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Polygenic Profile and Magnitude Impact of Inflammatory Response in Soccer Athletes

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Abstract

The practice of soccer involves carrying-out actions of high intensity, which demand a great generation of eccentric strength, which in turn results in an increase in the inflammatory response after training practice and game matches. The study aimed to investigate, in combination and individually, the association of 28 polymorphisms with the inflammatory responses of soccer athletes. The sample consisted of 47 male under-20 soccer athletes who belong to clubs in the first division of Brazilian soccer. Blood samples were collected at Pre, and 03, 24, and 48 hours after the training session to evaluate the inflammatory responses (hematological analyzes (hemogram), creatine phosphokinase (CK), high sensitivity quantitative C-reactive protein (CRP), tumor necrosis factor-alpha, (TNFα), interleukin 6 (IL-6) and insulin-like growth factor 1 (IGF-1)). DNA was obtained through scraping of buccal cells, where a sterile swab was rubbed on the inner side of the mouth of each participant 06 times. The database was built using the TruSeq DNA PCRFree kit (Illumina®) and the Covaris equipment for shearing genomic DNA (gDNA) by ultrasound. Of the analyzed SNPs, 09 (*ACTN3* rs1815739, *COL5A1* rs12722, *COL5A1* rs3196378, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795, *MMP3* rs679620, *SLC30A8* rs13266634, *SOX15* rs4227) were individually associated with biomarkers and 07 SNPs, (*COL5A1* rs12722, *COL5A1* rs3196378, *COL5A1* rs1800012, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795 and *MMP3* rs679620) analyzed in combination, explained 16% to 40% of the variation of inflammatory responses in soccer athletes. The results suggest that the genotypic profile can be taken into account for a more individualized distribution of the training load, along with the elaboration of recovery strategies for high-level athletes between training sessions and games of high physical

and physiological demand.

Keywords

Polymorphism, Genotype, Soccer, Inflammation

1. Introduction

Soccer is a sport characterized by intermittent actions of high intensity and short duration, interspersed with short recovery periods [1]. It has been suggested that approximately 80% - 90% of actions in a soccer game occur at low or moderate intensity, while the remaining 10% - 20% are high-intensity actions [2] [3]. Although high-intensity actions represent a low percentage of the total number of actions in a soccer match, they are generally associated with the most decisive moments and directly contribute to the game result [4] [5]. Castagna *et al.* (2019), found an average of 19 sprints during a match, which occurred every 4 - 5 minutes. In a study analyzing intense actions of short duration, [6] found that professional soccer players cover an average of 635 m accelerating and 611 m decelerating at a high intensity (>2 m/s^2) and 214 m accelerating and 209 m decelerating at a very high-intensity (>3 m/s^2). Thus, it is noted that the practice of soccer involves carrying out actions of high intensity, which demand a high generation of eccentric strength, which in turn is related to morphofunctional alterations of the muscular apparatus [7] resulting in increased muscle damage and inflammatory response after training and game matches.

Several investigations have revealed that actions that require unusual eccentric contractions, during which the muscle is stretched while active, can cause high loss of strength, higher pain levels, and increased blood concentrations of inflammatory biomarkers compared to concentric or isometric contractions [8] [9] [10] [11] [12]. These contractions are strongly associated with skeletal muscle damage consisting of structural disruption of sarcomeres, disturbances in excitation-contraction coupling, and calcium signaling, leading to a heightened inflammatory response and activation of muscle protein degradation pathways [13] [14].

Recent publications have shown that genetic factors have a significant influence not only on components of athletic performance, but also on the activation and resolution of inflammation, muscle tissue regeneration, and other phenotypes [15] [16] [17] [18]. Differences in the genetic-specific activity of some single nucleotide genetic polymorphisms (SNP) result in different expressions of several proteins involved in the inflammatory cascade, which may influence susceptibility to exercise-induced muscle damage [14] [18]. Individuals having an increased exercise-induced inflammatory response may have a greater predisposition to injury [19] [20]. This principle may explain, in part, the reason why athletes belonging to a squad, despite similar training routines, respond

with a high rate of exercise-induced muscle injuries. Thus, considering that multiple environmental, physiological, and psychological factors influence the formation of the athlete [21], among athletes of the highest competitive level, where the environment in which they are is very similar, it may be that the genomic profile characteristic of interindividual variation is a factor of differentiation between athletes [22].

The predisposition to muscle injuries and other phenotypes such as the strength/power status of athletes are considered of polygenic nature—that is, multiple genetic factors influence the observed phenotype [23] [24]. For example, Larruskain *et al.* (2018), using a Cox multivariate frailty model, demonstrated that a polygenic profile (composed of variations in the MMP3, TNC, IL6, NOS3, and HIF1A genes) was significantly associated with the increased risk of injury to the hamstrings. A recent review provided evidence that at least 69 genetic phenotypes are linked to the elite athlete status [25]. Of these, 11 polymorphisms (*AGT* rs699, *ACTN3* rs1815739, *CKM* rs8111989, *CNTFR* rs41274853, *GBF1* rs2273555, *HIF1A* rs11549465, *MLN* rs12055409, *MTHFR* rs1801131, *PPARG* rs1801282, *PPARGC1A* rs8192678 and *ZNF*608 rs4626333) have been associated with the ability to produce force in athletes [26]-[34].

The genetic profiles for muscle and ligament damages were analyzed by obtaining information on the incidence of muscle injuries, degree, and estimated recovery time [16] [35] [36]. Some studies [37] [38] have found associations between SNP of the inflammatory cascade protein genes with several diseases, however, very little is known about the potential genetic association with the inflammatory response resulting from sports practice [14]. Thus, identifying genes that may influence the levels of pro and anti-inflammatory and pro-regenerative molecules can help elucidate the factors and mechanisms related to the inflammatory process [16]. In addition, genotyping can be an important part of the multifactorial injury model—that is, genetic information can be used in conjunction with all other risk factors to identify those athletes at high risk of injury and to individualize preventive strategies, including load control [38] [39].

It is worth mentioning that the isolated evaluation of an SNP without considering its interaction with genotypes of other mediators can lead to misinterpretations since their combined action can result in differentiated effects [14] [16]. Therefore, studies about associations between genetic factors and inflammatory responses should seek broader evaluations in the genomic context, thus enabling better interpretations regarding these associations. Nonetheless, thus far, no study has attempted to quantify the impact of combined genetic variants on inflammatory responses in high-performance athletes. The aim of the present study, therefore, was to investigate, individually and in combination, the association of 28 previously identified polymorphisms with phenotypes related to inflammation resulting from sports practice with inflammatory responses of soccer athletes.

2. Methods

2.1. Ethical Concern

This study complied with all the norms established by the National Health Council (Resolution 466/12) involving research with human beings and was approved by the Research Ethics Committee (69253417.1.0000.5149). All procedures, risks, and benefits related to the research were duly passed on to the volunteers before signing the consent form for participation in the study.

2.2. Sample

The sample consisted of 44 male under-20 soccer players with at least 6 years of experience in systematized soccer training. The athletes belong to clubs in the first division of Brazilian football and compete in competitions organized and/or recognized by the Brazilian Football Confederation (CBF).

2.3. Procedures

The study was carried out in the third week of the pre-season of the participating teams. This choice was made so that the athletes are in an equalized physical condition and so that there is not a possible inflammatory response that is very different from the real situation in case of the athletes detraining due to the vacation period before the study.

On the first day of the study to characterize the sample, the subjects underwent a physical evaluation, in which the body mass, height, and skinfolds were measured to calculate the fat percentage [40]. On the same day, the first blood collection was taken, which was used for the genomic DNA extraction. The maximum values of oxygen consumption (VO_2max) were obtained through an evaluation carried out two weeks before the beginning of the study using the field test *YoYo Endurance Test* level 2 [1]. Subjects were genotyped for the ACTN3 gene and then divided into groups according to their genotype (**Table 1**).

After sample characterization, on the second day of the study, the second blood collection was taken to obtain the baseline of inflammatory biomarkers, and one hour after that the training session began. Still on the second day of the study, three hours after training, the third blood collection was taken for biomarkers analysis. The fourth collection of blood samples was on the next day (day 03), 24 hours after the end of the training session. Finally, 48 hours after training (day 04), the fourth, and final, blood sample was taken.

Throughout the study, from the sample characterization procedures until 48 hours after the training session, the subjects were accommodated in the club's facilities, where all meals followed the guidance of a sports dietary nutritionist. Participants were not taking any medication or dietary supplement with an anti-inflammatory action for at least 02 weeks before the study. Athletes able to perform training, but still in the recovering process from recent injuries were excluded from the sample as they are constantly submitted to treatments and

Table 1. Sample characterization.

	Average
Age	19 ± 1
Weight (Kg)	75.4 ± 8.6
Height (cm)	176 ± 7.1
Fat percentage %	10.4 ± 2.3
VO2 max (ml·Kg^{-1}·min^{-1})	56.8 ± 4.4

Data is presented in mean ± SD.

recovery protocols that alter inflammatory responses. **Figure 1** illustrates the experimental design of the present study.

2.4. Training Protocol

The subjects were familiar with the proposed training [41], but did not perform these types of exercises at least 1 month before the study, despite regularly training for 02 weeks, having participated in at least 07 training sessions per week, performing both cardiovascular and resistance exercises. After the warm-up exercises, the athletes were randomly divided and did, twice, a circuit consisting of five stations with intermittent exercises combining jumps, changes of direction, accelerations, and decelerations. Staying at each station lasted 03 minutes with 30-second intervals for changing stations. The activities were carried out at maximum speed and accompanied by verbal encouragement from the coaches. The total duration of training was 45 minutes. The analysis of the training session load was performed by recording the heart rate (HR) and other variables such as the total distance covered and high-intensity actions. These variables were recorded through GPS devices integrated with the Polar brand heart rate (Polar Team Pro®, Finland). The environmental conditions (temperature and relative humidity) of the entire training session were recorded using a digital thermo-hygrometer (Instrutherm® HT-260). The data referring to the physical demand of the session and the environmental conditions of the training are in **Table 2**.

2.5. Blood Sample Collection and Buccal Scrapings

Blood collections were taken at sample characterization moments, pre-training, 03, 24, and 48 hours after training and for analysis of inflammatory responses, the maximum values obtained for each marker in their respective kinetics were used. In each collection, 4 mL of blood was collected from each athlete in an EDTA tube (catalog: 454036-Greiner®) and 24 mL (six tubes) of serum (catalog: 454071-Greiner®). EDTA samples were refrigerated at 4˚C, and serological samples were centrifuged at 3000 g for 10 minutes and refrigerated at 4˚C.

For the collection of oral cell scrapings, a sterile swab was rubbed on the inner side of the mouth of each participant 6 times, after they remained for at least 30

min without ingesting solid food.

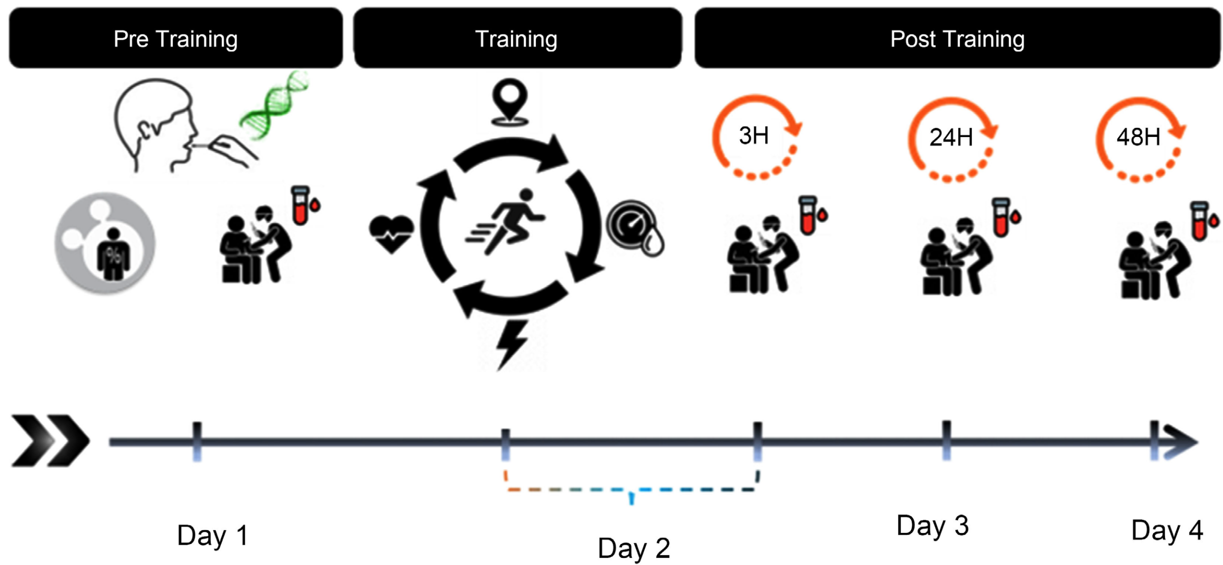

Figure 1. Experimental design. Source: Elaborated by the authors.

Table 2. Physical demand and environmental conditions of the training session.

	Average
Distance	3.102 ± 166.2
High intensity distance	160 ± 37.3
Sprints	20 ± 5
High intensity acceleration	61 ± 7
High intensity deceleration	52 ± 9
Total high-intensity actions	132 ± 18
% FC	79.8 ± 3.4

Data are presented as mean ± SD.

2.6. Hematological and Biochemical Analyses

Hematological analyzes (hemograms) were performed on the samples collected in an EDTA tube. In serum samples, biomarkers analyses were performed: creatine phosphokinase (CK), high sensitivity quantitative C-reactive protein (CRP), tumor necrosis factor alfa (TNFα), interleukin 6 (IL-6), and insulin-like growth factor type 1 (IGF-1). The hemogram was obtained by automated cell count through cytometry performed by the Roche XN® Series equipment (Roche Laboratories, Brazil). CK was measured using the ultraviolet kinetic method and CRP by turbidimetry, using the Atellica® Solution (Siemens Healthineers, Brazil). Cytokines (TNFα and IL-6) and IGF-1 were quantified by the Immulite® (Siemens Healthineers, Brazil) using chemiluminescence and radioimmunoassay methods, respectively.

2.7. Genotyping

The genetic material was extracted from the swabs using the magnetic bead technique with the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (catalog: A36570, Thermo Fisher®) according to the manufacturer's instructions. Samples were eluted with 50 μL of elution buffer and frozen at −80˚C until sequencing. All samples were evaluated for DNA quality and quantity by electrophoresis system using the tool TapeStation (Agilent®).

2.8. Whole-Genome Next Generation Sequencing (NGS)

Genomic libraries were constructed from 1 µg of DNA from each participant using the TruSeq DNA PCRFree Kit (Illumina®) and the Covaris equipment for shearing genomic DNA (gDNA) by ultrasound. Construction of the library began with the fragmentation of the gDNA to obtain nucleic acid fragments with adapters attached at both ends. Each sample had a unique index combination (a unique set of sequences in the adapter that allows data to be assigned to the indicated sample). The P5 and P7 sequences of the adapters were complementary to the oligos bound to the flow cell surface. In addition, the RD1 and RD2 SP sequences from the adapters were the primers used to initiate sequencing. The libraries were then mixed and prepared for the start of sequencing (cluster clonal amplification). Each cluster represents thousands of copies of the same strand of DNA at one point. Cluster generation took place in a flow cell (thick glass slide with channels randomly coated with oligos that are complementary to the library adapters). After denaturation, the single-stranded molecule flips over and forms a bridge by hybridizing with the adjacent complementary primer. The bridge amplification cycle was repeated until several bridges were formed. Then, sequencing by synthetic chemistry on the NovaSeq 6000 platform (Illumina, USA) took place with an average depth of 44*. Sequencing was previously analyzed in specific software using parameters such as number of reads, polyclonality, quality of reads and size histogram for evaluating samples and controls.

2.9. Bioinformatics Analysis

The mapping of the reads and assembly of the genome based on the GRCh38 version of the human genome was performed using the DRAGEN Germline App v3.7.5. platform available from the base space cloud archive (Illumina, USA). From files in FastaQ format, files in bam format were then obtained containing the sequenced genome, aligned, and assembled regarding the reference genome. To observe the variants of GRCh38, the variants were called using the *DRAGEN™ variant caller* tool to obtain files in vcf format.

Using the *Integrative Genomics Viewer* (IGV) tool (Broad Institute, University of California, USA) the genotypes of the SNPs chosen for each of the study participants were extracted.

2.10. Statistical Analysis

All analyses were performed using R Studio version 3.5.2. The Hardy-Weinberg equilibrium was used to check whether the genotype frequencies were in equilibrium. Subsequently, the assumptions of normality were verified using the Kolmogorov-Sminorv test. For descriptive statistics, minimum, maximum, mean, and standard deviation values were used.

The SNPassoc package [42] was used to verify the association between a polymorphism and the phenotype through dominance, codominance and recessiveness models. To verify the association between inflammatory biomarkers and polymorphisms in a combined way, multiple linear regression was used. The adjusted coefficient of determination (R^2) was used as a measure of explained variance. To evaluate the effect of the independent variables on the dependent variables, the f^2, effect size was used, classified as small ($0.02 < f^2 \leq 0.15$), medium ($0.15 < f^2 < 0.35$), and large ($f^2 > 0.35$) [43] [44]. Post-hoc power analysis was calculated for each generated regression model [43] [45]. The significance level adopted was $p < 0.05$.

3. Results

In this work, 28 polymorphisms previously identified in the literature with phenotypes related to inflammation resulting from sports practice were obtained and evaluated. **Table 3** presents the list of the 28 pre-selected SNPs and the Hardy-Weinberg equilibrium used to verify their allele frequency and distribution. Only two SNPs showed deviation from equilibrium (*CCL2* rs5857656 and *MMP3* rs 679620).

The association between genotypes and phenotypes was verified from the maximum values obtained in each blood biomarker and the SNP individually and in combination, generating codominance models; except the ACTN3 polymorphism with which a recessive model was generated. Of the analyzed SNPs, 09 (*ACTN3* rs1815739, *COL5A1* rs12722, *COL5A1* rs3196378, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795, *MMP3* rs679620, *SLC*30A8 rs13266634, *SOX15* rs4227) were associated with biomarkers (**Table 4**).

Seven SNPs (*COL5A1* rs12722, *COL5A1* rs3196378, *COL5A1* rs1800012, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795 and *MMP3* rs679620) in combination, explained 16% to 40% of the variation in inflammatory responses in soccer players (**Table 5**). The *HGF* rs5745697, *COL5A1* rs12722 and *COL5A1* rs3196378 SNPs together explained 22% of the variation in neutrophil activity ($R^2 = 0.22$, $F = 3.37$, $p = 0.01$, $f^2 = 0.28$, Power = 0.81). In combination, the IL-6 rs1800795 and MMP3 rs679620 SNPs explained 16% of monocyte activity ($R^2 = 0.16$, $F = 2.41$, $p = 0.04$, $f^2 = 0.19$, Power = 0.71). The *COL5A1* rs12722 and *COL5A1* rs319637 SNPs together explained 22% of the inflammatory response through IL-6 ($R^2 = 0.22$, $F = 6.92$, $p < 0.01$, $f^2 = 0.28$, Power = 0.87). Finally, combined, the SNPs *COL1A1* rs1800012 and IGF1 rs35767 explained 88% of TNFα expression ($R^2 = 0.40$, $F = 4.77$, $p < 0.01$, $f^2 = 6.76$, Power = 0.99).

Table 3. Pre-selected SNP and Hardy-Weinberg equilibrium.

HWE balance			
SNP	**Alleles**	**Allele Frequency**	**HWE**
ACTN3 rs1815739	C/T	61.4	1.000
CCL2 rs2857656	G/C	58	0.03*
CCL2 rs3917878	C/T	94.3	1.000
CK-M rs1803285	C/A	98.9	1.000
COL1A1 rs1800012	C/A	86.4	1.000
COL5A1 rs12722	C/T	65.9	0.74
COL5A1 rs3196378	C/A	67.4	0.49
EMILIN1 rs2289360	T/C	54.5	0.36
HGF rs5745697	G/T	90.9	0.29
HIF1A rs11549465	C/T	95.5	1.000
IGF1 rs35767	G/A	72.7	0.25
IGF2 rs3213221	C/G	53.4	0.77
IL-1 rs1143634	G/A	79.5	0.66
IL-6 rs1800795	G/C	73.9	0.13
LIN28A rs6598964	G/A	75	0.70
MLC rs2700352	G/A	80.7	1.000
MMP3 rs591058	C/T	71.6	0.46
MMP3 rs650108	G/A	61.4	0.35
MMP3 rs679620	C/T	64.8	0.04*
SLC26A10 rs11613457	G/A	98.9	1.000
SLC30A8 rs13266634	C/T	84.1	0.57
SOD rs4880	A/G	50	1.000
SOX15 rs4227	T/G	61.4	1.000
SPP1 rs28357094	T/G	87.5	0.51
TNC rs2014772	C/T	76.1	0.68
TNFα rs1800629	G/A	95.5	1.000
TRIM63 rs2275950	A/G	81.8	0.61
VEGFA rs2010963	G/C	55.7	0.77

Caption: SNP = polymorphism; HWE = Hardy-Weinberg equilibrium; *SNP out of HWE.

Table 4. SNPs individually associated with biomarkers.

SNP + A2:G34	Biomarker	Model	N	Average ± SD	p	AIC
ACTN3 rs1815739	IGF1	R/R R/X	38	19.18 ± 1.01	0.02	286.3
		X/X	6	13 ± 1.51		
COL5A1 rs12722	Neutrophils	C/C	18	4860 ± 399.6	0.02	765.6
		C/T	22	3679 ± 243.5		
		T/T	4	3373 ± 319.5		
		C/C	18	4.33 ± 0.39		

Continued

	IL-6	C/T	22	4.23 ± 0.37	0.02	271.9
		T/T	4	12.35 ± 4.10		
		C/C	18	4.32 ± 0.40		
COL5A1 rs3196378	IL-6	C/A	22	4.22 ± 0.43	0.01	262.9
		A/A	3	15.10 ± 3.72		
		G/G	37	3866 ± 199.8		
HGF rs5745697	Neutrophils	G/T	6	5971 ± 743.6	0.01	761.0
		T/T	1	3048 ± 0		
		C/C	25	7.91 ± 0.26		
IGF1 rs35767	TNFα	C/T	14	8.63 ± 0.41	0.01	236.9
		T/T	5	15.76 ± 4.46		
		G/G	26	432.62 ± 21.36		
	Monocytes	C/G	13	469.90 ± 39.75	0.04	556.3
IL-6 rs1800795		C/C	5	588.10 ± 78.15		
		G/G	26	384.30 ± 31.79		
	CK	C/G	13	433.72 ± 69.52	0.01	597.5
		C/C	5	698.81 ± 119.34		
		C/C	15	432.13 ± 23.23		
MMP3 rs679620	Monocytes	T/C	27	491.2 ± 27.98	0.04	556.2
		T/T	2	277.00 ± 9.00		
		G/G	27	1.34 ± 0.25		
SLC30A8 rs13266634	C-reactive protein	A/G	16	1.84 ± 0.44	0.01	164.5
		A/A	1	5.30 ± 0		
		T/T	16	16.12 ± 1.5		
SOX15 rs4227	IGF1	G/T	22	21.14 ± 1.32	0.01	283.2
		G/G	6	14.00 ± 0.37		

Caption: SNP = polymorphism. Data are presented in mean ± standard error (SE).

Table 5. Combined analyses of SNPs and biomarkers.

Biomarker	SNPs	R^2 adjusted	F	p	f^2	Power
Neutrophils	*HGF* rs5745697 *COL5A1* rs12722 *COL5A1* rs3196378	0.22	3.37	0.01	0.28	0.81
Monocytes	*IL-6* rs1800795 *MPP3* rs679620	0.16	2.41	0.04	0.19	0.71
IL-6	*COL5A1* rs12722 *COL5A1* rs3196378	0.22	6.92	<0.01	0.28	0.87
TNFα	*COL5A1* rs1800012 *IGF1* rs35767	0.40	7.44	<0.01	0.67	0.99

Caption: SNPs = polymorphisms.

4. Discussion

The present study aims to verify the influence of polymorphisms on inflammatory responses, individual and in combination, after a training session with the predominance of eccentric actions in soccer athletes. In summary, the results showed a significant association between *ACTN3* rs1815739, *HGF* rs5745697, *COL5A1* rs12722, *IL-6* rs1800795, *MMP3* rs679620, *SOX15* rs4227, *COL5A1* rs3196378, *IGF1* rs35767 and *SLC30A8* rs13266634 polymorphisms (individually and in combination) and inflammatory responses in soccer athletes after a training session.

The association between the polymorphisms with the phenotypes in the present study sought to identify in soccer athletes if the magnitude of the inflammatory responses can be explained in part by genetic factors. For instance, it is already well documented in the literature that genetic factors influence the concentrations of cytokines and other biomarkers involved in the inflammatory cascade and, consequently, in the activation and resolution of inflammation [14] [16]. Thus, the initial hypothesis considered that polymorphisms previously related to the inflammatory process (individually and in combination) would be associated with the inflammatory response in soccer players. In the present investigation, of the 28 SNPs analyzed, 09 were analyzed individually (*ACTN3* rs1815739, *COL5A1* rs12722, *COL5A1* rs3196378, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795, *MMP3* rs679620, *SLC30A8* rs13266634, *SOX15* rs4227) and 07 in different combinations (*COL5A1* rs12722, *COL5A1* rs3196378, *COL5A1* rs1800012, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795 and *MMP3* rs679620), showed significant associations with the magnitude of the inflammatory response.

4.1. ACTN3 rs1815739

In the results presented here, an isolated association of the SNP *ACTN3* rs1815739 with the maximum values of IGF-1 was observed. Of all the polymorphisms that have been associated with muscle damage and exercise-induced inflammatory responses, the most investigated is the SNP of *ACTN3* R577X [41] [46] [47]). North *et al.* (1999), identified in the *ACTN3* gene, the change from nucleotide C to T at position 1747 of exon 16, that is, a mutation resulting in the conversion of the amino acid arginine into a premature stop-codon in the residue 577 (R577X) [48]. The R577X variant results in two versions of ACTN3 gene in humans, a functional R allele and a null one that has the XX genotype. The individuals homozygous for the X allele are unable to express *α*-actinin-3, as opposed to individuals who have the RX or RR genotype [49] [50]. Interestingly, individuals with the ACTN3 XX genotype compensate for the *α*-actinin-3 deficiency with a higher expression of *α*-actinin-2 [17]. It is already well established in the literature that the ACTN3 XX genotype is associated with lower muscle volume and lower muscle strength [41] [51]. Furthermore, it is believed that individuals who did not express the *α*-actinin-3 protein demonstrated a shift in the

properties of fast twitch muscle fibers towards a more oxidative profile, lower muscle force production, reduced muscle mass, and reduced diameter of type IIb fibers [52] [53].

Seto *et al.* (2011) reported that as a consequence of α-actinin-2 overcompensation in XX homozygous individuals, more calsarcin-2 is bound to α-actinin-2 and less calcineurin. The binding affinity of calsarcin-2, which, in turn, functions as an inhibitor of calcineurin activation, is higher for α-actinin-2 compared to α-actinin [17] [54]. Thus, a higher level of free calcineurin can activate a signal for a slower myogenic process. Although calcineurin-dependent pathways are implied in muscle growth and adaptation to functional overload [55] [56], the α-actinins also interact directly with soluble signaling factors, phosphatidylinositol 3-kinase (PI3K), phosphatidylinositol 4,5-bisphosphate, and phosphatidylinositol 3,4,5-trisphosphate [57] [58]). All of these factors boost downstream pathways that regulate several cellular functions, including the PI3K/Akt/mTOR signaling cascade that regulates protein synthesis, cell proliferation, and protection of cells against apoptosis, as well as the increase of IGF-1 production [59] [60].

These data suggest that α-actinin-3 deficiency alters the regulation of muscle mass, modifying at the molecular level the signaling for protein synthesis and degradation, which may negatively influence training adaptations and the remodeling process of muscle tissue after high physical and physiological demand stimuli such as training and game matches. In this study, XX individuals for the SNP *ACTN3* rs1815739 had lower IGF-1 values compared to individuals carrying the R allele. A possible explanation for this may be the increase in circulating calcineurin in XX homozygotes, which, as previously mentioned, negatively influences the signaling of several important processes for tissue repair, including the decrease in IGF-1 concentrations.

4.2. COL5A1 rs12722

Collagens are a group of extracellular matrix proteins, and they are the most abundant proteins in mammals, constituting about 25% to 35% of the protein content of the whole body. Collagen, in the form of elongated fibrils, is found primarily in connective (fibrous) tissues such as tendons, ligaments, and skin and is abundant in the cornea, cartilage, bone, blood vessels, intestine, and intervertebral disc [55]. Specific SNPs of the COL5A1 gene (rs12722, rs1800012 and rs3196378) have been associated with the prevalence of ligament and tendon injuries (for example, tendinopathy/Achilles tendon rupture and anterior cruciate ligament rupture) [61] [62] [63]. When stratified by ethnicity, the pooled results indicated that the rs12722 polymorphism was significantly associated with soft tissue damage in Caucasians but not Asians [64]. However, very little is known about the mechanisms supporting this association and the potential genetic association of these SNPs with inflammatory responses in response to sports practice [65].

Massidda *et al.* (2015) identified that the TT genotype for the SNP rs12722 of

COL5A1 increases the risk of musculoskeletal injuries in Italian soccer players, and other studies have found a relationship between the C allele and resistance to soft tissue damage [66]. The results suggest that individuals who carry the C allele have a protective mechanism against muscle damage. Analyzing in isolation, the results presented here indicated that TT individuals stimulate less neutrophil activity and higher values of IL-6 compared to individuals carrying the C allele. However, for both biomarkers, the rs12722 variant significantly interacted with other SNPs (*COL5A1* rs3196378 and *HGF* rs5745697) and together explained 22% of the variations in neutrophil and IL-6 concentrations (**Table 5**). In the present study, the low number of cases of TT individuals is a limiting factor, requiring further studies with a more representative sample of the analyzed genotypes. It is possible that the C allele positively influences the properties of collagen tissue due to the influence of this SNP on the stability of the *COL5A1* mRNA, where the C allele is associated with a higher production of type V collagen than the T allele and may contribute to higher levels of resistance to traction and stiffness of collagen fibrils in skeletal muscle.

However, despite the T allele being associated with the severity of the musculoskeletal injury, higher risk of tendon injuries, and higher risk of anterior cruciate ligament (ACL) injury [65], Hall *et al.* (2021), reported that in athletes who have not yet passed puberty, the C allele of *COL5A1* rs12722 and the CC homozygotes suffered relatively more muscle and ligament injuries, respectively, compared to individuals carrying the T allele. This indicates that the association of genetic factors and muscle and ligament injuries may be dependent on the athlete's maturational status [67].

Heffernan *et al.* (2017), studied two SNPs in the *COL5A1* gene (rs12722 C/T and rs3196378 C/A) associated with tendon and ligament pathologies in 1105 rugby athletes and non-athletes. For rs12722, the injury-protective CC genotype and the C allele were more represented in athletes than non-athletes ($p < 0.01$). Similarly, for rs3196378, the CC genotype and the C allele were more common in athletes compared to non-athletes ($p = 0.02$). More significantly, when considering the two SNPs together, the combinations of inferred alleles were higher in the group of athletes (homozygous athletes CC = 18% and athletes who carry the C allele = 43%) than non-athletes (non-athletes homozygous CC = 13% and non-athletes who carry the C allele = 40%; $p < 0.01$) [68]. These data indicate that the C alleles, CC genotypes and the resulting haplotypes of both rs12722 and rs3196378 appear to be advantageous for athletes and may even provide a protective effect against soft tissue injuries despite exposure to the high-risk environment of high-performance sport [54] [68].

The *COL1A1* gene has been frequently investigated for its potential association with the risk of musculoskeletal, tendon and joint injuries. Studies suggest that there are associations of COL1A1 rs1800012 with chronic joint injuries [54]. Specifically, the rare TT genotype of the rs1800012 variant was associated with a decreased risk of acute injuries, including shoulder dislocations [69]. ACL rupture [70] and soft tissue rupture in combined analysis with other genetic factors

[71]. In this context, Wang *et al.* (2017) reported the association of the rs1800012 TT genotype with a reduced risk of ligament and tendon injuries related to sports practice. In contrast, the rs1800012 TT genotype was associated with an increased risk of lumbar disc herniation in young military recruits [72]. Furthermore, the GG genotype was associated with a reduced risk of ACL ruptures suffered during skiing [73] [74], however, the mechanisms that result in these injuries are proposed to be different from those that result in ACL ruptures sustained during land-based activities. In contrast to these associations, several studies failed to associate these variants with the risk of musculoskeletal soft tissue injuries [75]. In the present study, the SNP *COL1A1* rs1800012 individually was not associated with any biomarker, although, together with the SNP IGF-1 rs35767, they explained 40% of the expression of TNFα. The difference in the results of the studies can be explained by the differences in the studied populations, the physical conditioning levels of the subjects, and the investigated sports modalities. To date, this is the first study to globally analyze such SNPs and the magnitude of inflammatory responses through blood biomarkers.

4.3. HGF rs5745697

Hepatocyte growth factor (HGF) plays a role in regulating satellite cell proliferation and differentiation [76] and is encoded by HGF gene [54]. Active HGF is present within the extracellular matrix of the muscle [77] and for there to be migration of satellite cells to the injured muscle, HGF must signal with its receptor (Met protein) [78]. Due to the specific role of HGF in response to muscle injury, it could be considered an appropriate candidate gene for associations with inflammatory responses and injuries resulting from sports practice. However, to date, no study has sought to identify associations between HGF gene SNPs and inflammatory responses resulting from sports practice, and only one study has investigated the association between HGF gene SNPs and sports injuries [79]. In this investigation, the authors found associations between the SNP HGF rs5745697 non-contact muscle injuries, and recovery time in a sample of 73 elite soccer players. The results showed that athletes carrying the GG genotype for the rs5745697 SNP had a lower injury rate compared to those carrying the T allele (8.4 and 12.3/1000 h, respectively).

In addition, *HGF* rs5745697 was associated with recovery time from muscle injuries, where players carrying the rs5745697 G allele had a mean injury-related absence of 20.7 days compared to 27.5 days for TT homozygotes. Currently, there is little functional explanation for the associations described, and the authors cite previous research and suggest that an established interaction between HGF and its receptor allows for correct signaling in the cascade of the inflammatory process, resulting in the proliferation and migration of satellite cells and that the absence or reduction in the activity of the HGF and its receptor together (Met) leads to inadequate repair of skeletal muscle tissue [79]. Furthermore, HGF seems to play an important role in the interaction between the extracellular

matrix of muscle cells and the migration of myoblasts Nonetheless, the exact mechanism by which HGF gene SNPs may influence muscle injuries in soccer players remains unclear [14].

In the present study, the *HGF* rs5745697 SNP was associated (individually and in combination with other SNPs (*COL5A1* rs12722, *COL5A1* rs3196378) with blood neutrophil concentrations. The results suggest that GG subjects have lower neutrophil activity after a training session with a predominance of eccentric actions compared to individuals carrying the T allele. As previously mentioned, together with SNPs of the *COL5A1* gene, *HGF* rs5745697 explained the variation in blood concentrations of neutrophils by 22%. A possible explanation in relation to HGF and neutrophil activity is the theory that neutrophils, monocytes, and macrophages are involved in clearing away dead and damaged cells, and in signaling satellite cells to begin differentiating and rebuilding damaged muscle fibers. It is important that these processes occur accurately for successful muscle regeneration. It is worth noticing that in the sample analyzed here, only one athlete with TT genotype for the SNP *HGF* rs5745697 was observed, which limits the interpretation of the data. Future work should seek to confirm these associations in similar populations with more representative samples of the genotypes involved and investigate the molecular consequences of this and other *HGF* SNPs.

4.4. IGF1 rs35767

It is already well established in the literature that IGF-1 plays a fundamental role in the differentiation and activation of satellite cells and, consequently, in the processes of hypertrophy and repair of skeletal muscle tissue. The IGF-1 receptor (IGF1R) is a transmembrane receptor that regulates the effects of IGF-1. Animal model studies have shown that mice lacking IGF1R show a dramatic reduction in body mass, indicating the strong influence of this receptor on the growth and development of body mass (more specifically, skeletal muscle mass) [80]. Ben-Zaken *et al.* (2013), in a study with strength and power athletes, found that the T allele of the IGF1 SNP rs35767 was more frequent in high-level Israeli athletes (international and Olympic level) compared to national-level athletes, the T allele (rs35767 C/T) was found to be associated with higher levels of circulating IGF1, and possibly with increased muscle mass and optimized muscle recovery process. These results are not in agreement with the data presented here. Despite what has been observed, in general values, a higher average of IGF-1 for TT individuals (20.3 μg/L against 16.7 μg/L and 18.4 μg/L for CC and CT individuals, respectively), there was no significant association of the IGF1 rs35767 SNP with the concentrations of IGF. The number of athletes with the TT genotype in the sample was low, which may have been a limiting factor for the analyses [81].

Nonetheless, in our study, rs35767 showed a significant association with TNFα concentrations, both in the individual analysis, where TT individuals had

higher TNFα concentrations compared to CC and CT individuals, and in the analysis together with other genetic factors, where the SNP *IGF1* rs35767 together with *COL1A1* rs1800012, explained 40% of the variations in TNFα concentrations. The association of the SNP *IGF1* rs35767 with TNF-α and the biological effects on the inflammatory responses resulting from exercise still need clarification. To date, no studies were found that described the simultaneous relationship of the SNPs *IGF1* rs35767 and *COL1A1* rs1800012 with phenotypes related to sports practice [81].

In this context, considering that genes influence the production of their corresponding proteins, [82] found a direct relationship between IGF-1 and collagen in the first stages of tissue repair. IGF-1 promotes collagen synthesis by osteogenic cells, increasing bone matrix formation [83]. In humans, it is supposed that, even without alterations in the plasmatic concentrations, the local liberation of IGF-1 is responsible for the anabolic effect of exercise on the bone tissue [84]. New studies with a larger number of athletes could confirm the associations found here and may provide a better physiological understanding regarding the association between collagen and inflammatory responses after physical training.

4.5. IL-6 rs1800795

The *IL6* rs1800795 (-174 G/C) polymorphism appears to alter the transcriptional response. There is a genetically determined difference in the degree of IL-6 response to stressful stimuli between individuals, and the C allele is associated with significantly lower plasma concentrations of IL-6. Different studies have investigated the rs1800795 SNP in male Caucasian elite athletes (endurance and power athletes) and a control group of athletes. The frequencies of the GG genotype and the G allele were significantly higher in athletes from power sports compared to endurance athletes and the control group [85] indicating that the G allele could favor sporting performance in sports that demand great production of strength and power, such as soccer. Not consistent with these results, [86] reported that there were no differences in allele and genotype frequencies of the *IL6* rs1800795 polymorphism between elite athletes in endurance and power modalities and a control group of non-athletes (Israeli population).

In the present investigation, individually, *IL6* rs1800795 was associated with monocyte activity and CK values. Individuals with the CC genotype had higher monocyte activity ($p < 0.01$), and the presence of the G allele was associated with lower CK values ($p < 0.01$). After tissue damage, monocytes migrate to the injured tissue and differentiate into macrophages or dendritic cells [87]. Thus, higher monocyte activity indicates increased muscle damage in the active musculature [88] and, in turn, generates increased CK concentrations.

In agreement with our findings, studies in young individuals demonstrated that carriers of the C allele of the SNP rs1800795, presented higher CK values after eccentric exercise compared to GG homozygotes [89] [90]. In sports that

demand high muscle power, where muscle damage is high in training and competition, individuals who carry the G allele may benefit from faster recovery and low-grade chronic systemic inflammation. However, [91] who observed no association between the SNP *IL6* rs1800795 and rhabdomyolysis in response to exercise, challenge this conclusion. Thus, the influence of *IL6* rs1800795 on the inflammatory responses resulting from physical training is not entirely clear and needs further investigation, particularly in studies with a larger number of athletes and other sports.

4.6. MMP3 rs679620

Matrix metalloproteinases (MMP) play an important role in maintaining the functional integrity of muscle fibers through the degradation of extracellular matrix components, as well as in processes such as the regulation of migration and differentiation of satellite cells and regeneration of skeletal muscle cells [92] [93] [94]. An optimal balance between collagen deposition and degradation is important for scar tissue formation [92] and contributes to muscle stiffness [95] which supposedly increases tolerance to subsequent injuries [96]. Variations in the expression of the MMP gene are linked to several diseases, such as muscle pathologies [97], for, supposedly, these variations are capable of altering the function of the extracellular matrix and influencing the rate of muscle regeneration [98].

The rs679620 SNP of the *MMP3* gene is a mutation characterized by a T > C substitution with high linkage disequilibrium to the rs3025058 variant, a functional polymorphism located within the promoter region of the gene [99]. It is believed that the *MMP3* C allele rs679620 decreases MMP3 transcription [100] However, the activity that alleles promote seems to be population and tissue dependent [101]. There is no clear picture of whether the replacement of rs679620 T > C results in increased or decreased MMP3 expression [102] [103].

MMPs are produced by several types of cells including neutrophils, fibroblasts, and monocytes/macrophages [104]. This concept may help explain, in part, the results found here, which demonstrated associations between the *MMP3* SNP rs679620 and monocyte activity both in the individual analysis ($p = 0.04$) and in the combined analysis, where together with the *IL6* SNP rs35767, the generated regression model explained 16% of the variation in monocyte concentrations ($F = 2.41$, $p = 0.04$, $f^2 = 0.19$, Power = 0.71). We observed that individuals carrying the C allele had higher concentrations of monocytes than the TT homozygote ($p = 0.02$). Considering that a greater activity of neutrophils is related to a greater MMP3 production [105], our results contradict the findings of [100]. It is important to point out that in the present study, the frequency of individuals with the TT genotype was low (N = 2). Furthermore, plasma MMP3 concentrations were not used as an inflammatory marker, and the *MMP3* SNP rs679620 was not within the HWE, and these factors limit the interpretation of the data.

The few existing studies with the rs679620 SNP, refer mainly to the risk of tendon injury [106] [107], although it has been considered a role in muscle injury. The conclusions that emerge from the investigations of the rs679620 variant concerning sports injuries still show divergences. Pruna *et al.* (2017), in a study with 74 elite soccer players, found no association between the *MMP3* rs679620 SNP and non-contact muscle injuries. The study was carried out during 5 competitive seasons and the authors observed a total of 220 muscle injury [35]. On the other hand, [16] reported a significant association between the SNP rs679620 and tendon injuries in high-level soccer players, 160 tendon injuries were investigated in a sample of 107 players. These findings could be interpreted to suggest that elevated MMP3 expression, through the presence of both T alleles, could double the risk of hamstring tendon injury. Nevertheless, to date, these are the only existing studies on sport-related muscle injuries and *MMP3* gene SNPs, and the suggested physiological mechanisms for the reported associations remain speculative. More evidence on *MMP3* SNPs and the inflammatory process is needed before considering the gene as a marker related to athletes' inflammation patterns and increased injury risk.

4.7. SLC30A8 rs13266634

The immune system and macrophage function depend on zinc during an inflammatory event. Zinc homeostasis is regulated by the family of metal transporters, such as the SLC30A8 family [108]. The solute transporter family 30 (zinc transporter) protein gene member eight (SLC30A8) is expressed primarily in pancreatic islet beta cells and transports zinc from the cytoplasm into intracellular vesicles, which is crucial for the processes of crystallization, storage, and insulin secretion [109]. The *SLC30A8* rs13266634 SNP is associated with decreased systemic fasting insulin and attenuation of insulin secretion in response to glucose [110] [111].

Insulin signaling increases blood flow and protein synthesis at rest, and reduces catabolism after exercise, thus, improving net muscle protein balance by increasing amino acid availability [112]. In addition, exercise-induced muscle damage has been associated with a reduction in glucose synthesis and absorption [113] [114]. Probably, this occurs because muscle damage reduces muscle sensitivity to insulin [115], which, in turn, could be related to increased TNF expression attenuating insulin signaling, subsequently inducing insulin resistance in the skeletal muscle [116] by suppressing the activation of glucose transporter type 4 (GLUT-4) in muscle fibers [117].

The TT genotype of an SNP (C > T; rs13266634) within the *SLC30A8* gene was associated with lower CK plasma concentrations, lower pain levels, and attenuated myoglobin levels after muscle damage induced by eccentric exercise (elbow flexion) in physically active male individuals, apparently, the C allele can reduce zinc function leading to disturbed macrophage function and reduced insulin production. By increasing the catabolic pathway, lower insulin levels can

lead to a negative net protein balance [118]. Therefore, these results indicate that carriers of the C allele of the SNP *SLC30A8* rs13266634 may need longer recovery periods after strenuous exercise.

In the present study, we have found a significant association between rs13266634 and CRP concentrations ($p = 0.01$). Athletes with the C allele had lower CRP values compared to the TT genotype ($p < 0.01$). A hypothesis that supports these findings would be the relationship between CRP and the activity of immune system cells in the opsonization process after muscle damage. As an acute-phase protein, CRP is widely used in the context of muscle status analysis and can present elevations of up to 1000 times in inflammatory processes. This elevation occurs mainly due to its functionality as an opsonin binding to the chromatin of injured cells so that mainly neutrophils and macrophages (in the $LyC6^{pos}$ isoform) can engulf and phagocytose the damaged structure without exacerbating the microinjury [119] [120]. Thus, considering that lower CRP signaling may represent lower phagocytic activity, individuals with the TT genotype may present a protective factor against muscle damage after training with high physiological demand. However, in our sample, we observed only one individual with the TT genotype, which once again presents itself as a limiting factor in the interpretation of the results presented here. Other studies, with new blood markers and higher genotypic frequencies for *SLC30A8*, should investigate whether insulin production dependent on the *SLC30A8* genotype is associated with acute and chronic adaptations related to sports practice involving muscle protein synthesis and muscle hypertrophy, respectively.

4.8. SOX15 rs4227

The HMG-box protein (Sox15) which is encoded by the *SOX15* gene is inferred to assume an important role in myogenic differentiation, determining the fate of skeletal muscle cells during development [121]. A few *SOX15* polymorphisms have been identified, although it remains unclear whether these influence skeletal muscle function or muscle injury. The rs4227 SNP involves a G > T substitution, where the T allele represents the ancestral allele and the G is the minor allele. The functional and molecular consequences of this substitution are unclear. However, Pruna *et al.* (2017) reported an association between the rs4227 SNP and the incidence of muscle injuries, expressed per 1000 h of exposure to soccer (training and games). Athletes homozygous for the ancestral T allele (TT genotype) had the lowest incidence of lesions (7.8 lesions per 1000 h) compared to carriers of the G allele (10.2/1000 h and 14.8/1000 h for the GT and GG genotypes, respectively). While a mechanistic explanation remains unclear, Pruna *et al.* (2017) suggest in their results an indication that the T allele is necessary for the proper formation of skeletal muscle and that GG homozygous individuals have higher rates of injury due to a negative effect or inactivation of the SOX function. On the one hand showed that the muscles of rats with normal SOX15 action showed normal skeletal muscle development, on the other hand, cross-

sectional images of the skeletal muscle indicated a less pronounced extracellular matrix in rats with SOX15 deficiency. Although this theory is based on the suggestion that SOX15 inactivation decreases myogenic cell proliferation and disrupts muscle regeneration, further studies are needed to test the hypothesis [35].

Results of the present investigation have shown an association of the SNP *SOX15* rs4227 with IGF-1 concentrations. In general values, TT and GT subjects had higher concentrations of IGF-1 compared to GG subjects (16.12 μg/L and 21.14 μg/L against 14.00 μg/L respectively); however, only the GT genotype showed a significant difference to the GG genotype ($p < 0.01$). As previously discussed, assuming that IGF-1 directly influences the differentiation and activation of satellite cells for tissue repair, these findings may contribute to the suggestion by Pruna *et al.* (2017) that indicates that the T allele is related to a lower risk of injury due to SOX15 which in turn contributes to better muscle building. Therefore, the most appropriate muscle development mechanism through SOX15 activation may be related to higher plasma concentrations of IGF-1. However, future studies should seek to confirm this and other associations, to bring more mechanistic and physiological clarification of the effects of the SNP rs4227 on skeletal muscle [35].

Using genomic panels based on genetic polymorphisms still has somewhat controversial results [122]. The fact that the selected genes are not random, but selected from previous data regarding the phenotypes, justifies a deeper investigation. However, the high density of SNPs in the human genome and the possibility that many of them are in linkage disequilibrium and do not segregate independently make each of the SNPs panels represent distinctive haplotypes of many genetic markers simultaneously, making their application greater than the data alone show. Thus, it is expected that using genomic panels that are increasingly accurate and improved by new genomic technologies for massive data generation, such as Next Generation Sequencing, can bring exercise science closer to a real and efficient application of precision medicine in their sports results.

5. Conclusions

Individually, 09 SNPs (*ACTN*3 rs1815739, *COL5A1* rs12722, *COL5A1* rs3196378, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795, *MMP3* rs679620, *SLC30A8* rs13266634, *SOX15* rs4227) were significantly associated with inflammatory biomarkers. In combination, seven SNPs (*COL5A1* rs12722, *COL5A1* rs3196378, *COL5A1* rs1800012, *HGF* rs5745697, *IGF1* rs35767, *IL-6* rs1800795 and *MMP3* rs679620) explained, in four models, from 16% to 40% of the variation of inflammatory responses in soccer athletes.

Even though the mechanisms supporting the genetic associations of SNPs related to collagen production with inflammatory responses in response to sports practice are unclear, our results identified several associations involving the SNPs *COL5A1* rs12722, *COL5A1* rs3196378 and *COL5A1* rs1800012. Individu-

ally SNPs *COL5A1* rs12722 and COL5A1 rs3196378 were associated with peak concentrations of IL-6 and neutrophils. In combination, the collagen-related SNPs (*COL5A1* rs12722, *COL5A1* rs3196378 and *COL5A1* rs1800012) appeared as predictors in three of the four generated models. The SNP COL1A1 rs1800012 combined with the IGF1 rs35767, explained in the most robust model presented here, 40% of TNFα expression in soccer athletes. These results indicate that these polymorphisms may play an important role in the inflammatory cascade after sports practice. However, further studies are needed to confirm these associations and to elucidate the physiological processes that support these associations.

Such evidence can be used in the future for building an improved genetic panel that helps to understand the inflammatory demand of athletes for assisting the technical commissions in the decision-making for an optimized load control and elaboration of recovery strategies in a clear application of precision medicine concepts in sport science. Our objective was to bring a closer look at the influence of genetic factors on phenotypes related to the inflammatory process, notwithstanding, the inclusion of non-genetic variables (such as age, body composition, etc.) can improve the models presented here.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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