Genetics in Orthopaedic Practice

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ABSTRACT

DNA holds genetic information in the nucleus of eukaryotic cells; and has three different functions: replication, storage of hereditary information, and regulation of cell division. Most studies described the association of single nucleotide polymorphism (SNP) to common orthopaedics diseases and the susceptibility to develop musculoskeletal injuries. Several mutations are associated with osteoporosis, musculoskeletal ailments and other musculoskeletal deformity and conditions. Several strategies, including gene therapy and tissue engineering with mesenchymal stem cells (MSC), have been proposed to enhance healing of musculoskeletal tissues. Furthermore, a recent technique has revolutionized gene editing: clustered regulatory interspaced short palindromic repeat (CRISPR) technology is characterized by simplicity in target design, affordability, versatility, and high efficiency, but needs more studies to become the preferred platform for genome editing. Predictive genomics DNA profiling allows to understand which genetic advantage, if any, may be exploited, and why a given rehabilitation protocol can be more effective in some individual than others. In conclusion, a better understanding of the genetic influence on the function of the musculoskeletal system and healing of its ailments is needed to plan and develop patient specific management strategies.

Key Words: CRISP, DNA, Genetics, Muscles, Rehabilitation, Tendon.

INTRODUCTION

Cell biology and genetics are rapidly evolving basic science fields currently being explored to provide a better understanding of the defects underpinning musculoskeletal diseases. Much research and development pertains to orthopaedics, and the study of genomics is the foundation to personalized medicine.

DNA is composed of two nucleotide chains forming a double helix, each consisting of a deoxyribose sugar–phosphate (phosphodiester bonds) backbone with bases bonded with complementary bases on the opposite chain. Eukaryotic cells host many DNA types mostly located in the nucleus (Table 1). DNA has three cellular functions: replication (the two DNA strands separate, and each serves as a template for building a new complementary strand), hereditary information (every base pair and nucleotide sequence is necessary for build and maintain the organism), and regulation of cell division (through the expression of mRNA) (Figure 1).

Nucleotides are the structural units of RNA and DNA. The current human genome sequence contains 2.85 billion nucleotides interrupted by only 341 gaps. It covers 99% of the euchromatic genome, and is accurate to an error rate of one per 100 000 bases [1].

The 46 human chromosomes, consisting of both DNA and RNA, are located in the nucleus of every cell: 44 autosomes, determining somatic characteristics, and two allosomes, responsible for sexual characteristics [1].

The Human Genome Project, started in 1990, produced a complete sequence in 2003: there are only 20 000–25 000 protein-coding genes, contrary to the expectation of as high as 2 000 000[1].

GENOMICS OF ORTHOPAEDIC CONDITIONS

At the beginning of this millennium, orthogenomics was born [2–5]. The importance of genomics in future orthopaedic practice was mentioned, but implementation has been slow. Strategies were suggested to identify the genetic bases of diseases, such as those with a significant genetic component (osteoarthritis), with underdeveloped surgical or medical treatments (disk degeneration), and those affecting large population (infection) [2–4] (Table 2).

A recent review described the application of SNPs analysis in sports trauma, and discussed dosage effects between polymorphic collagen genes and Achilles tendinopathy or Ehlers-Danlos syndrome [6,7]. Most of the existing studies are published in non-orthopaedic journal. For example, information regarding bone-related cancers focuses on pathologic identification and chemotherapeutic management rather than on surgical management [5].

Paediatric osteosarcomas and Ewing sarcomas express platelet derived growth factor (PDGF) ligand and receptor and/or KIT kinase. Drugs designed to target PDGF or KIT kinase (eg, imatinib mesylate) demonstrated effectiveness only against gastrointestinal stromal tumors and chronic myeloid lymphomas. However, one phase II trial did not support this treatment in pediatric orthopaedic tumors, but it is possible than, in the future, PDGF or KIT will allow investigation into application of orthopaedic oncology related drugs [8].

Genotype may predict the risk for osteosarcoma, Paget disease and chondrosarcoma and the prognosis following diagnosis [9–11]. More mutations (eg, p53 gene) occur exclusively in high-grade but not low-grade disease, and some patients progress from low to high

grade, suggesting evolution simultaneously with progression. Additionally, SNPs in genes associated with osteosarcoma linked multiple biological processes with this cancer type [12].

Genetic contributions to the etiology and progression of common orthopaedic conditions are well studied in comparison with treatments and outcomes. Several mutations are associated with osteoporosis such as OPG genes, vitamin D receptor genes (VDR), LRP5 and others [13,14]. These genes are implicated in the inhibitions of osteoclast production and Wnt signalling, decreasing bone mineral density (BMD) and osteoporosis.

SNPs near OPG and Lrp5 increase the risk for osteoporotic fracture independent of decreased BMD. In particular, the prevalence of OPG related risk alleles in approximately 8,500 white women was 10-fold higher than the prevalence of glucocorticoid use [13]. This suggests that genomic profiles are more relevant.

A recent study [15] confirms the importance of 12 loci as risk factors for bone fracture (2p16.2 (*SPTBN1*), 7q21.3 (*SHFM1*), 10q21.1 (*MBL2*/*DKK1*), 11q13.2 (*LRP5*), and 18p11.21 (*FAM210A*), *SOST*, *CPED1*/*WNT16*, *FUPB3*, *DCDC5*, *RPS6KA5*, *STARD3NL*, and *CTNNB1*. Furthermore, in the same study, using a scale GWAS meta-analysis identified other 4 new genetic determinants of fracture, all of which also influence bone mineral density (6q22.33 (*RSPO3*), 6q25.1 (*ESR1*), 7p12.1 (*GRB10*/*COBL*), and 21q22.2 (*ETS2*)) [15]. Moreover, genetic predisposition to lower levels of vitamin D and estimated calcium intake from dairy sources were not associated with fracture risk [15].

Polymorphisms in the "disintegrin and metalloproteinase domain with thrombospondin motifs" 18 (ADAMTS18) gene encoding for antiangiogenic properties and transforming growth factor-β receptor type 3 (TGFBR3) genes, which regulates TGF-β signaling and extracellular matrix assembly, have been associated with BMD alterations, which have a heritability greater than 70% [16]. Associations between cortical BMD and SNPs near the OPG, RANK, and RANKL genes have been discovered both in adolescents and elderly [16,17].

Developmental dysplasia of the hip (DDH) and primary protrusio acetabuli (PPA) encompass the spectrum of acetabular development from a shallow acetabulum in DDH to a deep acetabulum in PPA. Both have an indeterminate aetiology and result in early onset osteoarthritis of the hip [18]. A genetic hormone-related aetiology has been proposed [19–21]. The association of developmental DDH and PPA with VDR polymorphisms Taq I and Fok I and oestrogen receptor (OR) polymorphisms Pvu II and Xba I suggest a possible correlation between gene polymorphisms and susceptibility and severity of DDH[22]. Indeed, the Taq I VDR polymorphisms may be associated with abnormal acetabular morphology while the Xba I OR XX genotype with an increased risk of developing DDH; no associations were found with PPA.

The contribution of genetics to osteoarthritis (OA) has been estimated at 65% for the knee, 60% for the hip, and 39% for the hand [23]. Association studies have detected two loci: growth differentiation factor-5 (GDF5), associated with bone and cartilage development, and component of oligomeric Golgi complex-5 (COG5) [11,24].

There are 56 SNPs from 50 genes or gene loci, which have been associated with OA or OA subtypes [25]. These genes affect Wnt-associated bone mass, bone changes in

response to compression, cartilage turnover, chondrogenic processes mediated by TGF-β1, and the development of type II cartilage [25–28].

However, the effect size of these loci is very small, and more factors are necessary to produce clinical OA. Some OA SNPs are risk factors in both sexes or in select ethnic populations[25]. For example, calmodulin-1 (CALM1) and asporin (ASPN) SNPs, identified in Japanese but not white and Greek patients with OA [26,29–31]; or frizzled-related protein-2 (FRZB2) and collagen type II alpha-1 (COL2A1) were associated with OA in females and males, respectively; cartilage oligomeric matrix protein (COMP) demonstrated differential effects in both sex [26,32].

The origin of chronic pain, whose presence defines symptomatic OA, is not clear: indeed, the presence of radiographic abnormalities is not always associated with pain [33]. The prevalence of radiographic knee degenerative joint disease was 19% and 28% among adults aged >45 years in the Framingham study and in the Johnston County OA Project, respectively, while the prevalence of symptomatic knee OA was 7% in the Framingham study and 17% in the Johnston County OA Project[34]. Initially, pain in OA occurs episodically during movement and loading [35], while constant pain may occur later [35]. Three relevant areas should be considered to explain OA pain: local processes in the joint, alterations of the nociceptive system, and general factors including comorbidities. Genetics is related to the second area, which is the most variable [36]. There is an increase of mechano-sensitivity [37], a downregulation of substance P in neurons[38] and a genetic contribution with the association of 400 genetic markers in the genome of patients with OA [39]. A genetic variant of catechol-O-methyltransferase (COMT) was associated with stronger hip OA pain[40], but not with knee OA pain [41].

Another report described the association of a TRPV1 gene variant and a SNP in the PCSK6 gene with a lower risk of symptomatic knee OA[42,43].

Annually, 1 million of total hip arthoplasties (THA) are implanted worldwide [44], and aseptic loosening (AL) has become more common [45], with high morbidity and mortality, especially in the elderly [46]. AL results in progressive bone loss and periprosthetic osteolysis, accounting for 75.7% of all THA revisions [47]. Pro-inflammatory mediators are implicated in aseptic osteolysis [48]. SNPs in TNF-238 A allele and TNF-a promoter [49,50], IL6-174G/597/572 [50–52], TGF-b1 [52], MBL [53,54], GNAS1 [55,56], OPG-163 [57,58], RANK [50,57,59] and MMP-1 [51,52,60] predispose to aseptic loosening. The mechanisms of regulation and gene activation are still unclear. In the future, this knowledge would allow better planning and anticipating the need for early intervention [61].

Congenital idiopathic talipes equinovarus (CTEV) has a prevalence of 1 to 5 per 1000 live births [62,63]. Its etiology remains unknown, but it has both genetic and environmental components [62–65], with extrinsic factors (e.g. congenital constriction bands, intrauterine poisoning), chromosomal abnormalities, and neuromuscular disorders. The role of inheritance in CTEV needs to be clarified [62,66,67].

GENOMICS IN SOFT TISSUE INJURIES

The limit of each individual to perform a given type of exercise depends on the nature of the task, and is influenced by a variety of factors, including genetic make-up [68,69]. Recently, the relationship between polymorphisms and susceptibility to develop ligament and tendon injuries has been explored [68–70].

Collagen type I is the major constituent of tendons and ligaments. An alteration of COLIA1 genotype with the polymorphism Sp1 TT was associated with reduction of 85% the risk of cruciate ligament tears and shoulder dislocation [71,72]. No significant association was found between this SNP and Achilles tendinopathy compared with healthy Caucasian controls [73].

Type V collagen, quantitatively minor fibrillar collagen which heterotypic fibrils, regulates the size and configuration of type I collagen. Polymorphisms of the COL5A1 gene have been associated with Achilles and quadriceps tendon injuries and anterior cruciate ligament tears [74–76].

Tenascin-C (TNC) plays a critical role in transmitting mechanical tendon force, and it is expressed in the myotendinous and osteotendinous junctions [77–79], controlling cell-matrix interactions[80]. The guanine-thymine (GT) dinucleotide repeat polymorphism was analyzed in association with Achilles tendon injuries [81], showing a significantly lower frequency of injuries between patients with 13 and 17 GT dinucleotide repeats, and control.

On the chromosome 9, between COL5A1 gene and TNC gene, lies the single gene determining the ABO blood group [82]. Individuals with blood group O are more susceptible to tendon injuries [83,84].

GENETICS AND REHABILITATION

Genetics determines the response of individuals to their surroundings [85]. Predictive genomics DNA profiling for athletic performance and injury rehabilitation allows to understand which genetic advantages should be exploited. These findings could partially explain why an individual is able to excel in one sport discipline, and why rehabilitation protocol can be more effective in some individuals than others. Genetic factors play a critical role in determining high levels of sport performances and satisfactory rehabilitation results [86,87]. The physical performance phenotypes for which a genetic basis can be suspected include endurance capacity, muscle performance, and determinants of the behaviour of tendons and ligaments.

Endurance is the ability to perform high level aerobic exercise for prolonged periods. It is supported by enhanced mitochondrial function, as suggested by increased mitochondrial gene expression, and mitochondrial enzyme activity [88]. The nuclear respiratory factor (NRF) 2 organizes the expression of nuclear and mitochondrial genes, explaining some of the inter-individual variance in endurance capacity [88].

Hemoglobin is a determinant of endurance performance, and SNPs in the hemoglobin gene could decrease the oxygen cost of running, explaining part of an individual variation in cardiorespiratory adaptation to endurance training [89]. The Arg16Gly polymorphism in the b2-adrenergic receptor (ADRB2) gene may be associated with endurance performance status in white men [90].

Some other gene polymorphisms have been associated with sport performance and rehabilitation, although results are still preliminary or controversial. These include

polymorphisms in the alpha2a-adrenoceptor gene [91], bradykinin beta 2 receptor, endothelial nitric oxide synthase 3 genes [92], vitamin D receptor gene [93], HIF-1 alpha [94].

Muscle performance is a direct consequence of the heterogeneity essential for its function, and is directed at optimizing the contractile responses [69]. For example, the creatine kinase isoenzyme MM (CM-MM) is responsible of the rapid regeneration of ATP during muscle contraction, the actin-binding protein [alpha]-actinin-3 (ACTN3) a component of fast skeletal muscle fibres, and the myosin light chain kinase (MLCK) plays a critical role in the regulation of smooth muscle contraction [95], in particular, the R577X polymorphism (premature stop codon) associated with complete ACTN3 deficiency is more prevalent among elite endurance athletes [101,102]. The humans CK-MM gene sequence variation show a significant association with maximal oxygen uptake following 20 weeks of training [98], peak performance and less decline in force generation [99].

The ACE gene has 'I' (insertion) and 'D' (deletion) alleles [100,101]. Controversy exists about the association of the ACE gene variation and many heritable traits, including skill parameters and physical performance [102]. For example, elite endurance athletes exhibit an increased frequency of the ACE I allele [103].

Other SNPs have been associated with muscle performance such as in the adenosine monophosphate deaminase 1 (AMPD1) gene or insulin-like growth factor 1 protein (IGF-1) gene [104]. In particular, sedentary subjects with the TT genotype at the C34T AMPD1 gene showed diminished cardiorespiratory response to rehabilitation exercise [105–107].

To the best of our knowledge, no published study suggests to identify these polymorphisms to guide rehabilitation after musculoskeletal injuries. More evidence is needed to evaluate the benefits of genomic screening in patients to improve the outcomes of specific rehabilitation protocols.

CAN WE INFLUENCE OUR GENETICS?

Tissue Engineering

In the last few decades, several strategies, including growth factors, gene therapy and tissue engineering with mesenchymal stem cells (MSC), have been proposed to enhance soft tissue healing [108].

Tissue engineering can be accomplished through the in vivo approach, which permits the self-regeneration of small tissue lesions, and the ex vivo, de novo approach, which produces functional tissue implantable in the body [109,110]. It is a multidisciplinary field founded on the use of healthy multipotent cells that are nonimmunogenic, the development of carrier scaffolds that provide short-term mechanical stability of the transplant, a template for spatial growth of the regenerate tissue and the delivery of growth factors that drive the process of cell differentiation and maturation [109,110].

Growth factors (GFs), the signaling molecules involved in cell proliferation and differentiation, play an important role in regulation of tendon healing [111], determining intracellular changes and DNA synthesis or expression [87,112–115]. They can improve the strength of the repair by promoting the formation of more scar tissue modulating stiffness and creep [111] and delivered to the site of injury by direct application, for example, via local injection, or by using impregnated sutures or scaffolds. The main disadvantage of direct application is that GFs only remain at the site for a short duration time.

Many other factors can be used, including cartilage-derived morphogenetic protein (CDMP) growth factor [116], PDGF [117], Interleukin-10 [118], VEGF [119], antibody to TGF-b1

[120] and IGF-1 [117,121]. Media consisting of PRP used for equine flexor digitorum superficialis tendon explants showed enhanced gene expression of collagen type I (COL1A1), collagen type III (COL3A1) and collagen oligomeric matrix protein (COMP), but no increase of catabolic molecules matrix metalloproteinase (MMP) 3 and 13 compared with other blood products tested [122]. A double-blind, placebo-controlled trial demonstrated no benefit of intramuscular PRP injections compared with placebo injections[123].

MSCs can differentiate into a variety of specialized mesenchymal tissues [108]. They can be applied directly to the site of injury or delivered on a suitable carrier matrix, which functions as a scaffold while tissue repair takes place [87,112–115]. Delivering MSC in organized collagen implants applied to large tendon defects can significantly improve the biomechanics, structure and probably the function of tendons after injury [124,125]. MSCs derived from synovium have a higher proliferation and differentiation potential than the other MSCs. Indeed, they can accelerate the early remodeling of tendon–bone healing producing more collagen fibers at 1 week and forming more oblique collagen fibers resembling Sharpey's fibers at 2 weeks [126]. MSCs have been investigated in the management of tendinopathy, showing significantly improved tendon histological scores when injected in tendinopathic equine flexor digitorum superficialis [127]. In rabbits, MSCs suspended in type I collagen gel and implanted into a surgically induced defect in the donor's patellar tendon demonstrated significant increases in maximum stress and strain energy density [128].

Gene Therapy

Gene therapy delivers genetic material to cells using viral or nonviral vectors or direct gene transfer, resolving the problem of short time permanence of GFs in the site of injury [109,110] (Table 3). The use of vectors is associated with loss of transgene expression and adhesion formation secondary to inflammation [129]. Gene transfer using vectors can be achieved via "in vivo", with direct application of the gene to the tissue, or "ex vivo" transfection, in which target cells are first removed and gene transfer is performed in the laboratory [129]. In vivo transfection is less invasive, but with the risk of nonspecific infection of cells adjacent to the target site.

Adenovirus-based gene therapy is an efficient means of gene delivery to rabbit flexor tendons, but the transduction efficiency of transgenes was dose dependent [130]. Rickert et al. [131] injected adenovirus particles into transected Achilles tendons of rats: in vitro, GDF-5 was secreted with a peak after 2 weeks, and in vivo after 4 weeks. The use of AAV vectors to transfer exogenous bFGF gene to proliferating tenocytes showed significantly increased levels of expression of type I and III collagen genes compared with those in the cells treated with sham vectors or in nontreatment controls [132].

The rate of transfection of a gene in rat patellar tendons using the HVJ liposome-mediated gene transfer method was significantly greater than controls [133], and, compared to adenoviral and AAV vectors it showed the most prominent healing response on injured flexor tendons of rabbit [134]. Injecting directly into the injured patellar tendon of rats a HVJ-liposome suspension containing PDGF-B cDNA enhances the expression of PDGF in healing ligaments with angiogenesis promotion and collagen deposition in the wound [135]. Gene therapy with BMPs may improve the healing ability of tissues. Achilles tendon transduced with BMP-14 exhibited less visible gapping, a greater number of neotenocytes and 70% greater tensile strength than controls at 2

weeks after repair [136]. Majewski et al. [137] evaluated the effects of BMP-12 gene transfer on the healing of rat Achilles tendons using a genetically modified muscle flap, reporting acceleration and improvement of tendon healing.

A plasmid carrying the lacZ marker gene was injected into the Achilles tendons of rats and mice and into the patellar tendons of rabbits showing at 48 h transduced cells, a minority of the tendon cells [138]. Kinetics study in rats showed a gradual decrease of β-gal-expressing cell number; at day 42, gene expression was no longer detected, without inflammatory reaction [138]. Wang et al. [139] transferred, using a plasmid, the PDGF-B gene to tenocytes obtained from explant cultures of rat intrasynovial tendons: RT-PCR showed significantly increased expression of type I collagen gene by tenocytes.

With the advent of clustered regulatory interspaced short palindromic repeat (CRISPR) technologies, AAV has shown promising therapeutic efficacy with good safety profile in animal and human clinical trials [140]. It revolutionized gene-editing techniques because of its simplicity in target design, affordability, versatility, and high efficiency [141]. CRISPR/Cas9-based RNA-guided DNA endonuclease has, rapidly, become the preferred platform of genome-editing for interrogating endogenous gene function in vivo [142,143].

The CRISPR/Cas9 complex can be introduced into the cell in forms of DNA, messenger RNA, or protein [144]. Because of the great potential of viral vectors, the major classes — lentiviruses [145], adenoviruses [146], retroviruses [147], AAVs [148], and baculoviruses [149] — have been employed to present CRISPR components into eukaryotic cells for genome editing. The AAV-CRISPR system has also been successfully used in mice to restore gene function in Duchenne muscular dystrophy [150–153] and other conditions. The AAV-

CRISPR system holds enormous translational potential to develop therapeutic treatments for patients with severe and life-threatening genetic diseases by editing disease-causing or risk genes in the human body. The AAV-CRISPR system needs more tests *in vivo* to become a successful human gene therapy [140].

CONCLUSIONS

A better understanding of musculoskeletal system function and healing will allow specific management strategies to be developed. Many interesting techniques, discussed in this article, are at an early stage of development. Although these emerging technologies may develop into substantial clinical management options, their full impact needs to be evaluated critically in a scientific fashion.

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FIGURES & TABLES

Table 1 | Rapid overview and description of DNA and RNA types.

Table 2 | Overview of major musculoskeletal-related disorders and their inheritance patterns.

Table 3 | Overview on the main vectors used in gene therapy. AAV: adeno-associated virus, HVJ: hemagglutinating virus of Japan.

Figure 1 | Central dogma of molecular biology: from DNA replication to protein synthesis. dNTP: deoxyribose nucleoside triphosphate, rNTP: ribonucleoside nucleoside triphosphate.