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Review

Osteoarthritis year 2013 in review: genetics and genomics

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SUMMARY

Progress in genetic research has delivered important highlights in the last year. One of the widest impact is the publication of the Encyclopedia of DNA Elements (ENCODE) project showing the impressive complexity of the human genome and providing information useful for all areas of genetics. More specific of osteoarthritis (OA) has been the incorporation of DOT1-like, histone H3 methyltransferase (*DOT1L*) to the list of 11 OA loci with genome-wide significant association, the demonstration of significant overlap between OA genetics and height or body mass index (BMI) genetics, and the tentative prioritization of HMG-box transcription factor 1 (*HBP1*) in the *7q22* locus based on functional analysis. In addition, the first large scale analysis of DNA methylation has found modest differences between OA and normal cartilage, but has identified a subgroup of OA patients with a very differentiated phenotype. The role of DNA methylation in regulation of *NOS2*, *SOX9*, *MMP13* and *IL1B* has been further clarified. MicroRNA expression studies in turn have shown some replication of differences between OA and control cartilage from previous profiling studies and have identified potential regulators of TGFβ signaling and of IL1β effects. In addition, non-coding RNAs showed promising results as serum biomarkers of cartilage damage. Gene expression microarray studies have found important differences between studies of hip or knee OA that reinforce the idea of joint specificity in OA. Expression differences between articular cartilage and other types of cartilage highlighted the WNT pathway whose regulation is proposed as critical for maintaining the articular cartilage phenotype. Many of these results need confirmation but they signal the exciting progress that is taking place in all areas of OA genetics, indicate questions requiring more study and augur further interesting discoveries.

I have covered in this annual review the fields of genetics, epigenetics and functional genomics of human OA. I only considered studies done in human OA samples and I did not intend to be exhaustive. Specifically, I have done searches only in PubMed and no work reported in abstracts is covered. The previous review of this series[1] is taken as baseline and published work is covered until the first weeks of April 2013.

Genetics of complex diseases today

Genetic research of OA has followed the common disease-common variant hypothesis that was behind the impetus leading to the Genome Wide Association Studies (GWAS)[2]. These studies have been very successful, leading to the identification of hundreds of loci associated with complex diseases[3] and of 11 loci associated with OA (Table I). However, GWAS have also made evident that the common disease-common variant hypothesis does not satisfactorily account for most complex diseases[4–7]. It had predicted that most of the risk to complex diseases will be attributable to a modest number of common variants[2]. In contrast, the effect sizes (the risk afforded by each susceptibility factor) of most loci are too small. Therefore, they are able to explain only a small fraction of the heritability of most diseases. Ongoing efforts to identify other sources of heritability have not yet been successful. In particular, rare variants with modest to large effect sizes have resulted very difficult to demonstrate in spite of the extraordinary development of sequencing technologies[8]. In addition, it seems increasingly likely that a substantial fraction of the unexplained heritability is due to a large number of loci of similar or even smaller effect sizes than the already known. This scenario of polygenic inheritance is consistent with GWAS data for many diseases including OA and it is likely to include as many as a few thousand loci for each complex disease[9–11].

The inadequacy of the common disease-common variant hypothesis was suspected by few researchers before the GWAS[12,13]. This led to the widespread expectation of loci allowing prediction of disease risks, prognostic assessment and tailoring of

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Table I
OA associated loci at the genome-wide significance level

Gene*	Joint†	Gender	Population‡
GDF5	Knee	All	EUR and Asian
HBP1 (7q22)	Knee	All	EUR
MCF2L	Knee + hip	All	EUR
DVWA	Knee	All	Asian; no EUR
HLA class II/III − BTNL2	Knee	All	Asian; no EUR
GNL3/GLT8D1	Knee + hip TJR	All	EUR
ASTN2	Hip TJR	Women	EUR
FILIP1/SENP6	Hip	All	EUR
KLHDC5/PTHLH	Hip	All	EUR
CHST11	Hip TJR	All	EUR
DOT1L	Hip	Men	EUR

* *BTNL2* = butyrophilin-like 2, *GNL3* = guanine nucleotide binding protein-like 3 (nucleolar), *GLT8D1* = glycosyltransferase 8 domain containing 1, *ASTN2* = astrotactin 2, *FILIP1* = filamin A interacting protein 1, *SENP6* = SUMO1/sentrin specific peptidase 6, *KLHDC5* = kelch domain containing 5, *CHST11* = carbohydrate (chondroitin 4) sulfotransferase 11.

† Joint or combination of joints in which genome-wide association was reported. Often association of lower strength was found in other joints.

‡ Most OA loci have not been studied in Asians or in other ethnic groups beyond EUR.

treatments[14]. Today, this view seems too optimistic for many diseases. It has become clear that the prediction potential of genetics in complex diseases will be much harder to obtain and that it is even doubtful if it will be of utility in a wide range of diseases[5,15,16]. However, some situations in which the genetic prediction is clinically useful have already been identified and have led to the development of clinical tests[17–19], but none in OA. Besides prediction, the susceptibility loci are expected to lead to new therapeutic targets. This expectation is as valid and strong today as it was before[5]. There are many loci that have opened or reinforced new areas of research. When the loci are understood, even incompletely, they become the experiments of nature that signal critically sensitive steps in the disease process. A limiting step in the path to tangible benefits has been the slow progress from association to mechanism, but powerful help has arrived in the form of large projects including the 1000 Genomes Project[20] and the Encyclopedia of DNA Elements (ENCODE) project[21].

The 1000 genomes and the ENCODE projects

The 1000 Genomes Project aims to identify rare polymorphisms and other variants like deletions and insertions through sequencing the genomes of more than 1000 individuals of up to 14 populations[20]. In this way, it is facilitating the study of rare variants by providing a catalog and a null expectation of the number of variants of each type in randomly selected individuals. This is very important, because it has shown that each individual bears a significant number of potentially functional variants, even in categories that seem damaging as loss of function variants. In addition, the 1000 Genomes Project is helping analysis of existing GWAS data by imputation of non-genotyped polymorphisms that allows fine mapping of associated loci or discovery of new signals. The first type of application, fine mapping of the locus around an isolated associated SNP identified by genotyping, was of utility for obtaining confidence in *MCF2L* as an OA susceptibility locus[22].

The ENCODE project aims to systematically uncover the function of the genome[21]. This is a monumental task that is still ongoing but that has already provided a huge amount of high quality information. Now it is possible to know how a sequence of interest behaves in up to 147 different cell types and in a wide array of experiments (Fig. 1). This information includes whether this sequence is transcribed or not and the kind of transcript it is producing. This has led to the unexpected discovery of wide (more than 60 % of the genome) and complex transcription including many alternative transcripts for the same sequence, genes that are co-transcribed, anti-sense transcripts and overlapping transcripts. Among these transcripts more than 8000 correspond to small RNAs, more than 9000 to long non-coding RNAs and near 1000 to pseudogenes. Thanks to ENCODE is also possible to know if a sequence of interest contains any of more than a dozen different chromatin marks including DNA methylation, histone modifications and its DNase sensitivity. These epigenetic marks are associated with stable

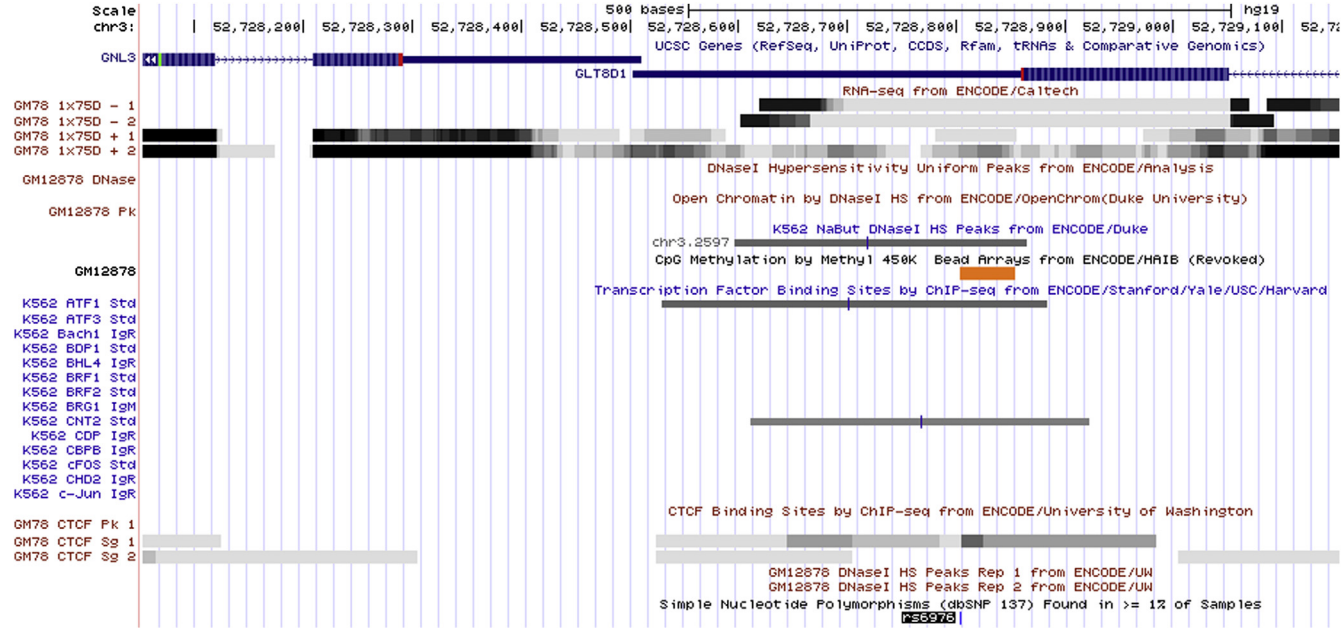

Fig. 1. Example of genome track with functional data from the ENCODE Project. The rs6976 OA associated SNP is highlighted in the bottom track. A very small fraction of all the available tracks is shown with information of, from top to bottom, position, genes, RNA-seq readings. DNA hypersensitivy, CpG methylation, selected transcription factor binding and CTCF binding.

functionality in ways that permit to predict, for example, if a promoter is active in a cell type or if the sequence is likely to be an enhancer. In addition, binding of more than one hundred transcription factors has been analyzed leading to a simplification of the analysis of regulatory elements at each site of interest. All this information, adding to 1640 genome data sets, is useful at multiple levels. One of them is to help identify the causal variant in a locus and its mechanism of action. Unfortunately, none of the cell lines that have been prioritized for study in ENCODE is from joint tissues. However, there are cartilage, bone, bone marrow stroma and muscle cell lines as well as mesenchymal stem cells in the, so called, tier 3 of the project. Some of these cells have already been studied for a few traits. More specific information will only become progressively available. Therefore, it will be necessary to extrapolate from other tissues in most situations. This approach will be especially useful in sites that show similar functionality in multiple cell types or in other mesoderm lineage cells. The first applications for the study of complex diseases have already started to appear[23,24].

The arcOGEN study

The arcOGEN study is the largest GWAS of OA to date[25]. It is also the most successful leading to the discovery of five loci with genome-wide significant association and of three near this level (Table I). Publication was preceded by presentation of its main findings in the last two OARSI meetings and they were already commented in last year review[1]. It is remarkable that most associations were specific of particular subgroups of patients, stratified either by joint, gender or severity. The heterogeneity of OA across these strata has also been found in studies reported this year.

The relationships between OA genetics and the genetics of overweight measured as BMI and adult height were analyzed in a new arcOGEN publication[26]. Motivation behind this analysis is the increased OA risk associated with high BMI and the dual association of the risk allele of rs143383 in growth differentiation factor 5 (*GDF5*) with OA and low height. Lack of height or BMI information for the arcOGEN subjects made necessary to compare association signals between studies, arcOGEN for OA and the GIANT Consortium for height and BMI. A significant overlap was found with both height and BMI. For height, excess overlap was observed with more than 3500 independent SNPs that showed $P < 0.1$ with OA and with height. For BMI, excess overlap was detected with 20 independent SNPs that showed $P < 0.005$ with OA and BMI. The level of association of the individual overlapping SNPs was too low to identify them with any confidence. However, some loci can be signaled thanks to information from other studies (Table II)[25]. The overlap does not appear as strong as to be a significant confounding factor for most SNPs in statistical analysis. This was already shown for BMI in the arcOGEN GWAS[25], and is shown in this new study for BMI and height[26]. Only association of fat mass and obesity associated (*FTO*) among 19 selected loci was significantly affected by adjusting them in a set of samples with complete information on OA, BMI and height. These results suggest pleiotropy of the loci, with different mechanisms behind association with each phenotype, but only elucidation of these mechanisms will solve this question.

Table II
Loci showing sound association with OA and with either height or BMI. No genome-wide significant association was requested for inclusion in this table

Gene	Joint	Gender	Height	BMI
GDF5	Knee	All	+	
PTHLH	Hip	All	+	
DOT1L	Hip	Men	+	
FTO	Knee + hip	Women		+
COL11A1	Hip	All	+	

In addition, the arcOGEN study has been used to explore association of mitochondrial haplogroups with OA but none was found[27] questioning the previously reported findings[28,29].

Genetic association studies

No new OA GWAS has been reported in this period but there have been some interesting association studies. One of them has brought DOT1-like, histone H3 methyltransferase (*DOT1L*), encoding a H3K79 methyltransferase with a role in chondrogenic differentiation and adult articular cartilage including interaction with TCF and WNT signaling[30], to genome-wide significant association with hip OA in men[31]. *DOT1L* was already associated at that level with minimal joint space width and at a lower level (10^{-4}) with hip OA in a previous study[30]. The inclusion of new sample collections, largely thanks to the Treat-OA consortium and the arcOGEN project, led to its strengthened association with hip OA and to discover that this association is specific of men, a gender specificity that was not observed in the association with minimal joint space width. This is one of the OA loci that are also associated with lower height (Table II)[32,33].

Other study analyzed the SNP in double von Willebrand factor A domains (*DVWA*) that is associated with knee OA in combined Japanese and Chinese patients[34] but not in European subjects[35,36]. The new study did not show association in a population cohort of Koreans (725 with radiographic knee OA and 1737 without OA)[37], further strengthening the evidence of ethnic heterogeneity of *DVWA* association. A bone morphogenetic protein 5 (*BMP5*) functional microsatellite previously associated with hip OA in women[38] was also associated with knee OA in a new study[39]. Association with the same alleles and showing also women specificity in the 1003 knee OA European patients (requiring total joint replacement (TJR)) and 1543 controls could indicate that it has escaped GWAS detection for lack of linkage disequilibrium (LD) with SNPs. Other association studies suggest new associations with *GREM1*, *SREBP-2* or *OPN*, but they require more evidence or replication[40–42]. Associations of purinergic receptor P2X, ligand-gated ion channel, 7 (*P2RX7*) and *PCKS6* with pain in OA[43,44] were already commented last year[1]. They together with the association of transient receptor potential cation channel, subfamily V, member 1 (*TRPV1*) are the first steps in an area of great interest and that needs to be further developed[45]. All previous association studies bring us to the following list of OA loci.

Lists of OA associated loci

Three OA loci with genome-wide level association in Europeans (EUR)[22,46,47]and two exclusive of East Asians[34,48] were known before publication of the arcOGEN study (Table I). The arcOGEN GWAS brought the number to 10[25]. *DOT1L* has been discovered since then[31]. This makes to a total of 11 loci in this first class. It is remarkable that many of these loci do not contain obvious OA candidate genes. This may make progress more difficult but will clarify new aspects of the pathogenesis of OA.

A second group of loci is more loosely defined and the list will vary depending on criteria (Table III). It contains those near the genome-wide level or found in large and sound studies. A significant fraction of these loci will eventually pass to the genome-wide significant category. Lack of genome-wide significance at this time could be consequence of insufficient power or of heterogeneity in OA. It is important to remark that the gene names we use to refer to these loci are in many cases only educated guesses because available evidence does not allow excluding alternative genes in the

Table III
Loci that have been associated with OA without genome-wide significance. The most clearly associated are near the top of the list. Uncertainty increases towards the bottom. Order is only approximate

Gene*	Joint	Gender	Population†
TP63	Knee TJR	Women	EUR
FTO	Knee + hip	Women	EUR
SUPT3H/CDC5L	Knee + hip	Men	EUR
PTGS2	Knee	All	EUR
MICAL3	Knee + hip	All	EUR
COL11A1	Hip	All	EUR
PCSK6	Pain knee	All	EUR
C6ORF130	Knee	All	EUR
MATN3	Hand	All	EUR and Asian
SMAD3	Knee	All	EUR and Asian
ASPN	Knee	All	EUR and Asian
TRPV1	Pain knee	All	EUR
RBFOX1	Hand	All	EUR
DIO2	Hip	Women	EUR
P2RX7	Pain knee	All	EUR
BMP5	Hip + knee	Women	EUR
CALM1	Hip	All	Asian; no EUR
FRZB	Hip	Women	EUR

* *TP63* = tumor protein p63, , *SUPT3H* = suppressor of Ty 3 homolog, *CDC5L* = CDC5 cell division cycle 5-like, *PTGS2* = prostaglandin-endoperoxide synthase 2, *MICAL3* = microtubule associated monoxygenase, calponin and LIM domain containing 3, , *PCSK6* = proprotein convertase subtilisin/kexin type 6, also known as *PACE4*, *C6ORF130* = chromosome 6 open reading frame 130, *MATN3* = matrilin 3, *ASPN* = aspirin, , *RBFOX1* = RNA binding protein, fox-1 homolog, also known as *A2BP1*, , *CALM1* = calmodulin 1.

† Populations that have been studied with, at least, a positive study.

same loci. Other studies, including functional studies, will be necessary to solve the ambiguities.

Functional studies of OA loci

A clear example of functional studies helping to discern between genes in a locus has been reported this year. The *7q22* OA locus is characterized by strong LD that prevents distinguishing between six genes. Functional studies in humans, mice and zebra fish had been done to differentiate between them without success[47,49]. Initial emphasis was put in *GPR22*, because this G protein-coupled receptor shows a *cis*-QTL in lymphoblastoid cells[49]. However, this year's study discards *GRP22* after showing that it is not expressed in any of the seven joint tissues analyzed[50]. The other five genes were expressed in all joint tissues and at lower levels in OA than in control hip cartilage. Low expression of three of them was correlated with the OA associated SNPs showing the difficulty of distinguishing between them also by expression analysis. Fortunately, allele expression imbalance analysis (AEI) identified only one of them, HMG-box transcription factor 1 (*HBP1*), as the gene to prioritize for further study without definitively excluding any of the other genes in this locus. *HBP1*, coding for HMG-box transcription factor 1, has been implicated in suppression of WNT signaling, a pathway of great interest in OA and prominent in multiple genetic studies of this year.

New studies have added interesting information about *GDF5*, which has become the best understood OA locus, and about other loci of less well established status. A new regulatory element in the *GDF5* promoter has been identified[51]. The site is affected by a variant so rare that only 1 was found among >1900 sequenced Europeans[52]. However, it led to a more complete understanding of *GDF5* regulation reinforcing the context specificity of the risk alleles effect. The variant modifies binding of the transcription factor YY1. Overexpression of YY1 stimulates *GDF5* expression and is able to compensate for the OA predisposing alleles. In a different sequencing study, signals of positive selection in East Asians have been found in the promoter and first exon of *GDF5*[53]. The OA/low height risk alleles are absent in Africans, very frequent out of Africa, and positive selected in East Asians. This study proposes that these alleles have selective advantages. To end with *GDF5*, the risk allele was associated with the severity of tibiofemoral OA (but not of patellofemoral OA) in a study analyzing more than 3400 knee OA cases with K/L scores $\geq$2[54]. No significant association was found with other two OA loci, *7q22* and *MCF2L*. Given the cross-sectional design of the study, this result can be due to a role in progression of OA or in its early onset.

Several studies have addressed deiodinase, iodothyronine, type II (*DIO2*). One of them has found that *DIO2* risk allele is associated with increased expression in AEI probably indicating that this nsSNP is in LD with a *cis*-acting polymorphism[55]. Overexpression of *DIO2*, which has been repeatedly shown in OA cartilage[55–57], is assumed to be damaging and it accelerates OA in a rat destabilization model[57]. However, a study suppressing *DIO2* in human chondrocytes showed an anti-inflammatory role of this enzyme, likely mediated by LRXα[58]. New studies will be needed to see if these apparent contradictory results fit in a consistent model. An analysis of collagen, type XI, alpha 1 (*COL11A1*) has found puzzling results: the OA associated SNP does not correlate with AEI in cartilage, whereas an SNP associated with lumbar disc herniation in the Japanese correlates with AEI but is not associated with OA[59]. Finally, two nsSNPs in frizzled-related protein (*FRZB*) that have been studied for association with hip OA[60] are now reported as associated with proximal femur shape[61]. This is an OA endophenotype of great interest that has already been shown to be genetically influenced[62].

DNA methylation

DNA methylation is the most studied epigenetic mark in the OA field[1,63]. Previous studies have been centered in the promoters of selected genes. Now, the first DNA methylation profiling studies in OA have been published[64,65]. They used the Illumina array interrogating more than 27,000 CpG sites predominantly placed in CpG islands of proximal promoters. As a general rule, hypermethylation of these sites signals stable repression of the gene downstream. One of the studies with the 27 K array compared full thickness cartilage of the central tibial plateau from OA patients with normal controls[65]. Modest differences, with only 91 differentially methylated sites, were observed. Gene enrichment analysis showed inflammatory response and positive regulation of transcriptional activity as significantly hypomethylated. The hypermethylated sites were enriched in regulation of phosphorylation and mitogen-activated protein kinase activity. The most hypomethylated site was in *RUNX1*, a transcription factor involved in chondrogenesis. The most hypermethylated site was in *MSX1*, a transcriptional repressor involved in development. Unfortunately the study did not include validation of methylation or analysis of expression of the differentially methylated genes in the same samples. An additional result of this study is the identification of a cluster of OA patients showing marked methylation differences with the rest of OA patients. Hypomethylation in this group was enriched in promoters of genes involved in inflammation, in particular chemokine and cytokine activities, and hypermethylation in genes of structural components of the extracellular matrix. Confirmation of the differences between patients will be necessary because the subdivision of OA patients could be of great relevance for OA research.

The second study profiling DNA methylation addressed differences between OA and osteoporosis (OP) in trabecular bone from the femoral head of women[64]. Differential methylation sites were enriched in functional groups fit for bone: development of the appendicular skeleton, limb morphogenesis and osteoblast

differentiation. Focusing on the 45 more differentially methylated sites, 43 were more methylated in OA bone than in OP bone, possibly reflecting the more prominent role of bone in OP than in OA. These 43 genes were from a wide array of functional groups, including eight homeobox genes. The two sites more methylated in OP bone than in OA were related with the immune system, lymphotoxin α (LTA) and LY9 that could indicate differential involvement of bone marrow cells. These are very suggestive results but further experiments will be required to appreciate their meaning.

The promoters of four genes have been specifically analyzed during this year. Two CpG promoter sites were identified by analysis of their sequences as possible binding sites for the cartilage catabolic transcription factor HIF-2α: the −110 site in the *MMP13* promoter that is hypomethylated in OA cartilage[66], and the −299 site in the *IL1B* promoter that is hypomethylated in chondrocytes after repeated stimulation in culture[67]. Now, the *MMP13* site has been demonstrated as a methylation modulated HIF-2α binding site whereas the *IL1B* site is not[68]. However, the *IL1B* site has been shown to be hypomethylated in OA chondrocytes and to regulate *IL1B* expression[68]. Also the inducible NO synthase gene, *NOS2*, is regulated by DNA methylation in OA cartilage[69]. This is the first example of methylation changes in an enhancer, not in the promoter. Two CpG sites more than 5 Kb upstream of the gene were hypomethylated in OA chondrocytes. These two sites are in the neighborhood of an NF-κB binding site and their methylation repressed transcription induced by the p65 subunit of NF-κB demonstrating their functionality. In contrast, the anabolic transcription factor *SOX9* has shown the opposite change in hip OA cartilage, promoter hypermethylation[70]. The widespread hypermethylation at this promoter inhibits binding of two transcription factors, NFYA and CREB, and correlates with decreased mRNA and protein levels of *SOX9*. In addition, this study addressed histone modifications in *SOX9*. The histone marks were characteristic of a repressed promoter: increased H3K9me3 and H3K27me3 and decreased H3K9ac.

These studies provide the first direct evidence of epigenetic regulation of *IL1B*, *SOX9* and *NOS2* in OA cartilage extending the reach of this type of epigenetic modification in OA. These stable changes are potential targets for manipulation as are the miRNAs regulating cartilage expression.

miRNA and other non-coding RNA studies

The study of miRNA is a burgeoning field that has produced many interesting findings. However, it is still early to understand their significance due to the diversity of miRNAs, the multiplicity of their targets and a certain lack of consistence between studies. Several excellent reviews have been published that give an introduction to the field and information on previous studies[63,71].

Two miRNA profiling studies in OA cartilage were published more than 2 years ago[72,73]. They identified several up- and down-regulated miRNA but none was coincident and one was found as upregulated in one study and down-regulated in the other. A new study analyzing RNA extracted from pellets of cultured chondrocytes has brought the first replications this year[74]. The single miRNA upregulated in OA according to this new study, *miR-483*, was also the most upregulated in one of the previous studies[72]. In addition, one of the 6 down-regulated miRNAs, *miR-149*, was also down-regulated in the other previous study[73]. More profiling studies are clearly required to establish the catalog of miRNA with consistent changes. Lack of reproducibility of results until now can be ascribed to differences in methodology, the small number of samples analyzed and confounding. This last possibility is supported by the strong correlations between BMI and five of the miRNAs associated with OA[72], and by changes of miRNA expression in cartilage with age[75].

miR-140 has been singled out as a critical miRNA in OA because of its specific expression in cartilage, its important role in cartilage development, the lower levels observed in OA and the accelerated OA in mice lacking *miR-140*[63,71]. However, a new study found increased *miR-140* in OA cartilage in contrast with previous reports[76]. The difference is unexplained but it is intriguing that this study used femoral heads, whereas previous studies included knees[72,77,78]. The same study has identified other miRNA, *miR-455*, that was also overexpressed in OA cartilage and that suppresses *SMAD2* expression[76]. This finding together with *miR-140* suppression of SMAD family member 3 (*SMAD3*), led the authors to propose that these two miRNAs play a synergistic role in OA favoring a catabolic phenotype[76]. The two are induced by TGFβ signaling and suppress the SMAD2/3 TGFβ pathway, which is anabolic and prevents terminal differentiation. In addition, by suppressing *SMAD2/3* the two miRNAs allow TGFβ signals follow the SMAD1/5/8 pathway without competition, with the subsequent differentiation of chondrocytes to a catabolic phenotype.

Other authors have addressed the specific regulation by miRNA of important genes after IL1β stimulation of chondrocytes. A very complete study has identified two miRNAs, *miR-199a* and *miR-101.3*, regulating the *COX2* gene[79]. The pathway that was analyzed involves IL1β stimulation of p38-MAPK activation followed by downregulation of *miR-199a* and *miR-101.3* and subsequent upregulation of *COX2*. *miR-199a*, but not *miR-101.3*, was found at lower levels in OA cartilage than in normal cartilage. A similar study identified *miR-101* as regulating *SOX9*[80]. *SOX9* is a target of *miR-145* in chondrocyte differentiation[63,81], but expression of *miR-145* was not altered by IL1β excluding its involvement in the chondrocyte changes[80]. This study also showed that overexpression of *miR-101* is able to abrogate ECM degradation induced by IL1β indicating its wide cartilage protection effects. It is interesting that the two studies have identified *miR-101* as a critical miRNA in chondrocytes cultured with IL1β[79,80].

There is some evidence indicating that miRNAs and other non-coding RNAs can be useful as OA biomarkers. A report described four miRNAs overexpressed in PBMCs of patients with OA[82]. This result is reminiscent of a previous study showing increased levels of a different miRNA in plasma of OA patients[83]. However, these two studies are small and need replication. Better powered than them is a third that did not address OA but a situation that can lead to OA, cartilage damage 1 year after anterior cruciate ligament injury. No change in miRNAs was found but serum levels of two small nucleolar RNAs, *U48* and *U38*, were associated with the injury antecedent and levels of *U38* correlated with the extent of cartilage damage[84]. The validity of these potential biomarkers in OA will surely be further explored.

Functional genomics

As reviewed last year[1], the microarray technology used for functional genomics is now accompanied by RNA sequencing (RNA-seq). RNA-seq has advantages over microarrays but it has not yet been used in the OA field. Therefore, all this year's studies addressing specific OA questions were done with microarrays.

The first whole genome microarray study comparing cartilage from hip OA patients with femoral neck fracture patients was reported[85]. It showed low concordance with a similar study previously done for knee OA[56]. Only 229 genes were associated with OA in the two studies among the 998 differently expressed in the hip and 1423 in the knee. In addition, 71 of the 229 common genes showed opposed changes in the two joints, therefore commonality was even smaller. Common between the two studies were ECM associated genes especially collagens. They were upregulated in what is interpreted as an attempt of cartilage repair. Opposed

changes were found in metalloproteinases, with ADAMTS5, ADAMTS1 and MMP1 increased in knee OA and decreased in hip OA. These results strengthen the concept of specificity of joint in the OA process that has been alluded above. In addition, this work has identified hub genes (showing $\geq$5 interactions with other genes) that are upregulated or down-regulated in hip OA. Collagen genes are predominant in the upregulated group, whereas signaling molecules are abundant among the down-regulated hub genes. In this latter group, the authors highlight two kinases, *AKT3* and *MAPK1*, and two transcription factors, *MYC* and *ATF3*.

A paper has found a surprising pattern of expression of OA synoviocytes[86]. It compared OA with RA and with healthy synovial cells. The most differentiated expression was found in OA FLS, with more changes between OA and healthy controls than between RA and healthy controls. These results show that synovial cells are involved in the OA disease process and that they cannot be considered as equivalent to healthy synoviocytes. The authors indicate that no proinflammatory phenotype or increased expression of ECM proteases was observed in OA synoviocytes. However, they observed a decreased expression of growth factors of the hepatocyte, bone morphogenetic protein and fibroblast families, as well as, dysregulation of cell adhesion and motility factors and increased expression of neurotransmitter receptors and signaling molecules. The authors interpret that these changes are suggestive of a widespread change in mesenchymal cell expression that could be common to chondrocytes, osteoblasts and bone marrow stromal cells. The advanced status of OA and RA in this study that obtained synovial tissue from knee replacement surgery should be considered.

Also of interest are two studies profiling expression differences between two types of cartilage. A first study compared articular and osteophytic cartilage from the same OA knee[87]. The two tissues were clearly different with a bias to higher expression in osteophytic cartilage (515 genes) over articular cartilage (85 genes). 34 transcripts were more than 20 fold different. Osteophytic cartilage was enriched in factors involved in terminal chondrocyte differentiation, endochondral ossification and in ECM enzymes. Articular cartilage showed increased levels in growth factor signaling genes. These changes are interpreted as showing the process of terminal differentiation in the osteophytic cartilage and the prevention of hypertrophy and terminal differentiation in articular cartilage. This prevention was observed in spite of severe OA and it involves inhibition of the BMP and WNT pathways by *GREM1*, *WISP3* and *FRZB*, and at other levels by *STC2*, *SOX9* and parathyroid hormone-like hormone (*PTHLH*), all of them genes upregulated in articular cartilage. A second study compared articular cartilage with growth plate cartilage from adolescents to identify mechanisms involved in hypertrophic differentiation[42]. Overexpression in the growth plate cartilage of cell cycle-related genes and of the WNT signaling pathway was highlighted by the authors. Articular cartilage showed higher expression of *TLE2*, a transcriptional inhibitor of the WNT pathway and three WNT antagonists *GREM1*, *FRZB* and *DKK1*. The three WNT antagonists were the most differentially expressed genes in this study. The results of these two studies support an active maintenance of the articular cartilage by downregulation of the WNT pathway. Based on this interpretation, the authors propose that potential regenerative approaches for OA should consider inhibitors of terminal differentiation in addition to anabolic factors[87].

Summary

We are now more conscious of the limitations and potential that genetics can provide. The limitations have not undermined excitement in the field caused by the discovery of eleven OA susceptibility loci at the genome-wide significance level and by quick and interesting progress in technology, genomic information resources, functional studies, epigenetics and functional genomics. New areas of research have been opened by new OA loci as *DOT1L* or the prioritization of *HBP1* for further confirmation in the *7q22* locus, by the first large genetic study of OA severity, the identification of a subgroup of OA patients with differentiated cartilage DNA methylation, the potential biomarker role of non-coding RNAs, the uncovering of multiple differences between OA in the hip and knee joints, and by the active downregulation of the WNT pathway in articular cartilage among other many findings. They are the starting points of as many interesting stories, many still require confirmation, but some will become useful for our understanding and management of this complex disease.

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Competing interest statement

The author has not declared competing interests.

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