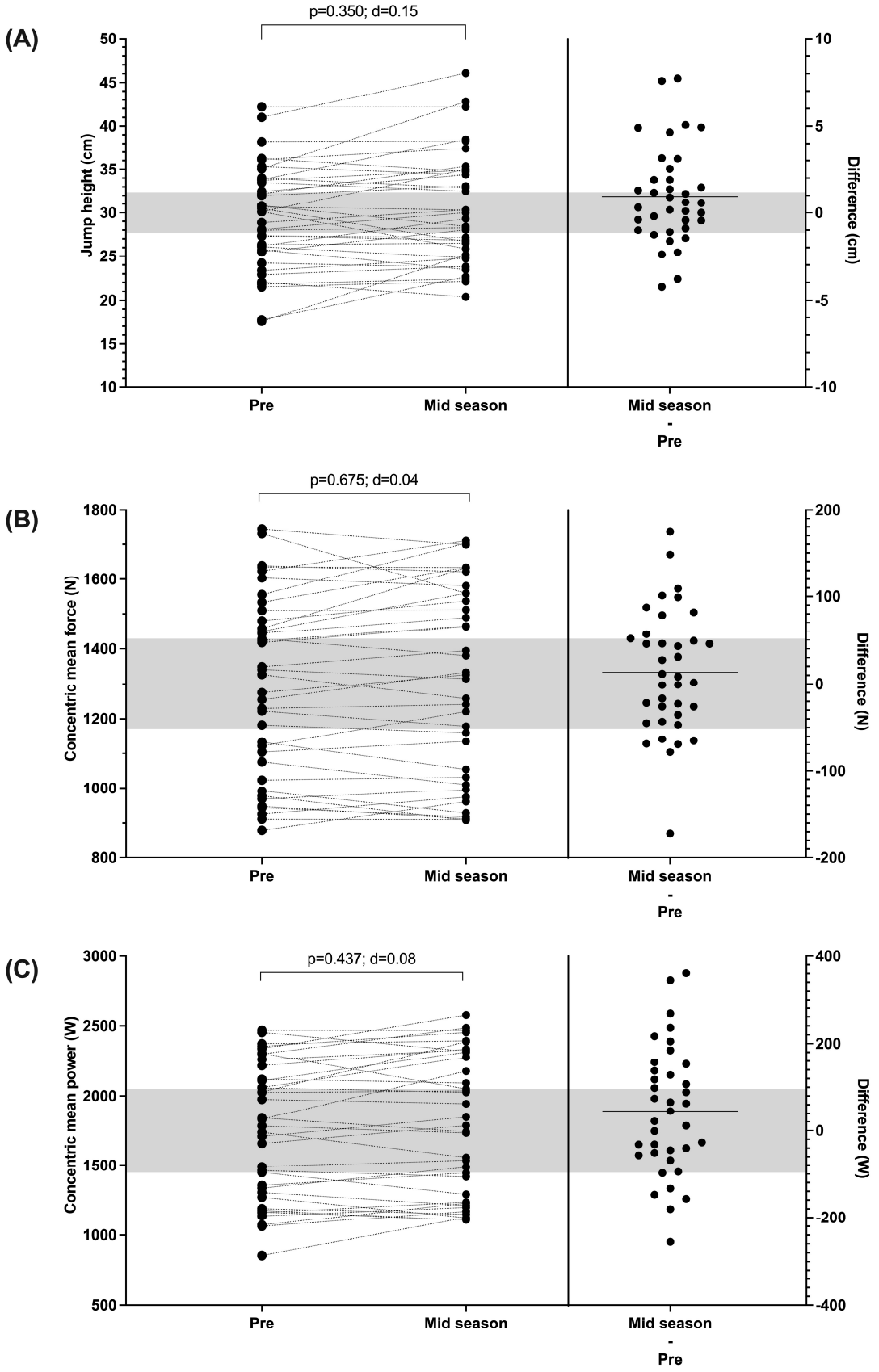

Figure 1. Individual changes and differences in jump height (**A**), concentric mean force (**B**), and concentric mean power (**C**) between the start of the season (September) and the mid-season (February). Gray area represents the SWC.

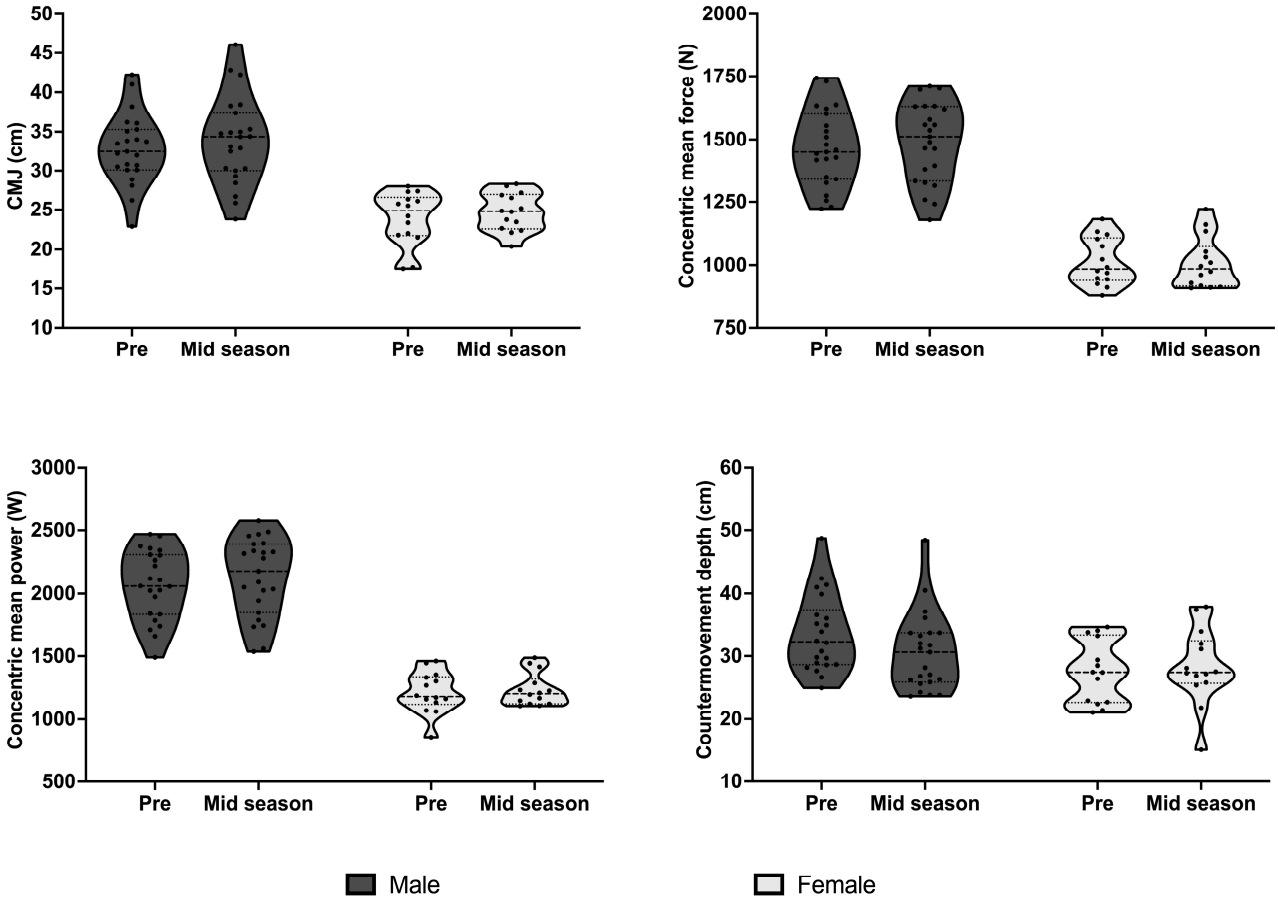

Figure 2. Differences between pre- and mid-season for CMJ-derived metrics as a function of gender.

Table 3. Genotype distribution in responder football players and non-responder football players in jump height for muscle performance polygenic profile.

Symbol	Gene	Polymorphism	dbSNP	Genotype	Responder	Non-Responder	*p* Value
ACE	Angiotensin-converting enzyme	I/D	rs4646994	DD ID II	9 (64.3%) 4 (28.6%) 1 (7.1%)	14 (60.9%) 8 (34.8%) 1 (4.3%)	0.884
ACTN3	Alpha-actinin-3	c.1729C>T	rs1815739	CC CT TT	5 (35.7%) 4 (28.6%) 5 (35.7%)	5 (21.7%) 4 (17.4%) 14 (60.9%)	0.332
AMPD1	Adenosine monophosphate deaminase 1	c.34C>T	rs17602729	CC CT TT	10 (71.4%) 4 (28.6%) 0 (0.0%)	16 (69.6%) 6 (26.1%) 1 (4.3%)	0.729
CKM	Muscle-specific creatine kinase	c.*800A>G	rs8111989	GG GA AA	0 (0.0%) 6 (42.9%) 8 (57.1%)	3 (13.0%) 6 (26.1%) 14 (60.9%)	0.272
MLCK	Myosin light chain kinase	c.49C>T	rs2700352	CC CT TT	9 (64.3%) 5 (35.7%) 0 (0.0%)	16 (69.6%) 7 (30.4%) 0 (0.0%)	0.739
		c.37885C>A	rs28497577	CC CA AA	0 (0.0%) 2 (14.3%) 12 (85.7%)	0 (0.0%) 2 (8.7%) 21 (91.3%)	0.595

Table 4. Genotype distribution in the responder and non-responder football players in concentric mean power for the muscle performance polygenic profile.

Symbol	Gene	Polymorphism	dbSNP	Genotype	Responder	Non-Responder	*p* Value
ACE	Angiotensin-converting enzyme	I/D	rs4646994	DD ID II	8 (57.1%) 5 (35.7%) 1 (7.1)%	15 (65.2%) 7 (30.4%) 1 (4.3)%	0.864
ACTN3	Alpha-actinin-3	c.1729C>T	rs1815739	CC CT TT	5 (35.7%) 4 (28.6%) 5 (35.7%)	5 (21.7%) 4 (17.4%) 14 (60.9%)	0.332
AMPD1	Adenosine monophosphate deaminase 1	c.34C>T	rs17602729	CC CT TT	8 (57.1%) 6 (42.9%) 0 (0.0%)	18 (78.3%) 4 (17.4%) 1 (4.3%)	0.197
CKM	Muscle-specific creatine kinase	c.*800A>G	rs8111989	GG GA AA	1 (7.1%) 5 (35.7%) 8 (57.1%)	2 (8.7%) 7 (30.4%) 14 (60.9%)	0.941
MLCK	Myosin light chain kinase	c.49C>T	rs2700352	CC CT TT	10 (71.4%) 4 (28.6%) 0 (0.0%)	15 (65.2%) 8 (34.8%) 0 (0.0%)	0.739
		c.37885C>A	rs28497577	CC CA AA	0 (0.0%) 3 (21.4%) ↑ 11 (78.6%) ↓	0 (0.0%) 1 (4.3%) ↓ 22 (91.3%) ↑	0.035

3.2.3. Concentric Mean Force

The genotype distribution of the muscle performance genes in the concentric mean force responder's football players group, when compared with the non-responder's players, was statistically significant for ACTN3 c.1729C>T polymorphism ($p = 0.042$), showing a higher frequency in the "worse" genotype than in non-responder's players (TT 63.0%) than the responder's (TT 20.0%), Also, statistical differences were found in AMPD1 c.334C>T polymorphism ($p = 0.022$), showing a higher frequency of the "optimal" genotype in the non-responder players (CC 81.5%) than in the responder players (CC 40.0%), as well as the responder players showing a higher frequency of the "heterozygous" genotype (CT 60.0%) than the non-responder players (CT 14.8%). Similar results were found in MLCK c.37885C>A polymorphism ($p = 0.022$), showing a higher frequency of the "heterozygous" genotype in responder players (CA 30.0%) than in non-responder players (CA 3.7%), as well as non-responder players showing a higher frequency of the "worse" genotype (AA 96.6%) than the responder players (AA 70.0%). No differences were shown in the other muscle performance polymorphisms between responder and non-responder players (Table 5).

Table 5. Genotype distribution in responder and non-responder football players in concentric mean force for muscle performance polygenic profile.

Symbol	Gene	Polymorphism	dbSNP	Genotype	Responder	Non-Responder	*p* Value
ACE	Angiotensin-converting enzyme	I/D	rs4646994	DD ID II	6 (60.0%) 3 (30.0%) 1 (10.0%)	17 (63.0%) 3 (33.3%) 1 (3.7%)	0.752
ACTN3	Alpha-actinin-3	c.1729C>T	rs1815739	CC CT TT	4 (40.0%) 4 (40.0%) 2 (20.0%) ↓	6 (22.2%) 4 (14.8%) 17 (63.0%) ↑	0.042
AMPD1	Adenosine monophosphate deaminase 1	c.34C>T	rs17602729	CC CT TT	4 (40.0%) ↓ 6 (60.0%) ↑ 0 (0.0%)	22 (81.5%) ↑ 4 (14.8%) ↓ 1 (3.7%)	0.022
CKM	Muscle-specific creatine kinase	c.*800A>G	rs8111989	GG GA AA	1 (10.0%) 3 (30.0%) 6 (60.0%)	2 (7.4%) 9 (33.3%) 16 (59.6%)	0.958
MLCK	Myosin Light Chain Kinase	c.49C>T	rs2700352	CC CT TT	7 (70.0%) 3 (30.0%) 0 (0.0%)	18 (66.7%) 9 (33.3%) 0 (0.0%)	0.847
		c.37885C>A	rs28497577	CC CA AA	0 (0.0%) 3 (30.0%) ↑ 7 (70.0%) ↓	0 (0.0%) 1 (3.7%) ↓ 26 (96.6%) ↑	0.022

4. Discussion

This is the first study providing information on seasonal changes in the performance, kinetics, kinematics, and strategy of the CMJ according to the genotype distribution of male and female footballers. There were no significant differences in jump height, mean concentric force, or power between the assessments. However, approximately 38% of the participants experienced an increase in concentric power and mean force. This indicates a need to analyze the individual characteristics of those players who did improve their performance. In this sense, positive responders exhibited a lower frequency of the AA genotype and a higher frequency of CA genotype for the c.37885C>A polymorphism of *MLCK*, as well as a lower frequency of the TT genotype of *ACTN3* and the CC genotype of *AMPD1*.

At a group level, our findings indicate that the jump height, concentric force, and power are similar between the end of the preseason (or start of the season) and the midway point of the season (Figure 1). Similar results were observed in team sports, where training periodization over a similar timeframe was not effective in improving either the vertical jump height or other athletic performance surrogates [4,6,18]. Specifically, neither jump height, RSImod, nor concentric peak power changed after 20 weeks of training in elite futsal players. In fact, these variables tended to decrease over the season, showing reductions between the preseason and mid-season evaluations (ES = −0.28 to −0.93) [4]. Loturco et al. [6] also showed that, over an 11-month period, there were no significant differences in the vertical jump height or 1RM in half squat, despite performing between 1.8 ± 1.9 to 4.0 ± 1.1 strength/power training sessions during the season. Despite the differences in training content and volume, the lack of improvement in neuromuscular performance could be explained by the structured microcycle periodization by the players of the present research. The concurrent nature of team sports and the limited time available for resistance training between competitions may hinder the generation of an optimal training stimulus. Specifically in soccer, recovery after a match can extend up to 72 h (MD+3) after the end of the game [19], and the next match can be played three days after that MD+3. Therefore, in contexts where players train once a day, there are only four training sessions available to stimulate all the physical capacities involved in soccer. It seems that current training periodization in soccer are not effective given the limited time to accumulate training load. However, in the studies that have analyzed the seasonal changes in CMJ, a high variability in training adaptations was observed, even with different training periodization and training loads [18,20–23]. Taken together, the training stimuli provided during the 20-week observed period may not be optimal for a generalized increase in the mechanical performance, as previously observed [3,4,6]. Nevertheless, this training regimen enabled athletes to sustain their CMJ height from the beginning to the midpoint of the competitive season.

Genetics may play a crucial role in determining an athlete's neuromuscular capabilities, affecting muscle fiber composition, protein production and repair, energy metabolism efficiency, neuromuscular function, training adaptation capacity, and injury susceptibility [24]. These genetic influences, combined with training and other environmental factors, contribute to the differences in sports performance among individuals [25]. Neuromuscular performance is regulated by a complex network of biological pathways that include muscle contraction, neuromuscular signaling, energy metabolism, mitochondrial biogenesis, protein synthesis and degradation, muscle repair, and the inflammatory and oxidative stress response [26,27]. The interaction and efficiency of genetics and these pathways largely determine an athlete's ability to perform and recover from intense physical activities [25,26]. While success in football is influenced by a wide variety of factors, including genetics, there is no specific "power and sprint gene" [28]. However, there are certain genes and genetic profiles that may be associated with the physical characteristics relevant to explosive strength in football that more accurately define the influence of genetics on performance and injury risk [13,28,29], as well as explosive strength [30]. Some of the genes that have been linked to these abilities to date include *ACTN3* and *ACE* polymorphisms, which can influence muscle composition, muscle injuries, and the ability to generate explosive force [12,31]. However, in our study, only the c.1729C>T polymorphism of the *ACTN3* gene

has shown an association with the response in concentric mean force, with the TT genotype showing a higher frequency in non-responders.

The *AMPD1* gene has been the subject of interest in relation to athletic ability and sports performance, particularly in sports that require explosiveness [32]. The c.34C>T polymorphism is associated with the increased activity of the AMPD enzyme. This higher activity could theoretically lead to a greater ability to produce ATP (adenosine triphosphate), which is the main source of energy used during explosive efforts, associating the CC genotype and C allele with a greater ability to produce ATP and sprint/power skills [32]. However, studies on the relationship between the *AMPD1* gene and athletic performance are inconclusive. Some studies suggest that athletes with the CC genotype of the *AMPD1* gene may have an advantage in sports that require rapid energy release [33], while others have found no significant associations [34]. In this study, the only association with neuromuscular performance presented by the *AMPD1* gene was in the concentric mean force in which there was a high frequency of non-responders in the CC and CT genotypes. The fact that there were amateur footballers could be one of the reasons for the lack of association and the small sample size presented, which justifies carrying out future studies in professional football players to study the results presented in greater depth. The *MLCK* gene encodes an enzyme that plays a key role in the regulation of muscle contraction, specifically myosin light chain phosphorylation, which activates myosin and triggers muscle contraction. Some studies have suggested that the *MLCK* c.37885C>A polymorphism may be associated with differences in the response to muscle damage after exercise, increasing susceptibility to muscle damage, especially in the AA genotype. In our study, it has been shown that the "worst" genotype for muscle performance (AA) showed a higher frequency among non-responders in the concentric mean force. In this case, the c.37885C>A polymorphism of the *MLCK* gene together with the c.1729C>T polymorphism of the *ACTN3* gene have been associated with muscle damage [35]. However, the results are still mixed and not all studies have found a significant association between the *MLCK* c.37885C>A polymorphism and muscle damage. As we found these results to be promising, it will be necessary to implement them in professional athletes in which the demands of training and competitions are higher and in which it has previously been shown that this muscle damage may be associated with muscle injuries and setting in professional footballers [29]. Although the association of the c.*800A>G polymorphism of the *CKM* gene in professional football players' injuries and performance has been previously presented [33], in our study, we did not find any relevant results indicating that this polymorphism is related to neuromuscular performance in amateur football players. The lack of genetic studies on the *CKM* gene and its association with muscle damage creates the need for further research to confirm the results found in this study.

The results presented in this study have not shown an association in any polymorphism of *ACE*, *ACTN3*, *AMPD1*, *CKM*, and *MLCK* in the response effect in the CMJ and the concentric mean power, even though 14 out of 38 players increased their jump height by more than 1.19 cm (SWC for jump height) without any modifications in their body weight. This leads us to confirm that these 14 participants were able to generate greater impulse (F·t) during the contact phase of the jump, which led to a higher velocity at takeoff and finally leading to a greater jump height. This is also confirmed by the higher concentric mean power observed in those same 14 participants (Figure 1). The knowledge of new variants and new profiles that affect these abilities can be found to deepen the knowledge of this new field of research until now unknown and which for the first time is presented together with genes involved in neuromuscular performance.

This investigation shows, for the first time, that genetics could be a significant factor in the predisposition to the response in terms of neuromuscular performance in amateur football players, especially in concentric mean force, showing new candidate genes associated with the etiology of the response to training and power capacities: *ACTN3*, *AMPD1*, and *MLCK*.

Despite the strengths of this investigation, this study presents several limitations: (i) the amateur standard of football players recruited for this study may well have confounded these results, as the neuromuscular performance have been influenced by lower

levels of strength/fitness and/or lower levels of scientific support in this population. Thus, the small effect of individual genetic polymorphisms (which is multifactorial even in professional football players) is more likely to be elucidated in a higher standard of football players; (ii) given the small effect size of an individual genetic polymorphism and the multifactorial nature of neuromuscular performance, the sample size is too low to provide any meaningful information regarding a genetic association with the strength/power. In the future, the authors will collect more data by significantly increasing the sample size and conducting a follow-up; (iii) this study has only presented two temporal evaluation points. This lack of intermediate evaluations hinders our understanding of whether there have been fluctuations in vertical jump capacity between measurement points and (iv) given the difference in strength and muscle mass values, future research should consider these variables as covariates and/or conduct these analyses based on the gender of the soccer players. Additionally, these potential fluctuations could be related to the training load experienced by the participants. This is also a limitation of this study, as the total training load (sRPE) suffered weekly by the participants is unknown, which could result in the different "training doses" experienced and have implications for the interpretation of our results.

After 20 weeks of training during the competitive period, there were no generalized increases or decreases in the neuromuscular performance of the lower body, measured through the CMJ test. There were positive responders to training who did increase jump height, although there was no significant relationship with any of the analyzed polymorphisms. However, positive responders in the concentric mean force exhibited a lower frequency of the AA genotype and a higher frequency of CA genotype for the c.37885C>A polymorphism of *MLCK*, as well as a lower frequency of the TT genotype of *ACTN3* and the CC genotype of *AMPD1*. These results suggest that genetic analysis could be a potential tool to anticipate individual training adaptations in amateur soccer players.

There were no changes in CMJ-derived metrics in amateur soccer players, suggesting that neuromuscular performance (at least for this performance marker) is not modified by 4 months of soccer training in amateur male and female players. Therefore, coaches and fitness trainers should include additional training methods beyond game-based training if they want to increase this neuromuscular performance marker. Individual analysis revealed that some athletes did increase jump height, power, and concentric force above the SWC. Given the variability in neuromuscular response, it is necessary to monitor the performance of soccer players individually to understand the training and recovery needs of each athlete. Genetic analyses are becoming accessible to athletes, coaching staffs, the medical staff of clubs, and sports federations. These genetic analyses allow for the identification of potential training responders, which in turn allow for the individualization and personalization of sports training. According to our findings, it is not necessary to present an optimal neuromuscular performance genotype to participate in amateur soccer categories. However, this is not the case at the high level of sport, where genetic characteristics do seem to discriminate between those who reach the highest level of competition and those who do not.

From a practical perspective, genetic analysis and individualized performance monitoring enables the personalization of training based on genetic insights and athlete-specific adaptations, representing a significant advancement in sports science. For coaches, this translates to an enhanced ability to design more effective and safer training programs tailored to the unique needs of each athlete. For athletes, this approach provides a clear roadmap to maximize their potential, improve performance, and reduce injury risk. Combining genetic data with continuous monitoring and adjustment ensures that both coaches and athletes can achieve optimal sporting performance in amateur environments sustainably and effectively, while acknowledging the limitations of such settings.

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