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Short Communication

Genetic Polymorphisms and Muscle Injuries among Italian Soccer Players

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Abstract

Introduction: Skeletal muscle injuries sustained during sport and their subsequent functional recoveries seem to be influenced by interactions between environmental stimuli (training, overload/overtraining, wrong techniques) and specific genotypes. Variations in DNA sequences in specific genes have been associated with specific phenotypes involved in athletic performance, including individual susceptibility to injury.

Aim: The aim of this pilot study was to analyze the influence of five genetic polymorphisms (COL5A1 rs12722, MTC1 rs1049434, VDR Apa I, VDR Bsm I, and VDR Fok I), to the development of skeletal muscle injuries among soccer players.

Methods: Sixty-four young male soccer players from three teams participating at the Official Italian Football Championship were recruited during the 2012-2013 season. The sample was genotyped for 5 SNPs (Single Nucleotide Polymorphisms) and data on injuries were collected in according to standard procedure. Using Total Genotype Score (TGS) the combined influence of the polymorphisms was analyzed.

Results: No significant correlations were found between injuries and single genotypes or between incidence of injuries and TGS. The regression model highlighted that the combined influence of training volume and TGS significantly predicted injury rate, explaining 10% of the variability in injury incidence (R2 = 10.36, df = 2.61 , F = 3.52 , P=0.03).

ABBREVIATIONS

VDR: Vitamin D Receptor; **MCT:** Monocarboxylate Transporter; **COL5A1:** Collagen Type V Alpha 1

INTRODUCTION

Muscle lesions are common in sports, especially among high demand and elite athletes.

Muscle injuries represent one of the major problems facing both professional and amateur football players. Some studies highlighted that this type of injury represents a high percentage (20% to 37%) of the time-lost because of accidents at the professional level among men [1,2].

The most common injuries that occur in soccer are muscle strains, joint ligament sprains (especially in the ankle), haematomas, and concussion. Muscle strains occur most often at the musculo tendinous junction as a consequence of indirect trauma. The primary site of injury is the musculo tendinous junction of the hamstring, rectus femoris, or medial head of the gastrocnemius. Two-joint muscles, those that contract eccentrically and with a high percentage of type II fibres are the most predisposed to injury. The most severe joint injury is rupture of the anterior cruciate ligament [3].

Researchers began to study the causes of injuries in soccer, with the aim of reducing their incidence and increasing the safety of the athletes. Muscle injuries and their subsequent functional recoveries seem likely to result from the interaction of environmental stimuli (e.g. training, overload/over-training, incorrect technique, competition, etc.) and genotypes.

Variations in the DNA sequence in candidate genes have been associated with specific phenotypes related to athletic performance, including predisposition to injuries [4]. In fact, the genetic predisposition to developing injury in sports is conferred by different genetic variants [5].

For example, the symptoms and signs of muscle damage during exercise, heat exposure, and abnormal erythrocyte lactate transport were correlated with the presence of two mutations in the Monocarboxylate transporter 11 (MCT 11) gene [6]. In particular, Merezhinskaya et al. [6] found an association between a single nucleotide variation (MTC1 rs1049434) from A to T that led to the conversion of glutamic acid to aspartic acid in the lactate transporter, causing a reduction in the rate of transport of the erythrocyte lactate by 35-40%.

A deficit in lactate transport in an intracellular acidic environment, created by muscle activity during exercise, leads to degeneration of the muscles and the release of myoglobin and creatine kinase [7,8]. This flaw could compromise extreme performance in healthy individuals. A deficit in lactate transport in skeletal muscle may provide an explanation for certain muscle injuries, including muscle fatigue, muscle cramps after exercise and delayed-onset muscle soreness, from the delayed removal of protons (H+) that accumulate during anaerobic work.

Some recent association studies suggested there is a genetic predisposition for muscle-tendon injuries. In fact, researchers identified specific genes that encode for the component of the extracellular matrix of musculoskeletal tissue, including collagen-specific genes [9, 10]. A common C to T SNP (rs12722) in COL5A1 turned out to be associated with sport injuries [9,10] and with phenotypes correlated to performance [10]. Laguette et al. [11] recently reported that COL5A1 rs12722 is associated with altered stability of mRNA. The same authors speculated that this variant could determine an altered quantity of type V collagen production, which may alter the fibril architecture and its mechanical properties.

Finally, some recent studies demonstrated that skeletal muscle is a target organ for vitamin D, whose metabolites directly affect muscle cell metabolism through various pathways [12]. Moreover, vitamin D deficiency is associated with muscle weakness [12], which in turn is a factors that causes the onset of muscle injuries [13]. More specifically, this vitamin acts through the VDR nuclear receptor, which plays a key role in regulating vitamin D transcription activity. Gene codifying for VDR contains several SNPs polymorphims, such as Fok I, Bsm I, and Apa I.

The aim of this research is to study and quantify the combined effect of five genetic polymorphisms (COL5A1 rs12722, MTC1 rs1049434, VDR ApaI, VDR BsmI, and VDR FokI), that turned out to be candidate markers for influencing the development of injuries among top-level soccer players [14- 16]. In this pilot study, for the first time, we used Total Genotype Score (TGS) [17] for estimating the risk of injuries.

MATERIALS AND METHODS

The recruitment, genotyping, and statistical analyses of this study were performed following the guidelines of "replicating genotype-phenotype associations" [18]. The study design specified data collection procedures according to studies of football injuries and outlined in the consensus document by the UEFA [19].

Sampling

The sample comprised 64 male professional soccer players recruited from three teams participating at the Official Italian Football Championship in different categories (Giovanissimi (G), n=17, age 14±2.7 yrs; Allievi (A), n=32, age 15.8±0.7 yrs; Primavera (P), n=26, age 17.6±0.7 yrs). All the athletes were Caucasian (≥3 generations).

Each soccer player trained approximately 6 weeks in the preseason and approximately 322 weeks during the competitive season (420' per week on average). Annual training volume was calculated for each soccer player as the number of hours of workout.

All participants provided informed written consent, the study protocol was approved by the Ethics Committee of the team, and it was in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000).

Injuries data collection

A muscle injury was defined as any physical muscle complaint occurring during practice that prevented participation in training or match play for at least 1 day after the day of the onset [20]. Muscles injuries were logged during the 2012-2013 season, based on a clinical examination by the team medical staff, following standard procedure. Indirect structural-mechanical injuries, such as muscle lesions (grade I, II, and III), and functional injuries, such as fatigue-induced or neurogenic muscle hardening (hypertonia), were included in the muscle injury group, whereas contusions and hematomas were excluded. Muscle Injuries Incidence (MII) was calculated per 1000 hours of exposure to training and matches ($(\sum$ injuries$/\sum$ hours of exposition) x 1.000).

DNA analysis

Genomic DNA was extracted from buccal swabs using QIAamp DNA Minikits (QIAGEN, Hilden, Germany), and was subsequently analyzed by Polymerase Chain Reaction (PCR) following the protocol previous published by Cupeiro et al. [21] for determining MCT1 rs1049434 polymorphism, by Galasso et al. [22] for determining COL5A1 rs12722, and by Rezende et al. [23] for determining VDR rs1544410, rs7975232, and rs2228570 polymorphisms.

Statistical analysis

The Hardy-Weinberg equilibrium was calculated using Markov Chain, and allele frequencies were calculated with gene counting using GENEPOP 4.0 software. One way ANOVA was used to examine the differences among genotypes and continuous data. Where appropriate, a Scheffé post-hoc analysis was used to determine which of the five genotypes were significantly different from each other. Data were analyzed using Statistica program ver. 7.0 (StatSoft, Inc., Tulsa, OK, USA).

Using Total Genotype Score (TGS) [16] it was possible to quantify the combined influences of the polymorphisms currently associated to muscle injuries. Correlation between TGS and injury incidences was tested with regression analysis. Specifically, each polymorphism genotype was scored based on its association with an unfavourable phenotype (i.e. muscle rupture). Specifically, the unfavorable homozygous genotype would receive a score of 0, the heterozygous genotype a score of 1 and the remaining homozygous genotype a score of 2. Total

genotype score was then found by mathematically transforming the total so that it would lie between 0 and 100. A TGS of 100 represented a "perfect" polygenic profile for a low risk of injury development while a TGS of 0 represented the worst polygenic profile with a high risk of injury development (Table 1).

RESULTS AND DISCUSSION

We first divided the sample into two subgroups: injured players and healthy players. Using the exact differentiation test, the statistical difference in genotype and allele distribution between the two groups was calculated. The two groups did not show significant difference (p-value>0.05) for the markers analyzed. As a consequence, we considered the general sample.

The estimated total incidence of injuries was 3.47 ± 3.7 per 1000 hours of exposure (G=2.8±3.1; A=3.3±3.9; P=4.9±3.6). Sixty three muscle injuries were recorded during the season, accounting for a loss of 2339 days from training in the pre-season and the regular competitive season. Genotype and allele frequencies of the five polymorphisms are presented in Table 2.

No significant genetic difference was found in the distribution of genotype and allele frequencies (data not shown) among the competitive level groups, so we considered the sample as a whole.

The sample exhibited a Hardy-Weinberg equilibrium distribution for the all markers.

Allelic frequencies fell within the range found in the Italian population [24-28].

A significant positive correlation was found between annual training volume and incidence of injuries (r = 0.27; P<0.05) (Figure 1).

Moreover, the annual training volume increased with increasing players age and their competitive level (Giovanissimi [Level I], Allievi [Level II], Primavera [Level III]). No significant differences were found between injuries incidence and any single genotypes (P=0.508).

Despite being previously highlighted in other studies, the effect of a single gene variant is usually too small to define a complex phenotypic trait [29- 31].

Moreover, no significant correlation was found between injury incidence and TGS (p=0.16). Figure 2 shows the variance of injuries explained by the regression model. The combined influence of training volume and TGS significantly predicted the injury rate, explaining 10% of the variability in injury incidence (R2 = 10.36, df = 2.61 , F = 3.52 , P=0.03).

Our preliminary results indicate that in spite of using a more comprehensive approach, combining the influence of some genetic markers, the model remained insufficient for predicting muscle injury incidence in young soccer players.

Table 1: Studied polymorphisms and score assigned for each genotype.

Gene	Polymorphism	Genotypes (0=high risk of injuries)
COL5A1	351 and 316 bp (T)/ 316 and 271 and 80 bp (C)	2=CC; 1=TC; 0=TT
MCT1	287 bp Ins(I)/Del(D) (peptidyl-dipeptidase A) 1	2=TT; 1=AT; 0=AA
VDR ApaI	740 bp (A) 515 bp and 225 bp (a)	2=AA; 1=Aa; 0=aa
VDR BsmI	825 bp (B) 650 bp and 170 bp (b)	2=BB; 1=Bb; 0=bb
VDR FokI	272 bp (B) 198 bp and 74 bp(b)	2=FF; 1=Ff; 0=ff

Abbreviations: **VDR:** Vitamin D receptor; **MCT:** Monocarboxylate transporter; **COL5A1:** collagen type V alpha 1

Table 2: Allele and Genotype frequencies of the studied polymorphisms in young Italian Soccer Players.

Gene	Genotypes	Frequency (%)
COL5A1	TT, TC, CC T=0.534	20, 65, 15
MCT1	AA, AT, TT A= 0.680	46, 46, 8
VDR ApaI	AA, Aa, aa A=0.609	37, 48, 15
VDR BsmI	BB, Bb, bb B= 0.438	22, 43, 35
VDR FokI	FF, Ff, ff F=0.664	46, 39, 15

Abbreviations: [**VDR:** Vitamin D receptor; **MCT:** Monocarboxylate transporter; **COL5A1:** collagen type V alpha 1]

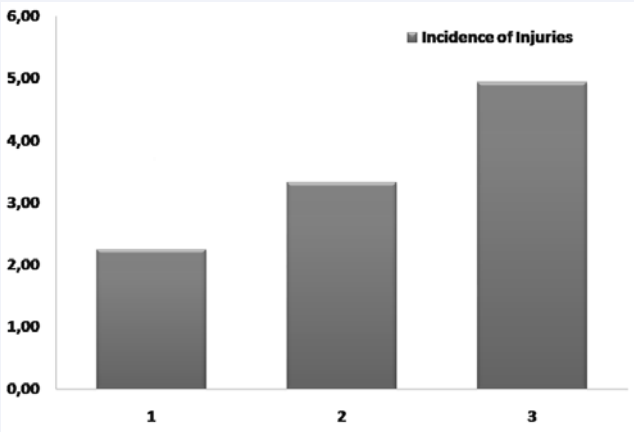

Figure 1 Relationship between annual training volume (1= 170 h/yr; 2=266 h/yr; 3=367 h/yr) and incidence of muscle injuries.

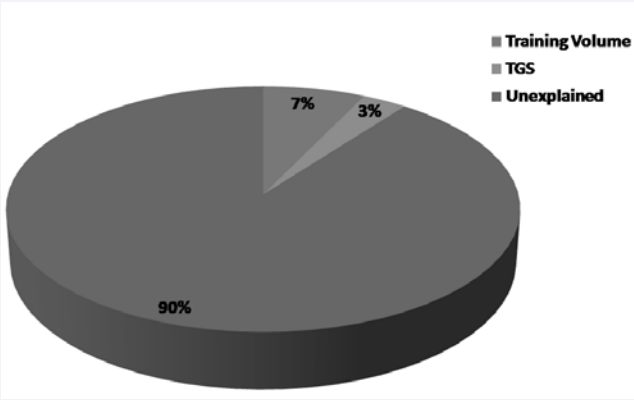

Figure 2 Incidence of injuries variance (%); p=0.003.

This result could be attributed to the various types of muscle injuries analyzed, thus creating a very complex phenotypic trait. Muscle injuries occur through a variety of mechanisms, including direct trauma (e.g., lacerations and strains) and indirect causes (e.g., ischemia and neurological dysfunction) [32], thus forming a very heterogeneous group of injuries.

Our result could be attributed also to the training volume of the young soccer players and this latter point could have played a determinant role in our results. In fact, we found an association between the annual training volume and muscle injury incidence showing a higher rate of injury when annual training volume was greater. Moreover, when the annual training volume of each soccer player is considered in the regression model, also the TGS entered the regression model explaining the 3% of the muscle injuries variance. Several studies have showed that the risk of injury seems to be influenced by player age [33-36] and level of play [37, 38].

Based on these results, we can speculate that combinations of particular polymorphisms, such as those included in our study, become predictive of the risk of injury only when the training volume exceeds a certain threshold.

CONCLUSION

In this study, for the first time, the polygenic influence on muscle injuries in young soccer players was analyzed.

The results suggest that the training volume together with the combined influence of the included polymorphisms seem to predict individual susceptibility to development of injuries in elite young football players, while the action of single genotypes do not appear to be sufficient to influence the incidence of injuries in the same group of athletes.

It remains to be established whether these genetic variants are directly involved in the development of these muscle soft tissue abnormalities or in strong linkage disequilibrium with actual disease-causing loci.

We propose that genetic risk factors will be included in multifactorial models in the future to understand the molecular mechanisms that cause musculoskeletal soft tissue injuries or related pathologies. Clinicians and coaches could eventually use these models to develop personalized training programs to reduce the risk of injury as well as develop treatment and rehabilitation regimens for injured individual. A larger cohort is required to fully understand the relationship between genetic predisposition and musculoskeletal injuries in soccer. Moreover, our future studies will be focused on a single type of muscle injury.

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