INFLUENCE ON INJURABILITY AND INJURY RECOVERY TIME OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN GENES INVOLVED IN CONNECTIVE TISSUE REPAIR

UEFA Research Grant

Programme

2012

Final Report

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March 2013

<u>Abstract</u>

Soft tissue injuries (in muscles, tendons and ligaments) are a key factor in "talent selection" due to their great importance in high-level sports. In fact, an athlete with a high injurability index may find that the process of natural selection has denied him/her the opportunity to compete.

Epidemiological studies, based on meticulous data collection, are the source of our current knowledge of injurability and have become the most reliable way to obtain objective information on injurability. However, few serious studies on the etiology of injuries have been performed, and no scientific evidence has yet conclusively identified potential risk factors related to injurability or interindividual differences in recovery times.

Muscle injuries represent 10-55% of all sports-related injuries. While it is possible for slight injuries to heal completely, serious muscle injuries lead to the formation of scar tissue, which weakens the injured muscle even after recovery and which is often associated with muscle contractions and chronic pain. Although there are different degrees of injury and different treatments for recovery, there is also a large interindividual variation in the speed of recovery to a specific kind of injury. This variation in recovery time may be due to the presence of single nucleotide polymorphisms (SNPs) that can affect an individual's response to a specific treatment. Moreover, it has recently been suggested that certain individuals are genetically predisposed to suffer certain kinds of injuries. Several studies have demonstrated that the presence of certain SNPs in genes involved in the repair of muscle tissue can enhance recovery after injury.

Our Research Group, located in the Unit of Anatomy and Human Embryology of the University of Barcelona School of Medicine (Casanova Campus), has a long-standing close relationship with the School of Sports Medicine, located in the same Unit. We have wide experience in the study of SNPs in genes involved in the repair and regeneration of connective tissues. We believe that further study of these SNPs may shed light on the tendency of certain individuals to suffer muscle injuries and may identify a gene signature to identify individuals with an enhanced injury repair system.

Keywords: genetic polymorphisms, connective tissue, injury, elastin, titin, Sox15, IGF2, CCL2.

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Introduction

Soft tissue injuries (in muscles, tendons and ligaments) are a key factor in "talent selection" due to their great importance in high-level sports. Few studies have focused on the cause or etiology of soft tissue injuries, and exhaustive epidemiological studies¹ are the source of our current knowledge of injurability. To date, no scientific evidence has conclusively identified potential risk factors to explain interprofessional injurability or interindividual variability despite similar programs of injury prevention. At present, although epidemiological studies are the best available tools for identifying the type of injury and injurability index¹⁻⁴, they do not allow us to establish a direct relation between an injury and its etiology, since there is no scientific evidence of risk factors to explain the predisposition of an individual to suffer an injury, the origin of the injury, or even the estimated recovery time. Depending on the kind of sport, muscle injuries represent 10-55% of all injuries² suffered by athletes. In spite of the fact that small injuries can be completely cured, recovery from more serious muscle injuries causes a scar in the muscle tissue that decreases muscle function and can lead to muscle contractions and chronic pain⁵. Variability in recovery time from the same type of injury with the same type of therapy may be due to the presence of single nucleotide polymorphisms (SNPs) that cause some individuals to respond better than others to a specific therapy. Moreover, some studies have suggested the existence of a genetic predisposition to suffer certain types of injuries^{4, 6-7}.

A SNP is a DNA sequence variation that is detectable in at least 1% of the population. This change gives rise to different alleles, which are the alternative forms of a specific gene. A SNP may or may not influence the phenotype, which may be a clinically useful marker. SNPs can be found in the intronic (non-codifying), exonic (codifying), or promoter regions of a gene. When located in the promoter region, they may or may not cause an increase or decrease in the gene mRNA expression levels. When they are located in an exonic region, they may or may not cause a change in the amino acid sequence of the protein that is produced (Figure 1).



Figure 1: Locations and effects of SNPs

Certain SNPs promote tissue repair and regeneration⁸⁻¹⁰. It has been observed that different athletes respond differently to the same treatment, with different recovery times; in other words, some athletes recover quickly and well, while others need a longer time and do not recover as completely. Recent studies have found that SNPs in genes involved in the repair of connective tissue may explain this variation in recovery time⁸⁻¹². Other studies have shown that the presence of certain SNPs in genes involved in the repair of muscle¹², tendon¹³⁻¹⁷ or ligament¹⁸ tissue may affect injury recovery time^{6,7}.

To date, however, two aspects of this issue have not been investigated: the importance of SNPs in genes associated with tissue repair and recovery and the frequency of these SNPs in different ethnic groups. In the present project, we have analyzed SNPs in genes related to tissue repair (elastin [ELN])^{19,20}, muscle assembly and force transmission (titin [TTN])²¹, skeletal muscle regeneration (SRY-related HMG-box [SOX15])²², muscle damage (insulin-like growth factor 2 [IGF2])⁹, response to muscle damage (chemokine, CC motif, ligand 2 [CCL2])¹⁰, ligament ruptures (Collagen type I alpha1 [COL1A1] and collagen type 5 alpha 1 [COL5A1])²³, and tendinopathy (COL5A1 and tenascin [TNC])²⁴. Our objective was to evaluate the association of these SNPs with

the degree of injury and to examine the possibility of ethnicity-related differences in their frequency or effect.

Objectives

Recent studies have found that SNPs in genes involved in the repair of connective tissue may explain interindividual variations in degree of injury and recovery time among athletes. Other studies have shown that the presence of certain SNPs in genes involved in the repair of muscle tissue may affect injury recovery time. We hypothesized that by analyzing a large number of SNPs in genes involved in tissue repair, we could identify a gene signature associated with lower risk of injurability and shorter recovery time. Based on this hypothesis, we identified the following objectives:

- 1. Select SNPs in genes involved in muscle tissue repair and rengeration.
- 2. Compare the frequency of these SNPs in our study population with that recorded in the NCBIdb SNPs
- Compare the frequency of these SNPs in individuals of different races in our study population.
- 4. Correlate the presence of these SNPs with the efficacy of injury repair.
- 5. Examine a potential association between a SNP-related injury behavior pattern and race.
- 6. Identify a potential genetic profile to select individuals with a longer or shorter recovery time for muscle injuries.

Material and methods

The study was approved by the Ethics Committee of the Hospital Clinic, Barcelona (registry no. 2012/7117), and all players gave their signed informed consent

1. Study population

Data was collected on injuries suffered by 73 elite football players from Futbol Club Barcelona (Barcelona, Catalonia, Spain) over the course of three consecutive football seasons. Blood was drawn from these players, and 4mL was used for the genetic analyses. Blood was drawn exactly every two months, and the same parameters were analyzed in every sample (Appendix I), primarily those related to fatigue, since this leads to a greater risk of injurability and a reduced performance.

All the players had the same amount of work, the same diet and the same ergogenic aids. The training field, the playing fields and the injury prevention protocols were also identical for all the players. In order to control for the variables that influence injury, data on injuries were collected in accordance with the Union of European Football Associations (UEFA) protocol (Appendix II). Ultrasound and magnetic resonance imaging were used to classify the injuries by anatomic region and severity. Injuries were classified as slight, moderate or serious according to the number of days the player needed to be absent from training and/or competition: slight, 1-15 days; moderate, 16-30 days; and serious, more than 30 days. All these parameters, including the evolution of the injury, were supervised by the same physician.

2. DNA extraction

Approximately 4 mL of whole blood was collected from each subject into EDTA vacutainer tubes, and stored at 4°C until total DNA extraction. Genomic DNA from whole blood was isolated using QIAmp DNA Blood Minikit (Qiagen, Valencia, CA) following the manufacturer's instructions (Appendix III). To measure DNA quantity, a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific INC, Waltham, MA) was used. DNA was stored at -20°C until analyzed.

3. SNP selection

SNPs were selected based on two criteria: 1) a frequency in the general population of 0.05-2) and 2) a possible association with tissue repair and regeneration. Table 1 shows the characteristics of all the SNPs analyzed.

GENE	ASSOCIATION WITH TISSUE REPAIR	rsNCBI	Reference
ELN	Tissue repair	rs2289360	19,20
ΤΤΝ	Muscle assembly	rs2742327	21
	Force transmission		
SOX15	Skeletal muscle regeneration	rs4227	22
IGF2	Muscle damage	rs3213221	9
CCL2	Response to muscle damage	rs2857656	10
COL1A1	Ligament ruptures	rs1800012	23
COL5A1	Ligament ruptures	rs12722	23,24
	Tendinopathy		
TNC	Tendinopathy	rs2104772	24

Table 1: The eight SNPs included in the study, with their NCBI identification

4. Allelic discrimination analysis

Primers and probes were obtained from Applied Biosystems (AB; Assays-on-Demand SNP genotyping product, Foster City, CA). FAM or VIC fluorophores were covalently bound at the 5' end of the probe with the MGB quencher attached at the 3' end (Figure 2). SNP analysis was performed using a real-time polymerase chain reaction (PCR) Allelic Discrimination TaqMan Assay (AB) with minor modifications. All PCR reactions were run in duplicate, and contained 50 ng of each individual's DNA; 6.25 μ L TaqMan Universal Master Mix (AB); 0.25 μ L primers and probes (AB) and water for a final volume of 12.5 μ L. Real-time PCR was performed on an ABIPrism 7500 Sequence Detection System (AB) using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of amplification (95°C for 15 seconds and 60°C for 1 minute) (Appendix III and IV). For each cycle, the software determined the fluorescent signal from the VIC- or FAM- labeled probe. Each PCR included a negative control.



Figure 2: Allelic discrimination analysis – detection of fluorescent signal.

The fluorescent signal from the VIC- or FAM- labeled probe distinguished between heterozygotes, wild-type homozygotes, and SNP homozygotes (Figure 3). For example, if the A allele occurred at a frequency of 70% and the T allele at a frequency of 30%, the blue dots in Figure 3 correspond to wild-type homozygous individuals with the AA genotype, the red dots to SNP homozygous individuals with the AT genotype, and the green dots to heterozygous individuals with the AT genotype.



Figure 3: Allelic discrimination analysis identified three populations.

5. Statistical analyses

The association between type and degree of injury and the SNPs (in ELN, TTN, SOX15, IGF2, CCL2, COL1A1, COL5A1 and TNC) was determined with the Chisquare test and Fisher's exact test when necessary. The association between SNPs and injury recovery time was evaluated using a multivariate analysis of variance.

For descriptive purposes, the SNPs evaluated and their distribution among ethnic groups was shown following the crosstabulation table technique. Polymorphism frequencies of each gene for each ethnic group were presented as percentages. Statistical differences among groups were determined by the Chi-square test and the Fisher's exact test when SNPs with values ≤0.05 were found. All statistical analyses were performed using SPSS version 14.0 or 15.0 for Windows (SPSS Inc., Chicago, IL). Significance was set at P<0.05.

<u>Results</u>

1. Blood analyses

Blood was drawn from the players on a regular basis, both to perform the genetic analyses and to record the parameters associated with fatigue. In general, hemoglobin levels of \geq 15gr/dl indicate that a player is stable, with no decrease in his performance or a higher risk of injurability. All the players included in the study met this requirement (Figure 4).



Figure 4: Hemoglobin levels in a representative sample of players included in the study

Ferritin levels are the basic index for evaluating iron metabolism. Slightly low levels are not well tolerated by high-level athletes since in a very short time, they could develop fatigue, leading to lower performance (Figure 5).



Figure 5: Ferritin levels in a representative sample of players included in the study

Levels of creatine kinase (CK) and transanimase levels are used to measure muscle overload and tolerance of muscle load (Figures 6 and 7).Low levels indicate the absence of muscle overload and hence a tolerance of the muscle load.



Figure 6: Creatine kinase levels in a representative sample of players included in the study





Figure 7: Transanimase levels in a representative sample of players included in the study

Alterations in protein and albumin levels could be cause for concern, since nitrogen imbalance is associated with decreased athletic performance and a higher risk of injurability (Figure 8).



Figure 8: Albumin levels in a representative sample of players included in the study

GGT levels can identify a liver problem (Figure 9).



Figure 9: GGT levels in a representative sample of players included in the study

2. Characteristics of the study population

Of the 73 soccer players included in the study, 43 (58.9%) were White, 11 (15.1%) were Black Africans, and 19 (26%) were Hispanics. The median age for all players was 26.2 years (range, 19-35), median weight was 75.6 kilos (range, 64-92), median height was 1.79 meters (range, 1.66-1.95), and median work load was 16026.55 minutes per year (267 hours/season/player), with no significant differences between ethnic groups for any of these characteristics (Table 2).

Characteristic	Whites	Black Africans	Hispanics
	n=43 (58.9%)	n=11 (15.1%)	n=19 (26%)
age	25.52 (19-35)	29.11 (20-35)	26.16 (25-34)
weight	76.44 (64-92)	77.6 (64-92)	72.63 (67-78.5)
height	179.62 (166-195)	181.3 (172-194)	176.3 (169-189)
% fat	8.66 (6.9-12.9)	6.5 (6-14.1)	10 (6.2-13.6)
work load *	15301	16224.3	16554.33

* minutes in competition or training per player and season

Table 2: Main characteristics of the study population

3. Epidemiology and types of injuries

Thanks to the UEFA protocol for collection of data on injuries (Appendix II), we were able to obtain a data base comprising all the injuries suffered by the players, including the date of injury, the type and location of the injury, whether it was a re-occurrence of a previous injury or a new injury, how the injury occurred (in training or an official match), the position of the player on the field at the time of injury, the type of playing surface, whether the injury was a result of contact with another player or not. Finally, the severity of the injury and the number of days the player was unable to train or play were also recorded

Over the course of the three seasons of the study, a total of 242 non-contact soft tissue injuries (NCSTIs) were recorded for all 73 players. Two hundred and three were muscle injuries, of which 129 (63.5%) were slight, 69 (34%) moderate, and 5 (2.5%) serious. Twenty-four were ligament injuries, of which 15 (62.5%) were slight, 3 (12.5%) moderate, and 6 (25%) serious. Fifteen were tendon injuries, of which 7 (46.7%) were slight, 7 (46.7%) moderate, and 1 (6.6%) serious (Table 3).

Type of injury	Degr	N		
-) , , , , , , , , , , , ,	slight	moderate	serious	
muscle	129 (63.5%)	69 (34%)	5 (2.5%)	203
ligament	15 (62.5%)	3 (12.5%)	6 (25%)	24
tendon	7 (46.7%)	7 (46.7%)	1 (6.6%)	15

Table 3: Total number of injuries suffered in three football seasons by all players included in thestudy

4. DNA extraction and quantification

DNA was extracted from the blood samples for the molecular analyses. DNA concentrations and sample purity can be seen in Figure 10 and Table 4, which show the results for 21 of the players included in the study.



Figure 10: Agarose gels showing DNA quality

			1			
SAMPLE	ng/µL	260/280		SAMPLE	ng/µL	260/280
1	143.11	1.88		15	56.37	1.85
2	177.01	1.86		16	220.11	1.85
3	155.45	1.88		17	136.64	1.88
4	93.19	1.85		18	187.82	1.84
5	146.27	1.87		19	189.46	1.87
8	181	1.83		21	87.18	1.81
9	229.41	1.85		22	54.57	1.74
10	182.91	1.85		24	176.05	1.86
11	164.63	1.81		28	175.67	1.87
13	171.61	1.83		35	40.24	1.71
14	184.95	1.87				

Table 4: DNA concentration obtained for each µl and the purity of the simple determined by NanoDrop ND1000 in a representative sample of players included in the study

5. Allele frequencies and genotypes

Table 5 shows the allele frequencies of the eight genes, both for the present study and according to the NCBI dbSNP. The frequency of the SNPs varied among the three subgroups in the present study (p<0.0001).

	POPULATION								
OFNE	OFNOTVE	TOTAL	WHITE	ES	BLACK AF	RICAN	HISPAN	NIC	- -
GENE	GENOTYPE	Present study	Present study	НарМар	Present study	НарМар	Present study	НарМар	p-value
		N=73	N=43	CEU	N=11	YRI	N=19	HISP1	_
	AA	27.40%	14%	15.50%	45.45%	40.20%	47.40%	n/a	
ELN	AG	56.20%	65.10%	65.11%	36.40%	44.20%	47.40%	n/a	<0.0001
	GG	16.40%	20.90%	36.40%	18.20%	15.20%	5.20%	n/a	
	AA	50.70%	58.10%	68.10%	27.30%	14.20%	47.40%	n/a	
TTN	AG	39.70%	37.20%	26.50%	45.50%	46.90%	42.10%	n/a	<0.0001
	GG	9.60%	4.70%	5.30%	27.30%	38.90%	10.50%	n/a	
	тт	58 00%	62 80%	58 /0%	36.40%	20.40%	63 20%	52 20%	
		30.90 %	02.00 %	30.40 %	50.40 %	20.4076	05.20 %	52.2076	_
SOX15	TG	31.50%	32.60%	33.60%	36.40%	49.60%	26.30%	34.80%	<0.0001
	GG	9.60%	4.70%	8.00%	27.30%	30.10%	10.50%	13.00%	
	GG	38.40%	30.20%	30.10%	45.45%	38.10%	52.60%	43.50%	
IGF2	GC	43.80%	53.50%	53.10%	27.30%	42.30%	31.60%	21.70%	<0.0001
	СС	17.80%	16.30%	16.80%	27.30%	9.20%	15.80%	34.80%	-
	GG	43.80%	39.50%	65.20%	54.50%	52.20%	47.40%	n/a	
CCL2	GC	46.60%	48.80%	30.40%	45.50%	34.80%	42.10%	n/a	<0.0001
	СС	9.60%	11.60%	4.03%	-	13%	10.50%	n/a	-
	AA	35.60%	30.20%	31.70%	45.45%	46.70%	42.10%	n/a	
тис	AT	43.80%	51.20%	53.30%	36.40%	41.70%	31.60%	n/a	<0.0001
	TT	20.50%	18.60%	15.00%	18.20%	11.70%	26.30%	n/a	_
	GG	71.20%	72.10%	-	81.80%	100%	63.20%	n/a	
COL1A1	GA	26%	23.30%	100%	18.20%	-	36.80%	n/a	<0.0001
	AA	2.70%	4.65%	-	-	-	-	n/a	_
	ТТ	-	-	24.50%	-	-	-	n/a	
COL5A1	TC	65.30%	76.20%	64.20%	18.20%	27.10%	68.40%	n/a	<0.0001
	CC	34.70%	23.80%	11.30%	81.80%	72.90%	31.60%	n/a	-

n/a, not available.

Table 5: Allele frequencies of the eight genes, both for the present study and according to the NCBI dbSNP for Whites (HapMap CEU), black Africans (HapMap YRI) and Hispanics (HISP1).

6. Association of SNPs with degree of injury and recovery time

When a professional athlete is injured, the most important question for both the attending physician and the athlete himself is how long it will take for the player to recover.

6.1. Muscle injuries and SNPs

SNPs in IGF2, CCL2 and COL5A1 were associated with severity of muscle injuries. The 93 muscle injuries associated with the IGF2 GC genotype were significantly less serious than those associated with the IGF2 CC or GG genotypes (p=0.032) (Figure 11).



Figure 11: Association between IGF2 and degree of muscle injury. Injuries suffered by individuals with the GG genotype were more serious, while those suffered by individuals with the GC genotype were slight and moderate.

The CCL2 genotypes CC and CG were also associated with less serious muscle injuries than the CCL2 GG genotype (p=0.013) (Figure 12).



Figure 12: Assocation between CCL2 and degree of muscle injury. The T allele was associated with more serious injuries.

Finally, the COL5A1 TC genotype showed trend towards an association with more serious muscle injuries (p=0.07) (Figure 13).



Figure 13: Association between COL5A1 and degree of muscle injury. The T allele was associated with more serious injuries.

The 10 ligament injuries associated with the ELN AA genotype were more serious than those associated with the ELN AG or GG genotypes (p=0.09) (Figure 14).



Figure 14: Association between ELN and degree of ligament injury.

SNPs in ELN also showed evidence of a significant association with recovery time. Injuries associated with the ELN AG genotype required a shorter mean recovery time (24.7days) than those associated with the ELN GG (37.5 days) or AA (83.2 days) genotypes (p=0.027) (Figure 15).



Figure 15: Association between ELN and recovery time for ligament injuries.

6.3 Tendon injuries and SNPs

Although other studies have found a relation between SNPs in COL5A1 and TNC and tendon injuries, in the present study, we have not found an association between SNPs and tendon injury or recovery time for tendon injuries. This may be due to the small number of tendon injuries suffered by the study population.

7. The potential impact of ethnicity on patterns of NCSTIs

7.1. Interethnic variability in injury patterns

A total of 242 injuries (203 muscle, 24 ligament, and 15 tendon injuries) were recorded. The 43 Whites suffered a total of 124 injuries, the 11 Black Africans 41 injuries, and the 19 Hispanics 77 injuries. Table 6 shows the distribution of each type of injury in each of the three ethnic groups. Interestingly, the Black Africans suffered no ligament injuries.

	SIT	TOTAL		
ETHNIC GROUP	MUSCLE	LIGAMENT	TENDON	IOTAL
Whites (n=43)	103 (83.06%)	15 (12.09%)	6 (4.85)	124
Black Africans (n=11)	36 (87.80%)	-	5 (12.2%)	41
Hispanics (n=19)	64 (83.11%)	9 (11.68%)	4 (5.21%)	77

Table 6: Number and site of injuries in the three ethnic groups studied.

7.2. Interethnic variability in genotypes and allelic frequencies

As shown in Table 5 above, the frequency of the SNPs varied among the three ethnic groups (p<0.0001). The minor genotype of the ELN gene was AA for Whites (14%) but GG for both Black Africans (18.1%) and Hispanics (5.3%). The minor genotype of the TTN gene was GG in both Whites (4.7%) and Hispanics (10.5%) but AA in Black Africans (27.3%). The minor genotype of the CCL2 gene was CC in both Whites (11.6%) and Hispanics (10.5%), while among Black Africans, the GG and CC genotypes had a similar frequency (54.5% and 45.5%, respectively. The minor genotype of the COL1A1 gene was AA for Whites (4.7%), while this genotype was not observed among the Black Africans or the Hispanics. Finally, the TT genotype of the COL5A1 gene was not detected in any of the subjects.

7.3. Interethnic variability in the relation between SNPs and injury patterns

Among the Whites, a near-significant correlation was observed between the pattern of muscle injuries and the IGF2 genotype (p=0.059). The presence or absence of the G allele identified two groups of individuals; those with the GC or GG genotype showed the same pattern of injuries, while those with the CC genotype had a different pattern. In addition, there was a correlation between the pattern of ligament injuries and the ELN genotype; the presence or absence of the A allele identified two clearly distinct groups of individuals: those with the AG or AA genotype showed the same pattern of injuries, while those had a different pattern of injuries, while those with the same pattern of injuries, a significant correlation was observed between the pattern of tendon injuries and ELN (p=0.05),

according to the presence or absence of the A allele, and IGF2 (p=0.05), according to the presence or absence of the C allele.

No relation between SNPs and injury patterns was observed among Black Africans.

Among Hispanics, there was a correlation between the pattern of muscle injuries and the ELN (p=0.032) and IGF2 (p=0.016) genotypes. The G allele of ELN and the C allele of IGF2 distinguished groups with different patterns of injuries.

Discussion

At present, epidemiological studies are the most reliable tool for classifying and describing the injurability index in a population of elite athletes ²⁵ and no other kinds of studies have been carried out to explain the etiology and recovery time of injuries.

The UEFA model of data collection and statistical validation has been used since 2001 in all the elite teams that participate in European competitions (UEFA-Champions League). In order to use the UEFA model and to participate in UEFA studies, it is necessary to have the approval of the UEFA-Nyon Medical Commission ²⁶ both as regards the level of commitment and the methodology of data collection. This model makes it possible to compare several variables among the 17 participating teams, including the total number of injuries, the number of muscle injuries, the number of ligament injuries, the severity of the injuries (light, moderate or serious) and the situations in which the injuries occur (training or competition).

To date, an index of injurability has generally been established based on the blood analyses of the players. Every two months, blood is drawn and analyzed for levels of hemoglobin, ferritin, creatinine, urea, transanimases, CK and other components. An athlete is considered to be in a "fragile" state when hemoglobin levels are below 15gr/dl, when ferritin levels are below 50ng/ml, when the levels of urea and creatinine are higher than the reference levels, when transanimase levels are higher than 60UI/L or when CK levels are higher than 600U/L. GGT levels are used to detect a possible muscle injury from liver damage. Our results indicate that normal GGT levels were found in the majority of the players included in the present study, except two players who suffered from hepatitis.

Nevertheless, this routine method of determining the physical state of an athlete is not reproducible, since the medical criteria used by physicians on different teams can vary. Therefore, we set ourselves the goal of establishing an objective method to relate the severity of an injury to the recovery time needed for the injury, both of which can vary greatly. These variations in severity and recovery time of an injury may be due to the presence of SNPs, which are variations in the genome of individuals,

Recent studies have shown that the presence of SNPs in certain genes can modulate response to treatment with certain drugs. From 2006 to 2008, several studies suggested that SNPs in DNA repair genes could influence response to chemotherapy ^{27,28}. Other studies have demonstrated that SNPs in genes involved in repair of muscle tissue could

influence injury recovery time^{8-11, 29}. All these studies indicate that genetic variations are important in the molecular mechanisms of action related to injury and response to treatment.

Physical activity and sports are normal activities today. Soft-tissue injuries, whether they be in muscles, tendons or ligaments, are connective tissue injuries³⁰ and are the most frequent type of injury in sports in general and in football in particular. It is well-known that interindividual – and even intraindividual – differences exist in the structure and function of connective tissue ³⁰. To date, various intrinsic factors have been identified as risk factors for injurability, including age ³¹⁻³³, sex ³³⁻³⁵ and the existence of a previous injury ^{33,36}.

All the players included in this study live within 30 kms of the training field and were thus subjected to the same climate and environmental conditions. All the players had the same work load, followed the same diet and received the same ergogenic aids. The training field, the playing fields and the injury prevention protocols were also the same for all the players. Finally, the treatment protocols for each type of injury, including medication and physical therapy, were the same for all the players, and all treatment was supervised by the same medical team. However, in spite of a strict control of these extrinsic factors, the players suffered different injuries, with different recovery times. For this reason, we have focused our investigation on genetic factors that could influence injurability and recovery time, since the interaction between extrinsic and intrinsic factors could be crucial ³⁷⁻³⁹.

We first analyzed eight genes related to tissue repair and regeneration to see the frequency of SNPs in these genes in the study population. ELN is a key extracellular matrix protein that is critical to the elasticity and resilience of many tissues ⁴⁰. TTN is the largest protein described in mammals and is expressed both in heart and skeletal muscles. The main role of TTN is to regulate myofibrillar assembly ⁴¹ and cell signaling ⁴². The SOX family of genes are transcription factors with an important role in determining cell destiny during development ⁴³. Several studies have found that SOX15 plays a fundamental role during myogenic differentiation ⁴⁴ and that it is crucial for skeletal muscle regeneration ²². IGF plays a role in growth and is increased during regeneration following injury, leading to activation of satellite cells ^{45,46}. CCL2 is a small chemokine produced by macrophages and satellite cells ⁴⁷. It plays a role in inflammation and immunoregulation ¹⁰. The expression of CCL2 increases rapidly after

muscle damage, and recent studies indicate that it is critical to muscle repair ^{10,12}. The major component of tendons and ligaments is collagen, especially type I and type V. COL1A1 and COL1A5 encode the large chains that make up collagen ^{18,23}. COL1A1 encodes the α 1 chain of type I collagen ²³ and is associated with ligament ruptures ^{14,30}. COL5A1 encodes the α 1 chain of type V collagen, which is a component of ligaments and tendons ⁴⁸. These molecules work with type I collagen to form heterotypic fibrillins in non-cartilage connective tissues ⁴⁹. TNC is a glycoprotein found in abundant amounts in tissues like tendons ^{14,50}. It is overexpressed in tendinopathies in the Achilles' tendon ⁵⁰.

In the present study, we have observed associations between some of the SNPs and muscle and ligament injuries but not tendon injuries. Although some of the SNPs included in our study had been described as markers for tendinopathy, we have examined the potential relation between the SNP and the degree of injury and recovery time, which had not been examined in previous studies.

We have observed a close relation between IGF2 and degree of muscle injury. IGF2 is located on chromosome 11p15 and plays an important role in growth. Along with fibroblastic growth factor, interleukin-1 β , interleukin-6 and transforming growth factor- β , IGF2 is increased following an injury as a result of the activation of satellite cells ⁴⁶. We found that the IGF genotype GC acted as a protector against serious injuries, while the homozygous GG or CC genotypes were associated with more serious injuries, although no relation with recovery time was observed.

We have also observed a relation between CCL2 and degree of injury. Hubal and colleagues had previously shown that SNPs in CCL2 and its receptor were related to markers of of muscle injury like CK and myoglobin levels, muscle pain and muscle function ¹⁰. Our results indicate that the presence of the C allele (CCL2 CC/CG) was associated with less serious injuries.

COL5A1 is a minor component of the collagen of tendons and ligaments ⁴⁸ and forms part of the extracellular matrix of other tissues, including skeletal muscle. These COL5A1 molecules connect with collagen type I fibers in non-cartilage connective tissue, modulating fibrillogenesis. However, in order for this process to be successful, both alleles must be present ⁴⁹, as shown in studies of tendinopathy, where the presence of the C allele has been associated with asymptomatic patients. Our results support this theory and indicate that a muscle injury can be more or less serious

depending on the composition of the collagen. We have observed that individuals with the COL5A1 CC genotype suffered less serious injuries.

ELN was associated with the degree and recovery time of ligament injuries. In a situation of tissue injury, repair and regeneration, the contractile function is lost and cellular differentiation leads to an immature phenotype capable of proliferation and translocation to the extracellular matrix. The lack of elastin distorts the stability of other components of the extracellular matrix, while the presence of the wild-type genotype prevents this from occurring. In our study, the wild-type genotype was associated with less serious injuries. Individuals homozygous for the A allele had less serious injuries. We also observed that individuals with the G allele (GG/AG) had shorter recovery times, which could be due to a more efficient elastin function, since altered elastin affects elastogenesis and the function of elastic fibers in vivo ²⁰.

TNC is a glycoprotein found in abundant amounts in tissues subjected to a high tensile and compressive stress^{50,14}, such as tendons. It is highly expressed in tendinopathies of the Achilles' tendon ⁵⁰. However, we found no significant association between TNC and degree of injury or recovery time.

Based on the large interindividual variation in recovery time for the same type and severity of injury, we felt that the effect of race on recovery time could be crucial. We therefore analyzed the frequencies of SNPs in different ethnic groups included in the study: Whites, Black Africans and Hispanics. A recent study comparing allele frequencies in genes related to drug metabolism in Caucasian, Japanese and Chilean populations concluded that ethnic variability must be taken into account when assessing polymorphisms in order to personalize cancer therapy⁵⁴. Chowbay and collaborators⁵⁵ analyzed polymorphisms in genes encoding drug-metabolizing and drug transporters in three distinct Asian populations compared with Whites and Africans. They concluded that the pharmacogenetics of drug-metabolizing enzymes were different in Asians compared to Whites and Africans, indicating that drug dose requirements for Whites and Africans may not be optimal for an Asian population. Another study⁵⁶ found that the different frequencies of CYP1A1 alleles with different types of cancer, including lung, breast and colon, also depends on racial origin.

We have extended this work on interethnic genotypic variability related to the pathogenesis and treatment of several diseases by examining the potential differential effect of SNPs on patterns of NCMSTIs in three ethnic groups. SNPs in ELN and IGF2 were related to injurability both in Whites and Hispanics. In the present study, IGF2 was related to both muscle and tendon injuries among Whites. However, muscle injuries were related to the presence or absence of the G allele, while tendon injuries were related to the presence or absence of the C allele.

Our study has two major limitations. Firstly, the scarcity of genetic studies in sports medicine makes it difficult to determine the correct cause-and-effect relationship of our findings. Secondly, although there are many genes involved in tissue repair and regeneration, logistic limitations obliged us to select only a few for this study. Further studies are warranted to determine the clinical significance of the associations between SNPs and degree of injury and recovery time observed in our study. The prevention, diagnosis and treatment of NCSTIs are key factors in both the daily practice of sports medicine and in "talent selection", due to their great importance in high-level sports, especially in football.

Conclusions

- The different frequencies of SNPs in the three populations included in the study (p<0.0001) could explain why certain individuals are more predisposed to suffer serious injuries and why they have longer recovery times.
- 2. IGF2 y CCL2 are associated with the degree of muscle injury and ELN with recovery time for ligament injuries.
- Among Whites, there was a significant correlation between the pattern of ligament injuries and the ELN genotype and (p=0.001). Moreover a significant correlation was observed between the pattern of tendon injuries and ELN (p=0.05), according to the presence or absence of the A allele, and IGF2 (p=0.05), according to the presence or absence of the C allele.
- 4. Among Hispanics, there was a correlation between the pattern of muscle injuries and the ELN (p=0.032) and IGF2 (p=0.016) genotypes.
- 5. Our results indicate that interracial genotypic differences may be important in the study of NCSTIs.
- 6. The genetic profile based on the SNPs identified in the present study can be used to better define the risk of injury of a given individual, possibly helping in player selection and allowing more specific treatment and prevention. It may also help to identify individuals who will require a longer recovery time following injuries.
- 7. **Clinical relevance:** The study of SNPs will help identify individuals with greater predisposition to injury, who may benefit from targeted preventive measures, and those who will require a longer recovery time following a muscle or ligament injury.

Benefits to UEFA

- The members of a professional football team can be genotyped in order to identify players with lower risk of injurability and shorter recovery times.
- Measures of injury prevention and methods of talent selection can be improved.
- Increasingly, teams are composed of players with different ethnic backgrounds. This study shows that patterns of injury differ according to race. With this information, teams can customize measures of injury prevention to the specific needs of their players.
- Ethnicity could be included in the epidemiological data collected by UEFA every year from all the member teams in order to further investigate the relationship of ethnicity with the types of injury suffered by the players.

Thanks to the support of the UEFA, this first ground-breaking study of genetics in professional athletes has been made possible.

Publications and presentations derived from this research project

- We presented our results in the XXXII World Congress of Sports Medicine: "Sports Medicine, the challenge for global health: Quo Vadis?" held in Rome, Italy on 27-30 September 2012. Appendix V shows the two abstracts and, the acceptance letters, as well as an excerpt from an invited lecture by Dr Y Pitsiladis, where he referenced our work.
- We also presented our results in the IV Muscletech Workshop held in Barcelona on 2-3 October 2012 (Appendix VI).
- 3. We currently have two manuscripts under review in different journals:
 - Single nucleotide polymorphisms in non-contact soft tissue injuries.Influence on degree of injury and recovery time
 - The potential impact of ethnicity on patterns of non-contact musculoskeletal soft tissue injuries
- 4. **4th European meeting on Sports Medicine and Exercise**, Florence, 22-23 March 2013:

"The importance of the genetic component in injury" (Appendix VII).

- We have submitted an abstract to ECOSEP 2013 to be held in Frankfurt, Germany, entitled "Importance of genetic variants and ethnicity in noncontact musculoskeletal soft tissue injuries. (Appendix VIII).
- Our work was unanimously awarded first prize in the 15th National Awards in Sports Medicine of the University of Oviedo (ppendix IX)

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Appendix I: Parameters analyzed in blood

SERVEIS MEDICS FCBarcelona							
Sang Hemostologia Hemograma complet Retirulocits ¹ Plaquetas T. Protrombina Fibrinògen TTP Siderenia BxSat Transferrina Parrilina Cap Fixferro Ac Fólic hurserit. Re Sol Transferrina Haptoglobina ¹ Transferrinenia Mioglobina ¹ VSG ¹⁻⁴ PCR ¹⁻⁴³ Gott Gett ¹⁻⁴ Ford Ringuinica Biogeninica Biogeninica Foof. Alcalines ¹ Pof. Alcalines ¹	Triglicènil s ¹⁻¹ Colesteral Total ¹⁻¹ Lípids Urea ¹⁻¹ Creatinina ¹⁻¹ Creatinina ¹⁻¹ Ac. Uric ¹ Proteinograma ¹ Magnesi ¹⁻¹ Magnesi ¹⁻² Caki Clor Pôsfor Equilibri Ac-Base Acidos grasos Omega 3	Estriol Bitradiol Projectiva Catecolamina Aldosterora ACTH IGF-1' Hitropoyetina Serologies Monanucleosis HIV Hegatilis (A,B,C) CMV Paretiditis Tétanos PPD					
Parámetros básicos ' Sediment ' Cultiu / Antibiograma ' Motiu de la petició: Metge: ' Fitzatge profesionals, ² Basir –	Sig natura: se guiment, ² Hemático-hemólisi.						

Appendix II: UEFA protocol for collection of data on injuries

Thigh Injury Card CHAMPIONS 4 Date of injury: •••••• Date of return to full participation: ••••• (Send-Injury-card-even-If-player-Is-still-In-rehabilitation)¶ Location -
 Anterior thigh (specify muscle(s)). τ. Posterior thigh (specify muscle(s)) ***** Injury-side¶ ■ Right → Type of injury 🔸 🛶 📋 Haematoma/contusion/bruise 🛶 🔲 Muscle-rupture/tear/strain 🛛 🛶 🔲 Tendon-rupture/tendinopathy¶ ■·Nerve injury→ → →
Overuse/hypertonia→ →
Other type ¶ Was this a re-injury?¶ □·No→ → → □·Yes·(give·date·of·return-from·previous·injury)·***** Was-the-injury-caused-by-overuse-(gradual-onset)-or-trauma-(acute-onset)?¶ Overuse -+ → 🔲 Trauma → Not applicable -+ When-did-the-injury-occur?¶ ■·Match· *** (min. of injury) → ■·Not applicable Training → -(overuse-Injury)¶ Indicate type of training or match where injury occurred □·Football training (F) → → □·Other training (O)·········· → Friendly match (F) --+ -+ -□·League·match(Ľ) → → → → □·UEFA·Champions·League·match·(CL)· + + Reserve/youth team training (R) + -+ UEFA Cup match (UC) → →
Other Cup match (C) → → National team training (N) -+ --+ -+ --+ + + -+ --+ -+ Reserve/youth team match (R)¶ -+ -+ + + National team match (N)¶ Not applicable — 1 Indicate-playing-position-at-time-of-injury¶ □·Goalkeeper→ → □·Defender → → □·Midfielder → → □·Forward¶ 1 Indicate surface where injury occurred Grass·(G) → → □·Artificial turf·(A) → □·Other surface·(O) → □·Not applicable¶ (overuse-Injury)¶ Was-the-injury-caused-by-contact-or-collision?¶ → □ Yes, with other player→□ Yes, with object (specify) ■ No→ → Injury-mechanism --□·Running/sprinting → □·Jumping/landing → □·Twisting/turning → □·Falling/diving• → ■ Kicked by other player Blocked → → Hit by ball ---+ □ Collision → + Heading → → □ Use of arm/elbow¶
 Tackled by other player □ Other acute mechanism¶
 Tackling other player □ Unknown mechanism¶ `**→** Stretching -+ Shooting -+ + -□ · Sliding → □ · Overuse → Passing/crossing -+ -+ Dribbling -+ --Injury-mechanism (describe in own words) Opponent foul Own-foul¶ -Red-card¶ Yellow-card + + (acute-match-injuries-only)-+ + Examination → → ¶ □·Clinical only → → □·X-rayı → → □·Ultrasonography¶ + ■·MRI·(enclose MRI·form) → □ Other (specify) •••••¶ Diagnosis (specify results of examination): ****** Treatment[.] Was injection therapy used? → 🗖 · No→ 🗖 · Yes (specify) ***** ¶

			Injury Ca
Name: Code no:	Team:		
Date of injury: Date of	of return to full participa	tion: er is still in rehabilitation)	
Injured body part Head/face Neck/cervical spine Sternum/upper back Abdomen Lower back/pelvis 	Shoulder/clavicula Upper arm Elbow Forearm Wrist Hand/finger/thumb	Hip/groin Thigh Knee Lower leg/ Ankle	Achilles tendon
Injury side Right	Left	Bilateral/c	entral
Type of injury Concussion Fracture Other bone injury Dislocation/subluxatic Sprain/ligament injury Other type (specify):	Lesion of meniscus/ca Muscle rupture/tear/st Tendon rupture/tendin Synovitis/effusion Overuse symptoms ut	artilage Haemator rain Abrasion opathy Laceratior Nerve inju nspecified Dental inju	na/contusion/bruise n ry ry
Diagnosis:			
Was this a re-injury?	Yes (give date of return Yes (give date of return)	n from previous injury)	
Was the injury caused by ove Overuse	eruse (gradual onset) or Trauma/acute	trauma (acute onset Not applic)? able
When did the injury occur?	🗖 Match 🔤 (min. of inju	iry) Diversion	able
Indicate type of training or m Football training (F) Other training (O) Football & other trainin Reserve/youth team t National team training	atch where injury occur ng (FO) raining (R) (N)	(breidsein) rred Friendly match (F) League match (L) UEFA Champions i UEFA Europa Leag Other Cup match ((Reserve/youth teal National team match	League match (CL) jue (EL) C) m match (R) ch (N)
Indicate playing position at ti	me of injury	Midfielder	Forward
Indicate surface where injury Grass (G)	occurred ☐ Artificial turf (A)	Other surface (O)	Not applicable
Was the injury caused by con	ntact or collision?	Yes, with object (sp	(overuse Injury) pecify)
Injury mechanism Running/sprinting Twisting/turning Shooting Passing/crossing Dribbling	 Jumping/landing Falling/diving Stretching Sliding Overuse 	 Hit by ball Collision Heading Tackled by other play Tackling other play 	☐ Kicked by other player ☐ Blocked ☐ Use of arm/elbow ayer ☐ Other acute mechanis er ☐ Unknown mechanism
Injury mechanism (describe ir	n own words)		
Referee's Sanction IN No f (acute match Injuries only)	oul Opp Vello	onent foul Cow card R	Dwn foul Red card
Other comments			

Appendix III: Protocol for DNA extraction from blood

- Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube
- Add 200 μl sample to the microcentrifuge tube. Use up to 200 μl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 106 lymphocytes in 200 μl PBS.
- 3. Add 200 μ I Buffer AL to the sample. Mix by pulse-vortexing for 15 s.
- 4. Incubate at 56°C for 10 min
- 5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
- Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
- 7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection ube (provided), and discard the tube containing the filtrate.*
- Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without etting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min.
- Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and iscard the collection tube containing the filtrate.*
- 10.Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g;14,000 rpm) for 3 min.
- 11.Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
- 12.Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water.
- 13.Incubate at room temperature (15–25°C) for 1 min, and then centrifuge 6000 x g (8000 rpm) for 1 min.



Appendix IV: Protocol for genotyping a sample

Appendix V: XXXII World Congress of Sports Medicine.

Single nucleotide polymorphisms (SNPs) in non-contact soft-tissue injuries: influence on degree of injury and recovery time

R. Artells¹, R. Pruna², J. Ribas^{1,3}, B. Montoro⁴, F. Cos⁵, C. Muñoz¹, G. Rodas³, M. Monzo¹.

 Unitat d'Anatomia i Embriologia Humana-Facultat de Medicina-UB. 2. Serveis Mèdics del Futbol Club Barcelona. 3. Escuela de Medicina Deportiva-Facultat de Medicina-UB.4. Departament de Farmacia i Tecnología Farmacèutica-Facultat de Farmàcia-UB. 5. INEF-UB

Introduction: In recent years, studies have highlighted the importance of genetic factors in the pathogenesis of non-contact soft tissue injuries. We have analyzed the relationship between SNPs in genes related to tissue repair and regeneration and the frequency and recovery time of non-contact tissue injuries

Material and Methods: Data was collected on injuries in 73 professional European football players, including type and degree of injury and recovery time. In blood extracted from the 73 football players, SNPs in the following 8 genes were analyzed: Elastin (Eln); Titin (TTN); SRY-related HMG-box (SOX15); Insulin-like growth factor 2 (IGF2); Chemokine, CC motif, ligand 2 (CCL2); Collagen type 1 alpha 1(COL1A1); Collagen type 5 alpha 1 (COL5A1) and Tenascin C (TNC). SNP analysis was performed using a real-time polymerase chain reaction (PCR) Allelic Discrimination TaqMan Assay.

Results: 242 injuries were recorded (203 were muscle, 24 were joint, and 15 were tendon). The degree of muscle injury was related to IGF2 (P=0.032). Moreover, we observed a close but non-significant relation between degree of muscle injury and CCL2 (P=0.1) and COL5A1 (P=0.07) and between Eln and degree (P=0.09) and recovery time (P=0.089) of joint injuries. Non-significant relation was observed between genes and degree or recovery time for tendon.

Conclusion: These results may be due to several factors. Eln is a major source of tissue elasticity, and IGF plays an important role in mitogenesis and myogenesis during muscular development, regeneration and hypertrophy and in readaptation processes. COL5A1 modulates fibrillogenesis, and CCL2 expression is known to increase after muscle injury, indicating a key role in inflammatory processes.



of SPORTS MEDICINE ROMA 27-30 September 2012

Sports Medicine, the challenge for global health: Quo Vadis?

The Italian Sports Medicine Federation (FMSD)

Congress Presidents Naurizio CASASCO - Fabio PIGOZZE

Organising Committee Executive Board FMSI

Local Scientific Committee The Chairman and Members of the FMSI Scientific Commission

International Scientific Committee The Chairman and Members

of the FIMS Scientific Commission

fmS

XXXII World Congress of Sports Medicine "Sports Medicine, the challenge for global health: Quo Vadis?" (Rome 27-30 September 2012)

Dear Mrs. ARTELLS,

We are pleased to inform you that your abstract entitled "SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN NON-CONTACT SOFT-TISSUE INJURIES: INFLUENCE ON DEGREE OF INJURY AND RECOVERY TIME" has been accepted for presentation in a **Poster Session** at the "XXXII World Congress of Sports Medicine".

Your presentation is scheduled as follows:

Presenting Author: ARTELLS R. Session: POSTER SESSION 2 Session Date/Time: 28-09-2012 from 14:30 to 15:30 Board Number: 232

Session Details:

Attendees consider the poster sessions an important and valuable part of the educational program of the World Congress of Sports Medicine. Therefore, the Scientific Committee has assigned a Chairperson to oversee the poster sessions.

You must hang your poster on the assigned board from 9 a.m. to 2 p.m. and remove your material from 3:30 p.m. to 5:30 p.m. Authors must strictly respect the above mentioned timing for hanging and removing posters, otherwise the Organizing Secretariat should not be addressed for any responsibility in posters' loss.

Please prepare your poster according to the "Poster Presentation - Session Format" as follows. The Poster size is height 120 cm, width 90 cm. Poster format is portrait.

You are required to be present at your poster during the entire session, which is listed above.

Please be advised, per Scientific Committee policy, only the first author may withdraw the abstract.

Failure to present is considered a no-show. Any "no shows" among posters disappoints attendees and detracts from the program.

Organizing Secretariat: AIM GROUP INTERNATIONAL Rome Office: Via Flaminia 1068 - 00189 Rome - Tel +39 06 330531 - Fax +39 06 33053229

Rome, July 19th 2012

The effect of ethnicity on the distribution of single nucleotide polymorphisms (SNPs) in genes related to tissue damage, repair and recovery in sport

J. Ribas^{1,2}, R. Artells¹, R. Pruna³, B. Montoro⁴, F. Cos⁵, C. Muñoz¹, G. Rođas³, M. Monzo¹.

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Introduction: The interaction between extrinsic and intrinsic variables, including genetic factors, can have an important effect on non-contact tissue injuries. We have analyzed for the first time the frequency of SNPs in genes related to tissue repair and regeneration and compared the frequencies observed in different ethnic groups. **Material and Methods**: SNPs in the following 8 genes were analyzed in blood extracted from 73 professional European footbl1 players: Elastin (Eln); Titin (TTN); SRY-related HMG-box (SOX15); Insulin-like growth factor 2 (IGF2); Chemokine, CC motif, ligand 2 (CCL2); Collagen type 1 alpha 1(COL1A1); Collagen type 5 alpha 1 (COL5A1) and Tenascin C (TNC). SNP analysis was performed using a real-time polymerase chain reaction (PCR) Allelic Discrimination TaqMan Assay. **Results**: The subjects included 43 Caucasians, 11 Blacks and 9 Hispanics. Significant (P<0.001) inter-racial differences were observed in the frequencies of the SNPs of each of the 8 genes.

Conclusion: Epidemiological data have demonstrated the existence of interindividual differences both in the degree of injury and in recovery time. The significant inter-racial differences for the 8 genes observed in the present study should be considered when studying these interindividual differences. Further studies with a larger sample size are warranted to correlate these inter-racial differences with degree of injury and recovery time.



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Despite the preliminary nature of our results, they were presented at the **XXXII World Congress of Sports Medicine** and were well received. Dr. Y. Pitsiladis cited our work in his invited oral presentation and encouraged us to continue this novel line of research.



Appendix VI. IV Muscletech Workshop



	Wednesday October 3r
14:30 - 17:00 Next Challenges	•
 CHAIRS: Enric Caceres. Clinical Director Trauma Centre Hospital Vall d'Hebron. Chair Autonomous I Henning Langberg. Associate Professor and lecturer at the Institute of Sports Medicine, Bisp Geoffrey Verrall. Professor at the University of Adelaide, Australia and Sports Physician S 	Jniversity of Barcelona, Associate Professor Johns Hopkins University. Baltimore ebjerg Hospital, School of Health Sciences, University of Copenhagen, Copenhagen, Denm specialist in hip and groin injuries, muscle injuries and sports medicine, Australio
MTN 2012 projects	
PRESENTATION OF THE MAGAZINE FEM	
Xavier Gassó, Director of Health Management at "Mútua d'accidents EGARSAT"	
MUSCLE ENERGY EFFICIENCY DURING SPRINT EXERCISE	
José A. López Calbet, Professor of the Department of Physical Education. University of Las Palm	nas de Gran Conaria, Las Palmas de Gran Conaria, Spain
INTERMITTENT HYPOBARIC HYPOXIA AND INDUCED MUSCLE DAMAGE IN RATS	
Ginés Viscor, Professor at the Physiology Department, School of Biological Sciences, University of E	larcelona, Barcelona, Spain
MUSCLE AND TENDON INJURY BIOMARKERS	
 Roser Cussó, Professor at the Department of Physiological Sciences I. Biochemistry and Molecular E University of Barcelona. Barcelona. Spain 	ialogy Unit. Metabolic Regulation and Molecular Pathology Group. School of Medici
LONGITUDINAL STUDIES OF HAMSTRING MUSCLE INJURIES IN ATHLETES.	
• Ramon Balius, Research Leader at the High Performance Sports Research Centre (CEARE), Catala	n Sports Council, Barcelona, Spain
 M^a Isabel Miguel, Professor at the Department of Pathology and Experimental Therapeutics, Res University of Barcelona, Barcelona, Spain 	earch Unit on Muscle Anatomy and Pathology, School of Medicine (Campus Bellvitg
Xavier Alomar, Director of the Diagnostic Imaging Department at Creu Blanca Clinic, Barcelona, Sp.	nain
INFLUENCE ON INIURY LIKELIHOOD AND INIURY RECOVERY TIME OF SINGLE NUCLEOTIDE POLYMORPHIS	IMS (SNPS) IN GENES INVOLVED IN CONNECTIVE TISSUE REPAIR
Rosa Artells. Senior Researcher at the Unit of Human Anatomy and Human Embryology. School of	Medicine, University of Barcelona, Barcelona, Spain

Our results were reported in the sports newspapers Mundo Deportivo (04/10/2012) and Diario Marca.com

- 1. <u>http://www.mundodeportivo.mobi/slowdevice/20121003/fc-barcelona/ricard-</u> pruna-conferencia-perfiles-geneticos_54352249857.html
- 2. <u>http://www.marca.com/2012/10/03/futbol/equipos/barcelona/1349279192.html?a</u> =2a78e20c3fea7561d1e734ed36afe334&t=1349344684



Appendix VII. European meeting on Sports Medicine and Exercise





Importance of genetic variants and ethnicity in non-contact musculoskeletal soft tissue injuries.

Ricard Pruna¹; Rosa Artells²; Jordi Ribas^{2,3}; Bruno Montoro⁴; Mariano Monzo²

¹Serveis Mèdics del Futbol Club Barcelona, ²Unitat d'Anatomia i Embriologia Humana-Facultat de Medicina-UB, ³Escola de Medicina Esportiva-Facultat de Medicina-UB, ⁴Departament de Farmacia i Tecnologia Farmacèutica-Facultat de Farmacia-UB.

Background: The prevention, diagnosis, and management of noncontact musculoskeletal soft tissue injuries (NCMSTI) related to participation in sport are key components of sport and exercise medicine physician. Epidemiological data have demonstrated the existence of interindividual differences in NCMSTI severity indicating that those injuries occur as a consequence of a combination of both, extrinsic and intrinsic factors, including genetic variations.

Methodology: We examined 8 single nucleotide polymorphisms (SNPs) in the following genes (ELN, TTN, SOX15, IGF2, CCL2, COL1A1, COL5A1 and TNC) related to tissue recovery and tissue repair in a total of 73 elite european football players population from white, black-african and hispanic origin and, at the same time, we have collected NCMSTI suffered by these population during three consecutive seasons. Finally we have correlated results with type, degree and recovery time in injuries collected and then with the distribution of injuries in the three studied populations.

Results: 242 injuries were recorded (203 were muscle, 24 were ligament, and 15 were tendon). The degree of muscle injury was related to IGF2 (P=0.032). Moreover, we observed a close but non-significant relation between degree of muscle injury and CCL2 (P=0.1) and COL5A1 (P=0.07). We also found a close relation between ELN and degree (P=0.09) and a significant relation between ELN and recovery time (P=0.027) of ligament injuries.

The frequency of the SNPs varied among the three sub-groups in the present study (p<0.0001). Our results shown a significant relation between ligament injuries and ELN (p=0.001) and a significant relation between tendinous injuries and ELN (p=0.05) and IGF2 (p=0.05) in white

population. Moreover, we have observed a significant relation between muscle injuries and ELN (p=0.032) and IGF2 (p=0.016) in Hispanic population.

Conclusions: SNPs study constitutes a new field of investigation in Sports Medicine which will help us to identify individuals with shorter recovery and those with greater risk of injury. Our results are only the pave to demonstrate that these genotyping interracial differences are important when studying injuries and let us to indicate that the genetic profile based on the SNPs can be use to describe, as objectively as possible each individuals injurability risk and may well be a useful tool for football players to receive a more specific treatment and preventive care options.

Appendix IX: XV Premio Nacional de Investigación en Medicina Deportiva de la Universidad de Oviedo

http://www.uniovi.es/prensa/actualidad/-/asset_publisher/0001/content/la-universidad-de-oviedo-concede-el-premioen-medicina-del-deporte-a-una-investigacion-sobre-

lesione?redirect = %2Fweb%2Funiovi%2Fprensa%2Factualidad%2Ftags%252

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Todos 2013 2012 2011 2010	2009 2008 2007	2006 2005 2004	
			« <u>Atrás</u>
La Universidad concede el Premio en Medio una investigación sobre lesiones en futboli	cina del Deporte a stas profesionales		18/2/2013

El trabajo ganador, que recibirá un galardón de 6.000 euros, establece la predisposición a padecer determinadas lesiones y el tiempo de recuperación



Los investigadores <u>Rosa Artells i Prats, Jordi Lluis Ribas Fernández y Ricard Pruna Grivé</u>, que desarrollan su labor profesional en la Universidad de Barcelona y en el F. C. Barcelona, han resultado ganadores del XV Premio Nacional de Investigación en Medicina del Deporte, que concede la Universidad de Oviedo a través de la Escuela de Medicina del Deporte, con el patrocinio de Cajastur. El trabajo que les ha hecho valedores del Premio, dotado con 6.000 euros, establece la predisposición a padecer determinadas lesiones y el tiempo de

recuperación. El estudio se realizó sobre muestras de sangre de futbolistas profesionales, y se centró en las lesiones sin contacto, no traumáticas.

El jurado, presidido por Mª Paz Suárez Rendueles, vicerrectora de Investigación y CEI de la Universidad de Oviedo, concedió el segundo galardón, dotado con 3.000 euros, a una investigación sobre la condición física y la calidad de vida en pacientes con anorexia nerviosa restrictiva, elaborado por un equipo de la Universidad Europea de Madrid y del Hospital Infantil Universitario Niño Jesús de la capital. Por su parte, el tercer premio fue para un estudio sobre la influencia negativa que tiene la fatiga del personal de salvamento (tras un rescate acuático) sobre la calidad de la reanimación cardiopulmonar, que contó con participación de la Universidad de Oviedo, entre otras instituciones.

A la convocatoria de este año del Premio se presentaron 26 trabajos de diferentes grupos de investigación, tanto nacionales como internacionales. Entre los criterios de valoración se tuvo en cuenta el esfuerzo científico, la calidad y el impacto de la investigación, y el establecimiento de puentes entre la investigación básica y la aplicada en beneficio de los pacientes.