

New Tendencies in Risk Factors for Soft Tissue Injuries: Genetic Biomarkers

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Received: February 22, 2015

Revised: March 17, 2015

Accepted: March 32, 2015

Published online: June 23, 2015

ABSTRACT

The prevention, diagnosis and treatment of injuries are key factors in the daily practice of Sports Medicine. Prevention programs are the main objective, in order to minimize the effect of risk factors and avoid a high incidence of injury and decrease injury severity. The multifactorial origin of injuries complicates the identification of risk factors both, extrinsic and intrinsic. In recent years it has begun to emerge the importance of the genetic component (as an intrinsic factor) of each individual as a possible cause of injury predisposition. We have reviewed genetic studies related to connective tissue repair and regeneration. This information could be very useful in order to individualize the preventive strategies and to optimize the therapeutic and rehabilitation process.

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Key words: Polymorphisms; Sport injuries; Genetic biomarker

Pruna R, Artells R. New Tendencies in Risk Factors for Soft Tissue Injuries: Genetic Biomarkers. *International Journal of Orthopaedics* 2015; 2(3): 307-311 Available from: URL: <http://www.ghrnet.org/index.php/ijo/article/view/1055>

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 studies 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 index^[1-4], 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 injuries^[3] suffered by athletes. In spite of the fact that small injuries can be completely cured, recovery from more serious muscle injuries causes a scarring in the muscle tissue that decreases muscle function and can lead to muscle contractions and chronic pain^[2].

The prevention, diagnosis and treatment of injuries are key factors in the daily practice of sports medicine due to their great importance in high-level sports^[5]. Although in clinical practice we see different responses to identical treatments, we continue to apply static prevention protocols and these prevention programs become the main objective, in order to minimize the effect of risk factors and avoid a high incidence of injury and decrease injury severity, but they would have to be specific as well.

The multifactorial origin of injuries complicates the identification of risk factors, (extrinsic and intrinsic factors)^[6-9] and taken together, these factors and their interaction predisposes the athlete to injuries. Despite rigorous control over many extrinsic and intrinsic factors, a wide range of interindividual differences exists in number and degree

of injuries and in recovery time^[10] suggesting that other factors, including genetic variations may exert an important influence on these differences^[11-13].

CONNECTIVE TISSUE

Nowadays soft tissue injuries are considered as connective tissue injuries, where collagen is the common structural basis in muscles, tendons and ligaments, forming a microenvironment where interactions with other environmental factors will determine the injury.

Normal ligaments and tendons are collagen structures, having a similar composition with slight variations. Both tissues are hypocellular, with <5% of total volume occupied by cells, and relatively hypovascular^[14]. Tendons and ligaments are composed of almost exclusively of collagen (COL): type 1, 3 and 5. The different groups and densities of these subtypes allow us to distinguish between cordonal and band-like ligaments, or tendons. Tendons are composed mainly by COL1, highly resistant to the loads. The COL3 is characterized by being slightly resistant to stress. The tendon degeneration result of inadequate assimilation of loads due to the transformation of COL1 to COL3^[15].

Skeletal muscle consists of slow twitch type I, rich in COL1 (resistant) and type II or fast fibers, rich in COL3 (brittle)^[16]. We suspect that this histological tissue constitution has a connection with the highest incidence of the second type lesional fiber, and even the quality collagen itself, which we know is genetically determined^[17,18], and it's a direct cause in the production of the lesion.

Again note that genetic factors may explain the cause of the injurability. One of the most widely studied genetic characteristics is the presence of genetic polymorphisms (SNPs).

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS)

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-coding), exonic (coding), 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).

Certain SNPs promote tissue repair and regeneration^[19-21]. 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 fully. Recent studies have found that SNPs in genes involved in the repair of connective tissue may explain this variation in recovery time^[19-23].

SEARCH OF NEW BIOMARKERS

As reported previously, epidemiological studies are the most reliable tool to classify and describe the injurability index in a group of elite athletes^[24] and no other kind of studies have been carried out to explain the etiology and recovery time of injuries.

To date, an variable index of injurability has generally been established based on the blood analysis of the players. Every two months, blood is taken and analyzed for levels of hemoglobin, ferritin, creatinine, urea, transaminases, CK levels and other

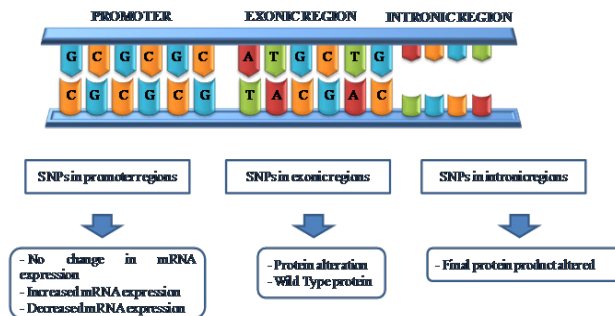


Figure 1 Possible consequences of the presence of SNPs.

components. An athlete is considered to be in a fragile state when hemoglobin levels are below 15 gr/dL, when ferritin levels are below 50 ng/mL, when urea and creatinine levels are higher than the reference levels, when transaminase levels are higher than 60 U/L or when CK levels are higher than 600 U/L. GGT levels are used to detect a possible muscle injury caused by damaged liver.

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 correlate the severity of an injury and the recovery time needed for the injury (both of which can vary greatly).

The study of SNPs in genes related to connective tissue repair and regeneration 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.

NEW GENETIC BIOMARKERS: STATE OF THE ART

Muscle Injuries

Recently have been described a number of genetic polymorphisms associated with variability of muscle damage^[25] caused by the stress, including kinase genes of the myosin light chain (MYLK), the α -actinin-3 (ACTN3), and the factor II insulin-like growth (IGF2)^[26,27].

α -Actinins (ACTN) are one of the most commonly studied SNPs. The major role of ACTN is its effect on muscle metabolism^[28]. The presence of an SNP in ACTN3 gene (rs1815739) encodes a premature stop codon leaving individuals with 2 copies of the G allele (homozygous GG) creating a non-functional protein which influences the function of human skeletal muscle^[29] (Table 1).

In a previous work from our group^[18] we have observed a significant relation between IGF-II (rs3213221) and CCL2 (rs2857656) and the severity of muscle injuries. Football players with GC IGFs genotype were protected to suffer severe muscle injuries ($p=0.034$). Players with CC/GC CCL2 genotype suffered less severe injuries than players with GG CCL ($p=0.026$) (Table 1). IGFs play a role in soft tissues growth, and increase their expression in response to degeneration and regeneration following an injury, when IGFs work with other genes, such as fibroblastic growth factor, interleukin 1 β , interleukin 6, and transforming growth factor- β , to influence satellite cell activation^[30-32]. CCL2 is a small chemokine produced by both macrophages and satellite cells^[33], and plays key roles in inflammation and immunoregulation^[21]. CCL2 expression increases dramatically following muscle damage, and recent data suggest that it plays significant roles in muscle damage, muscle repair and adaptation^[21,23]. SNPs in CCL2 have been related to markers of muscle injury, such as creatine kinase and myoglobin levels and muscle pain.

Table 1 SNPs in described genes with associated alteration in soft-tissues.

| GEN | RS IDENTIFICATION | INDICATOR OF |
|--------|-------------------|--|
| ACTN 3 | rs1815739 | muscle hypertrophy |
| IGF-II | rs3213221 | muscle damage |
| CCL2 | rs2857656 | muscle damage response |
| COL5A1 | rs12722 | ligament ruptures/risk of tendinopathy |
| TNC | rs2104772 | risk of tendinopathy |
| ELN | rs2289360 | tissue repair and elasticity |

Ligament and Tendon Injuries

Well it is known about the genetic component that causes Aquilles tendinopathy, especially those SNPs in COL5A1 (rs12722) and Tenascin (TNC) in physically active populations^[34-36] (Tabla 1).

TNC is a glycoprotein initially discovered in ECM mitotendinous union and is abundantly represented in tissues subjected to high tension and compression^[37]. TNC is found abundantly in the tendon, but not only around pericellular collagen fibers of the tendon belly and its expression is strongly up-regulated in degenerative diseases of the tendon, especially at sites subjected to excessive mechanical forces^[38].

Mokone *et al*^[36] observed that a GT dinucleotide repeat polymorphism in intron 17 of the TNC gene is associated with both chronic tendinitis, as with the Achilles tendon rupture

Type V collagen (COL5A1) makes up approximately 10% of the collagen content in ligaments and intercalates with the COL1 and COL3 to form heterotypic fibrils, where it plays an important role in regulating fibrillogenesis and lateral growth^[39]. Tendinopathy studies indicate that two copies of C allele (CC) are necessary in COL5A1 for this process to be successful^[40].

SNPs in the COL5A1 are associated with an increased risk of ACL tears in female athletes, where the CC genotype decrease the risk of this type of injury^[41].

In a previous work from our group^[18] we have found a SNP in elastin (ELN) gene that correlates with a significant statistical association with degree and recovery time for ligament injuries. ELN (Table 1) is a self-assembling extracellular matrix protein which is the major source of tissue elasticity. We observed that individuals with the ELN AA gentye suffered more severe injuries and longer recovery times tahn those with AG or GG genotypes. A previous study^[42] evaluating the relationship of the ELN SNP with age and arterial compliance in 320 individuals with no symptoms of cardiovascular disease found that carriers of A allele had decreased arterial compliance compared to those with the GG variant, due to an altered elastogenesis. In this line, the shorter recovery time observed in individulas that present G allele (AG/GG) may be related to a more efficient elastin function^[43].

Efficacy of Growth Factors in the treatment of Sport Injuries

Tissue repair in musculoskeletal injuries is often a slow and sometimes incomplete process. Musculoskeletal injuries have

Table 2 Growth Factors and associated function*.

| GROWTH FACTOR | COMPLETE NAME | FUNCTION |
|---------------|---|--|
| PDGF | platelet- derived growth factor | angiogenesis collagen synthesis |
| TGFB | transforming growth factor β | fibroblast proliferation collagen synthesis |
| PDEGF | platelet- derived epidermal growth factor | cellular proliferation cellular differentiation |
| VEGF | vascular endothelial growth factor | vascularization |
| IGF-I | insulin growth factor 1 | soft-tissue growth |
| FGF | fibroblastic growth factor | angiogenesis wound healing |
| EGF | epidermal growth factor | cell growth cell proliferation |

*modified from Pruna et al, 2014. MTLJ.

a great impact on athletes – especially elite athletes – and a rapid recovery of full efficiency and return to competition is of primary importance. The search for a minimally invasive treatment of these injuries is of great importance, especially in the world of sports. The use of growth factors is thought to be useful in clinical practice because it promotes rapid healing with a high-quality tissue and allows an early and safe return to unrestricted activity. Platelet-rich plasma (PRP) is a simple and minimally invasive way to obtain a natural concentration of autologous growth factors, including IGF, EGF, TGF β 1, FGF2 (Table 2). PRP is currently being widely experimented in different fields of medicine due to its ability to help in the regeneration of tissue with low healing potential.

Since PRP was first introduced as topical adjuvant therapy to treat chronic leg ulcers in the late 1980s^[44], it use has been extended to many fields of medicine, such as dermatology, ophthalmology^[45], dentistry^[46] and maxillofacial surgery⁴⁶. Recently, PRP injections have emerged as a fashionable non-invasive treatment also in sports medicine^[47], where they are used to treat acute or chronic tendinopathy^[48-51] and muscle^[52,53] and ligament^[54,55] injuries, because PRP provides numerous growth factors needed to promote the healing process.

In recent years there has been increasing research into genes related to the healing of soft tissue. Cytokines and growth factors are soluble signaling proteins that affect the process of normal wound healing^[56]. The inflammatory stage is guided by inflammatory cells that release multiple cytokines that induces the expression of fibroblasts and endothelial cells and cause macrophage expression of more cytokines. Early wound repair includes three steps which occurs simultaneously; epithelization, neoangiogenesis and prematrix formation and is controlled by growth factors^[57]. Due to the high implication of these molecules in this important process, it is important to analyze SNPs in genes that codify for these growth factors to elucidate if they could influence treatment effectiveness and explain differences observed in recovery times^[58] (Figure 2).

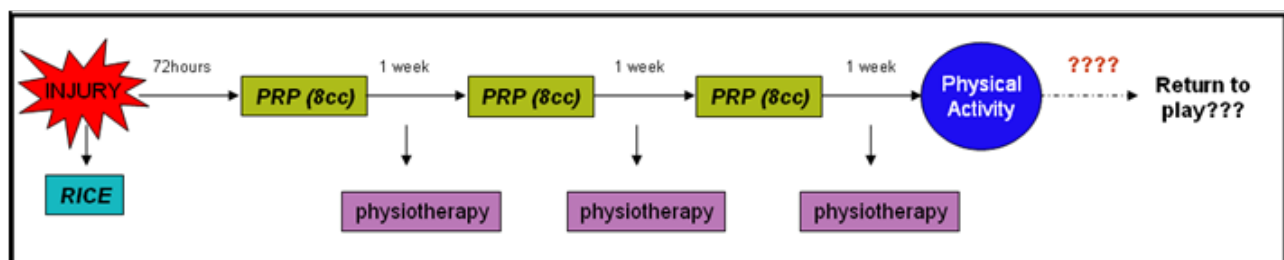


Figure 2 Example of a possible PRP treatment protocol.

TAKEHOME MESSAGE

The genetic profile based on the SNPs identified can be used to better define the risk of injury of a given individual, possibly helping in players' selection, and allowing more specific treatment and prevention. It may also help to identify individuals who will require a longer recovery following injuries.

In this editorial we have focused our attention in genetic studies related to connective tissue repair or regeneration and pave the way to future Sports Medicine research. This information could be very useful in order to individualize the preventive strategies to prevent injury and to optimize the therapeutic and rehabilitation process after injuries.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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