Injury, Int. J. Care Injured 48S6 (2017) S12–S25

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/Injury

Inter-individual gene variants associated with trabecular bone plasticity: A step forward in the personal genomics of degenerative bone disease

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KEYWORDS

femoral neck
fractures
osteoarthritis
osteoarthrosis
coxarthrosis
trabecular bone
polymorphism
whole-Exome sequencing
gene variants
bioinformatics analysis
INDEL mutation
genome
computational biology

ABSTRACT

Continuing tissue destruction in osteoarthrosis is maintained by molecular pathways related to an unbalanced chondrocyte metabolism, the loss of reactive oxygen species (ROS) homeostasis, increase catabolism in a degraded matrix and the limited response to growth factors due to cell aging. Rare deleterious gene variants driving relevant molecular pathways may play a key role in the pathogenesis and genetic control of common diseases and may also influence the common gene variants observed in GWAS. We use molecular profiling technologies based on massive sequencing of genes to interrogate clinical samples for a variety of molecules involved in the pathogenesis pathways of OA and also to derive new insights for drug targeting discovery at an early stage of the disease. By whole-exome sequencing performed in OA patients with extreme phenotypes and in non-related individuals without clinical evidence of OA, the most predominant of the rare gene variants found were non-synonymous single-nucleotide variants (SNV) from exonic DNA regions and with missense functional effects predicting a moderate impact on protein function. A total of 629, 577, and 639 gene variants for the TPF, COA, and ANHNF patients, respectively, were found not to be shared with the 20 non-disease-related individuals. After subtraction of the 306 variants shared among the OA patients, we obtained the individual profiles of 323, 271, and 333 gene variants, for the TPF, COA, and ANHNF patients, respectively. After filtering by the bioinformatics, genetic, and biological criteria established to assess the clinical consequences, comparative analysis of trio sequences using integrative genome visualization tool clearly demonstrate the differences between patients. Analysis of the collagen gene variants identified 78, 20, and 43 genetic collagen variants for the three extreme phenotypes. Rare gene variants encoding for proteins that are less abundant in the trabecular bone matrix, together with those responsible for the control and regulation of bone turnover and plasticity of subchondral trabecular bone, play important roles in OA and help to define the clinical phenotype.

Introduction

Although in the near future treatment options for early osteoarthritis (OA) of the hip will be available [1], hip replacement surgery by artificial joint, to reduce pain and improve hip function, remains the current treatment of choice to replace damaged joint tissue in patients suffering from degenerative OA. Destruction and loss of articular tissue involving degradation of hyaline cartilage, sclerosis of subchondral bone, osteophyte formation and/or synovial inflammation are the main features of OA [2]. Various clinical phenotypes of OA, including primary coxarthrosis, developmental dysplasia of the hip (DDH), avascular necrosis, rheumatoid diseases, slipped capital femoral epiphysis, posttraumatic coxarthrosis, pathologic coxarthrosis and Legg–Calvé–Perthes disease (LCPD) present underlying differences related to molecular pathways involved in the tissue-damaging process. Apart from the effects of mechanical forces and the predilection of specific joints, continuing tissue destruction is maintained by molecular pathways related to an unbalanced chondrocyte metabolism, the loss of reactive oxygen species (ROS) homeostasis, increase catabolism in a degraded matrix and the limited response to growth factors due to

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cell aging [2]. The up-regulation of Runx2, the co-participation of transforming growth factors, molecules of Wnt3a, Hedgehog signalling pathways and metalloproteinase (MMP) family members [3–5] have all been suggested as factors driving the molecular mechanisms for the initiation and progression of articular cartilage destruction [6].

Thickening of the subchondral bone and the formation of osteophytes characterize the bone phenotype at the proximal end of the femur in OA patients. The collagen of bone matrix (90% type I collagen) [7] provides structural support and flexibility [8] and adds plasticity and ductility to the trabecular bone [9] at the proximal end of the femur. In addition to the architecture and connectivity of individual trabeculae, properties at the molecular level influence a large part of the remodelling process that takes place on the surface of the trabecular structure. In this respect, the cellular activity of subchondral bone [10–13], which is responsible for the control of bone metabolism changes [14,15], may underlie the initiation and progression of OA. Moreover, bone resorption appears to be the initial remodelling process influencing the morphology and metabolism of subchondral bone in OA [16]. In fact, the modification of levels of several molecules has been described, including pro-inflammatory molecules [17,18], alkaline phosphatase, osteocalcin, urokinase plasminogen-activator, prostaglandin E_2 [10,18–20], nitric oxide (NO) as a stimulator of bone formation or bone lysis and also cartilage degradation [21–25]. In OA experimental models, bone remodelling appears to take place in both subchondral and trabecular bone metabolism [26,27].

The analysis of genetic predisposition to OA, based on single-nucleotide polymorphisms (SNPs) is clearly of great value, but it is a complex issue, involving several signalling and metabolic pathways in chondrocytes, osteocytes and the extracellular matrix [28,29]. Although genome-wide association studies (GWAS) of the common SNPs for genetic susceptibility to common diseases have identified consistent numbers of gene variants shared by large cohorts of patients, including OA, the disease-causing variant effect is not as significant as expected. On the other hand, rare deleterious variants driving relevant molecular pathways may play a key role in the pathogenesis and genetic control of common diseases [30] and may also influence the common gene variants observed in genome-wide association study (GWAS). This is an important issue, one that could enable the transfer of outcomes from personal genomics and precision medicine into personalized medical practice, through the employment of massive sequencing techniques in a very small number of pathological samples, possibly obtained from a single patient.

Mapping the molecular interaction and reaction networks driven by specific molecules within the cell, leading to a certain product, change, end point or cell function, will facilitate a more precise identification of the underlying genetic cause of OA. At present, known large-scale datasets are used in genomics, transcriptomics, proteomics, and metabolomics to assist in biological interpretation, to calculate the impact on pathogenesis and ultimately to determine the clinical consequences of the molecular changes accumulated in these networks. The use of molecular profiling technologies based on massive sequencing of genes to interrogate clinical samples for a variety of molecules involved in the pathogenesis pathways of OA is already possible in this postgenomic era. Whole-exome sequencing (WES) covering the exome (most of the protein-encoding genes) may help to evidence the proof-of-concept for disease-causing rare variants, especially in individuals with extreme phenotypes, and also to corroborate or refute an unclear diagnosis. Indeed, with WES it may be possible to determine the exomic variation profile detected in patients with OA, thus enhancing the process by which causal genetic variations are identified against the background of individual genetic variability, in a small number of samples, and at the same time reducing the difficulty in making a clinical interpretation of the consequences of the gene variants in OA. In view of these considerations, we carried out a WES analysis on OA patients in order to define individual exomic profiles, seeking to derive new insights for drug targeting discovery at an early stage of the disease.

Patients and methods

Patients

The following patients with extreme phenotypes of OA treated by unilateral hip replacement surgery were included for WES analysis and personal exome profiling; A 27-year-old male with hip OA at five years after traumatic femoral neck fracture (TPF) of the right femur, closed and non-displaced and treated by internal fixation (Figure 1). A 37-year-old premenopausal female, with bilateral avascular necrosis of the head and neck of the femur (ANHNF), grade I (right) and grade II (left) (Figure 2), and a family medical history of ANHNF (brother). A 72-year-old male diagnosed with unilateral primary coxarthrosis (COA) and no other medical record of musculoskeletal disease.

Whole-exome sequencing

Informed consent for genetic testing by exome sequencing was obtained from all these patients. Prior WES analysis, of 20 non-related individuals, without any medical evidence of OA or hip abnormalities,

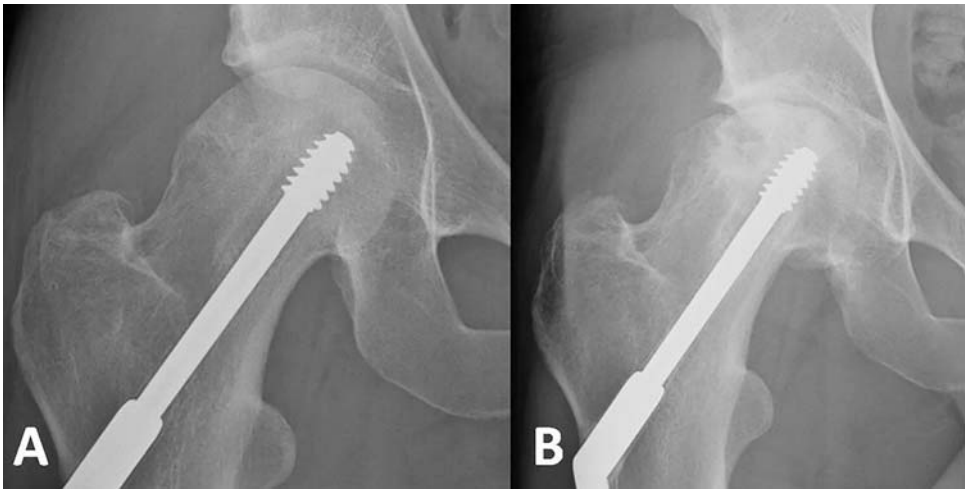

Fig. 1. X-ray of patient with unilateral osteonecrosis of femoral head secondary to femoral neck fracture. A: After internal fixation of femoral neck fracture; B: At 5 years after internal fixation.