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Summary of the OA Biomarkers Workshop 2010 - Genetics and Genomics: New Targets in OA

I. Meulenbelt^{†,‡,*}, V.B Kraus[§], L.J. Sandell^{||,¶}, and J. Loughlin^{#,*}

[†] Department of Molecular Epidemiology, LUMC, Leiden, The Netherlands [‡] Netherlands Consortium for Healthy Ageing, Leiden, The Netherlands [§] Department of Medicine, Duke University, Durham, NC, United States ^{||} Department of Orthopaedic Surgery, Washington University, St. Louis, MO, United States [¶] Department of Cell Biology and Physiology, Washington University, St. Louis, MO, United States [#] Newcastle University, Institute of Cellular Medicine, Newcastle, UK

Summary

On November 4th and 5th 2010 a group of more than 100 international investigators gathered in Atlanta for the 2nd Osteoarthritis (OA) Biomarkers Global Initiative workshop titled “Genetics and Genomics: New Targets in OA”. The 1st workshop took place in April 2009 and focused on in vitro (soluble) biomarkers whilst the 3rd and final workshop will take place in 2012 and will focus on imaging biomarkers. The Osteoarthritis Research Society International (OARSI) has organized the workshops. In addition to OARSI, the National Institute of Arthritis, Musculoskeletal and Skin Diseases, the Arthritis Foundation, Amgen, Genzyme, the American Orthopaedic Society for Sports Medicine and Pfizer sponsored the 2nd meeting. It was clear from this meeting that experiments in the genetics, epigenetics and genomics of OA, are yielding valuable insights into the etiology of this heterogeneous disease but that much still needs to be learnt. Combining genetic insights with conventional biomarkers and imaging modalities may provide scientists with the enhanced tools to understand this complex disease. With those tools in hand, clinicians and industry can develop protocols to ultimately improve patient care.

Osteoarthritis (OA) and the need for novel biomarkers

Over the last 20 years OA has come to be recognized as a complex disease involving most, if not all, tissues of the joint. OA has a major heritable component, confirmed by

*Address correspondence and reprint requests to: John Loughlin, Newcastle University, Institute of Cellular Medicine, Musculoskeletal Research Group, 4th Floor Catherine Cookson Building, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK. john.loughlin@ncl.ac.uk. Ingrid Meulenbelt, Department of Molecular Epidemiology, Postzone S-05-P, Leiden University Medical Center, Leiden, The Netherlands. i.meulenbelt@lumc.nl.

Author contributions

Ingrid Meulenbelt, co-chair of meeting

Virginia Byers-Kraus, co-chair of meeting

Linda J. Sandell, co-chair of meeting

John Loughlin, co-chair of meeting

All authors participated in writing and editing the Meeting Report

Conflict of interest

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epidemiological studies and now by molecular investigations. No disease modifying therapies have yet been developed, severely hampering disease management. Furthermore, OA is still principally diagnosed once radiographic changes in joint tissues are detected, often reflecting irreversible damage.

Like most common, complex diseases, the genetic architecture of OA remains to be clarified. Molecular studies have, however, generated promising information about the genetic underpinnings; such novel insights may provide useful information on how the disease begins and progresses. Moreover, determining pre-clinical changes or abnormalities that reveal the disease closer to its starting point could be accomplished at the molecular level with biomarkers. Strategies are required to detect and intervene early in the course of OA and to monitor disease progression after treatment. Ultimately these strategies will help scientists understand the differences between diseased and normal joint tissues and thus satisfy the needs of clinicians, industry, and patients. The OARSI OA Biomarker Global Initiative aims to help develop such biomarkers through a series of workshops designed to encourage international participation on a selection of relevant topics.

The OARSI OA Biomarker Global Initiative

In 2009 funding for a series of three workshops was awarded to the OA Biomarker Global Initiative by the National Institute of Arthritis, Musculoskeletal and Skin Diseases (NIAMS). These meetings provide a forum for interchange of information and ideas among members of the OA biomarker community by providing structure for biomarker discovery and use; providing resources to move the field forward; providing white papers, guidelines, and validation for use in the field; and maintaining momentum in this critical area. The first workshop entitled “Biochemical Biomarker: Biology, Validation and Clinical Studies” was held in April 2009 in Bethesda, Maryland. Attendees focused on biochemical biomarkers of OA (1).

Since the first workshop, the Global Initiative defined the kind of partnerships needed by investigators and funding organizations. The group also developed guidelines for sample collection, developed a website for sharing information, and began an initiative between the United States Food and Drug Administration (FDA) and OARSI. From this latter initiative came guidelines for changes in the structure for FDA clinical studies. Guidance related to the OA field in general include biomarker quantification; unmet needs in the biomarker field; and information on patient research.

The Second Workshop

In November 2010 over 100 delegates from across the globe gathered in Atlanta for the second OARSI Biomarkers workshop titled, “Genetics and Genomics: New Targets in OA”. In addition to NIAMS, the Arthritis Foundation, Amgen, Genzyme, the American Orthopaedic Society for Sports Medicine, and Pfizer sponsored the meeting. The workshop focused on current research in genetic, epigenetic, and genomic studies of OA. Sessions assessed whether biomarkers derived from these approaches can realistically be used now or in the near future to identify and monitor people who are at increased risk for OA, or even to identify those with enhanced protection from the disease.

Cohort Studies

Ongoing debate has centered on how best to select cases for OA genetic studies. Because OA is a heterogeneous disease, more refined clinical phenotyping may help stratify the disease into homogeneous genetic and environmental subsets, thereby enhancing power of such genetic studies. Such subsets are perhaps not resolvable until unambiguous association data emerges that allows the identification of the phenotypes of carriers of risk DNA variant alleles.

The workshop therefore started with a discussion on current issues regarding case selection. In the first OARSI biomarkers workshop on biochemical markers, the anterior cruciate ligament (ACL) injury model was proposed to track the onset of OA since it provides a clear starting point from which to monitor events and progression to pre-radiographic and radiographic OA. For example, refined reconstruction techniques for ACL tears allow athletes to return to the playing field. However, 10 years post-surgery, X-rays show evidence of arthritis in only some individuals, suggesting mechanical factors alone cannot account for OA. Such studies may reveal, in a reasonable timeframe, who is most at risk for developing joint pathology and progression to OA and whether genetic factors play a modifying role.

In a preliminary study of West Point cadets with and without ACL injury in the pre- and post-clinical state, commercially available biomarkers of cartilage degradation and synthesis were measured. This unique cohort study was possible due to serial collections, since 1985, of medical and physical activity data from cadets, beginning at recruitment. Furthermore, ACL tears occur in this population at approximately 45 per 1000. Perhaps most significant was the difference in pre-clinical levels of both CPII and C2C in the ACL injured group as compared to controls, indicating that characteristics of joint metabolism may predispose, when physically challenged, to such an ACL injury.

Finally, a study was presented that compared serum and synovial fluid biomarkers in the first several weeks after acute trauma to the ACL. In this pilot study, a large panel of biomarkers was analyzed following injury and treatment with intra-articular IL-1R α . Data showed high initial levels of inflammatory proteoglycans and other matrix molecules followed by delayed collagen release. Perhaps indicating that early pro-inflammatory response to injury leaves a crucial impact on long-term health consequences of the joint integrity. As collagen loss is considered irreversible very early treatment with agents to reduce collagen loss may be necessary to prevent the onset of post-traumatic OA (2).

Candidate Genes and GWAS

Candidate gene and genome wide analyses studies (GWAS) aim to provide insights into genes that may confer genetic risk or protection from OA. Thus far, OA appears to be highly polygenic with multiple risk alleles conferring small effects. Finding loci under these conditions requires large sample sizes. Current large-scale consortia such as arcOGEN and TREAT~OA are converging towards robust new OA targets with genome wide significance ($P < 10^{-8}$).

Genetic studies in OA have so far provided only a handful of robust signals, such as polymorphism at 7q22 (3,4), *DIO2* (5) and *GDF5* (6,7). Two of these are known to have some effect on the skeleton. *DIO2*, a selenoprotein that converts intracellular inactive thyroid hormone to its active form, regulates the growth plate through thyroid hormone. *GDF5*, a member of the TGF β superfamily of signaling molecules, is involved in the development, maintenance, and repair of bone and cartilage. Additional functional studies are necessary to elucidate the underlying molecular pathways, which may provide clues on possible druggable targets and /or biomarkers that allow early preclinical diagnosis of disease in carriers of these risk alleles (8,9). Gene markers used to predict the trajectory of OA don't necessarily have to be polymorphisms. A session describing the role of epigenetics in common disease provided insights into how differences in epigenetic profiles of genes encoding proteinases, interleukins and growth factors may influence OA progression. Overall differences in gene expression or epigenetic profiles could be useful markers or diagnostic tools.

More candidate genes and polymorphisms are expected as ongoing GWAS studies reach completion (10). Each gene can potentially confer allelic heterogeneity (common and rare pathogenic variants), with rare genetic variants potentially having stronger and possibly distinctive effects on phenotype, and therefore offering greater potential for intervention. Identifying robust polymorphisms associated with OA won't provide a complete story. Many SNPs likely reside outside genes, may not be disease specific, nor be relevant to transcripts expressed in joints. Also, associated SNPs may reside inside genes whose function is not yet understood. Thus, functional studies are critical. Functional genomic pipelines will elucidate molecular pathway underlying OA etiology and thus facilitate the discovery of therapeutic targets. Functional genomic approaches will also provide insight into the molecular background of these OA susceptibility loci and hopefully uncover disease mechanisms. Lastly, genetic contributions to the formation of joint shape may add to information provided by functional genetic approaches.

Challenges (The next step forward)

Data presented at the workshop showed progress in robust candidate genes, such as *GDF5*, and reinforced the need to understand the complex interplay between genetic and environmental causes of OA. To date, too few signals reach genome-wide significance whilst even fewer show compelling association across ethnic groups. Current OA susceptibility alleles are also not providing enhanced risk prediction when combined with conventional risk factors such as age, gender and BMI. The elucidation of underlying pathways can supply such information as will collecting more data relevant to genetics, clinical features, and environmental risk factors. These new data will allow for complete analyses of OA associated genetic variants.

The field of complex trait genetics is moving towards determining the role of low frequency and rare variants. The 1000 Genomes Project (11) and the UK10K Project can provide OA studies with additional DNA variants to test whilst the emphasis must remain on large sample sizes to allow study replication and to also provide stratified analyses to both

increase and specify attributable risk. Such second generation sequencing efforts aim to uncover rare genetic changes in tens of thousands of people across the globe.

Challenges remain in many areas; such as gene-environment interactions that are rarely captured in gene association studies and which complicate clinical utility. Furthermore, a large part of heritability remains unexplained. Deep sequencing (whole genome, exome, and RNA-sequencing) may uncover additional rare genetic variation specific to OA susceptibility and may fill-in some of the heritability gap. Also, biochemical markers or markers that denote joint shape are needed to provide quantitative phenotyping for genome-wide analyses of OA endophenotypes.

As the field moves towards sequence-based studies across the whole genome, a better idea of the full spectrum of genetic variants underlying OA may provide a therapeutic path for early intervention. Microarray expression profiles in cartilage have already provided insight into OA pathophysiology (12,13) whilst proteomic studies may provide insight into biomarkers with a synovial or cartilage origin (14). The inaccessibility of joint tissue and the invasiveness of drawing synovial fluid, however, limits their use as routine biomarkers for OA unless they are released into urine or blood. Expression profiles in blood may provide an accessible new source of sensitive genomic biomarkers so long as what is occurring in the damaged joint is reasonably mirrored in blood cells or serum. A similar conundrum applies to epigenetic analyses - what is the correct tissue/s and time point in disease development to target?

Future genetic and genomic approaches will need to address disease heterogeneity; small effect sizes ($OR < 1.2$); rare variants of possible large-effects; and epistatic and epigenetic effects. Functional studies will need to be performed on robust and replicated signals.

Lastly, the question of combining multiple markers to assign risk for a single individual needs to be addressed. Markers that might be combined include a single risk entity (e.g. SNP), haplotypes for multiple variations in a single gene, pathways for multiple expressed genes, metabolites, and proteins. The challenge will occur when multiple markers cannot be “easily” adapted to provide an overall indicator of disease risk.

To meet all challenges put forth in Atlanta, the Global Initiative will establish a central clearance house on the OARSI website to allow an overview of current available studies and data, for example upcoming GWAS of the NIH Osteoarthritis Initiative OAI biospecimens (<http://oai.epi-ucsf.org/datarelease/>). This clearance house will provide an overview on available cohorts and thereby encourage collaboration so cohorts can be used in different settings.

In addition, the Global Initiative will identify parameters in areas of common phenotypes, such as OA in multiple joints, and varying age groups. Diverse phenotypes will likely require equally diverse biomarkers for susceptibility, severity, and progression in combination with imaging approaches. Such an approach will help identify and follow OA, beginning with the earliest molecular changes.

The 3rd and final Biomarkers Workshop will take place in 2012, led by Professor David Hunter. This meeting will focus on imaging biomarkers. Like the 2nd meeting, the final workshop will also try and weave in what we have learnt from previous meetings to create an overall view of the current state of art of OA biomarkers. It will be of interest to know whether our understanding of the genetic, epigenetic, and genomic basis of OA has substantially progressed and whether it can integrate with conventional and imaging biomarkers to enhance our ability to improve clinical treatment of OA patients.

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