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The impact of single nucleotide polymorphisms on patterns of non-contact musculoskeletal soft tissue injuries in a football player population according to ethnicity

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ABSTRACT

Background and objective: The prevention, diagnosis, and management of non-contact musculoskeletal soft tissue injuries (NCMSTIs) related to participation in sports are key components of sport and exercise medicine. Epidemiological data have demonstrated the existence of interindividual differences in the severity of NCMSTIs, indicating that these injuries occur as a consequence of both extrinsic and intrinsic factors, including genetic variations.
Subjects and methods: We have collected data on NCMSTIs suffered by 73 elite players of White, black African and Hispanic ethnicity of European football over the course of three consecutive seasons. We have also examined eight single nucleotide polymorphisms (SNPs) in genes related to tissue recovery and tissue repair in blood drawn from the players and correlated our findings with type and severity of injuries in each ethnic group.
Results: The frequency of the SNPs varied among the three ethnic sub-groups ($p < 0.0001$). Among Whites, a significant relationship was observed between ligament injuries and ELN ($p = 0.001$) and between tendinous injuries and ELN ($p = 0.05$) and IGF2 ($p = 0.05$). Among Hispanics, there was a significant relation between muscle injuries and ELN ($p = 0.032$) and IGF2 ($p = 0.016$).
Conclusions: Interracial genotypic differences may be important in the study of NCMSTIs. A genetic profile based on SNPs may be useful tool to describe each individual's injuribility risk and provide specific treatment and preventive care for football players.

Impacto de los polimorfismos de nucleótido único en los patrones de lesiones de tejido muscular esqueletico, sin mecanismo de contacto, en una población de jugadores de fútbol según la etnia

Palabras clave:
Polimorfismos genéticos de un solo nucleótico
Raza
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RESUMEN

Fundamento y objetivo: La prevención, el diagnóstico y el tratamiento de las *non-contact musculoskeletal soft tissue injuries* (NCMSTI, «lesiones musculoesqueléticas producidas por el mecanismo de no contacto») son factores clave en el deporte y en la medicina deportiva. La interacción entre factores extrínsecos e intrínsecos, incluyendo en estos últimos los factores genéticos, es determinante en la causalidad de las NCMSTI.
Sujetos y métodos: Se han recogido las lesiones sufridas por 73 jugadores de fútbol profesional de diferentes razas (caucásicos, africanos subsaharianos e hispánicos), ocurridas durante 3 temporadas consecutivas. Se analizó la presencia de *single nucleotide polymorphisms* (SNP, «polimorfismos genéticos

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de un solo nucleótido») en un conjunto de genes relacionados con la reparación y la regeneración del tejido a partir de sangre obtenida de los jugadores, y se correlacionó con el tipo y grado de lesión en cada grupo racial.
Resultados: La frecuencia de aparición de los SNP varía en las 3 poblaciones estudiadas ($p < 0{,}0001$). En cuanto a la población caucásica, se observa una relación estadísticamente significativa entre lesiones ligamentosas y ELN ($p = 0{,}001$) y entre lesión tendinosa y ELN ($p = 0{,}05$) e IGF2 ($p = 0{,}05$). En cuanto a la población hispánica, existe una relación estadísticamente significativa entre la lesión muscular y ELN ($p = 0{,}032$) e IGF2 ($p = 0{,}016$).
Conclusiones: Las diferencias genotípicas interraciales pueden ser importantes en el estudio de las NCMSTI. Un perfil genético basado en los SNP podría ser una herramienta útil para describir el riesgo individual de un individuo a lesionarse y poder aplicar de esta manera los tratamientos preventivos adecuados.

Introduction

Epidemiological studies are the most reliable source of our current knowledge of injuribility.[1–3] However, few serious studies on the etiology of injuries have been performed,[4] and no scientific evidence has yet conclusively identified potential risk factors related to injuribility. In recent decades, studies have begun to focus on the importance of the genetic component in the pathogenesis of non-contact musculoskeletal soft tissue injuries (NCMSTIs).[5–7] There is great interindividual variation in degree of injury, response to treatment and recovery time to a specific kind of injury.[8] Several studies have demonstrated that the presence of certain single nucleotide polymorphisms (SNPs) in genes involved in tissue repair of muscles,[9] tendons[10,11] and ligaments[12] can enhance recovery after injury.[13,14]

Recent studies have shown that some diseases follow different courses in different ethnic groups.[15,16] For example, in a study examining differences in response to EPO therapy according to hemoglobin levels in patients with kidney disease and anemic complications, significant differences were observed in hemoglobin levels between the African Caribbean and the White populations. The authors concluded that these differences could affect treatment efficacy.[15] In addition, the frequency of certain SNPs varies among different ethnic groups.[17–19] In the world of professional sports, and especially in professional football, the constant search for the best players for each playing position has led to teams being made up of players from different countries and different ethnic groups.

In our previous study on the relation between SNPs and NCMSTIs in professional European football players,[20] we had recorded a total of 242 injuries (203 muscle, 24 ligament, and 15 tendon injuries) and found a statistically significant association between degree of injury and the IGF2 genotype ($p = 0.032$). In addition, there was evidence of a statistically significant association between degree of muscle injury and CCL2 ($p = 0.013$). Finally, we also found a significant relationship between ELN and recovery time ($p = 0.027$).

Based on these earlier results and given the importance of ethnicity in determining the frequency of certain SNPs, we chose to extend our previous work by examining the relationship between ethnicity, SNPs and NCMSTIs. We have examined eight SNPs in genes related to soft tissue repair and regeneration in blood drawn from the players and correlated our findings with type and severity of injuries in each ethnic group.

Materials and methods

Ethics statement

The study was approved by the Ethics Committee of the Hospital Clinic, Barcelona (registry no. 2012/7117).

Subjects and epidemiological data on injuries

The study comprised a total of 73 players of White, Black-African and Hispanic origin from Futbol Club Barcelona (Barcelona, Catalonia, Spain). Data were collected on NCMSTIs suffered by these football players over the course of three consecutive seasons (2009–2012) in accordance with the Union of European Football Association (UEFA) protocols.[21] Imaging techniques, such as ultrasounds and nuclear magnetic resonance (NMR), were used to morphologically classify the injuries by anatomic region. In addition, injuries were classified as mild, moderate or severe[22] according to their severity and the number of days that a player needed to be absent from training and/or competition.[23,24]

DNA extraction

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

Allelic discrimination analysis

We genotyped eight genes related to tissue repair (elastin [ELN]),[25] muscle assembly and force transmission (titin [TTN]),[26] skeletal muscle regeneration (SRY-related HMG-box [SOX15]),[27] muscle damage (insulin-like growth factor 2 [IGF2]),[28] response to muscle damage (chemokine, CC motif, ligand 2 [CCL2]),[29] ligament ruptures ([COL1A1] and collagen type 5 alpha 1 [COL5A1]).[30] and tendinopathy (COL5A1 and tenascin [TNC]).[7,31] Table 1 shows the position of each SNP in its gene.

Primers and probes were designed for the region of DNA according to the supplier's instructions and were obtained from Applied Biosystems (Applied Biosystems, Assays-on-Demand SNP genotyping product, Foster City, CA, USA).

Table 1
Genes examined with the position of each SNP.

Gene	SNP position
ELN	6124052T>C
TTN	89464A>G
SOX15	392C>T
IGF2	13790C>G
CCL2	G7319001G>C
TNC	46973317T>A
COL1A1	6252G>T
COL5A1	643223C>T