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Genome Wide Association Studies and Next Generation Sequencing Technologies in Osteoarthritis

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Abstract

Osteoarthritis is a commonly age-related degenerative joint disorder characterized by articular cartilage degradation, subchondral bone sclerosis, osteophyte formation, and synovial membrane inflammation. Various environmental, biomechanical, and genetic factors have been recognized as playing essential roles in OA development. A number of studies have endeavored to decipher the pathogenesis of osteoarthritis. In an attempt to identify the genetic markers of complex diseases such as osteoarthritis, there has been a paradigm shift away from traditional linkage mapping studies and candidate gene association studies to higher-density genome-wide association studies. This introduction to genome-wide association studies and next-generation sequencing technologies provides of these areas and then considers their relevance to in osteoarthritis. High-throughput genomic and transcriptomic methods have resulted in a paradigm shift in the way osteoarthritis is perceived and have changed the way translational research is performed. This review presents an overview of high-throughput genome wide association and next generation sequencing in osteoarthritis and discusses clinical applications of these technologies.

Keywords: Genome wide association studies; next generation sequencing; osteoarthritis

Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease that is characterised by articular cartilage loss, osteophyte formation, subchondral bone sclerosis, and synovial inflammation. OA is associated with joint space narrowing and osteophyte formation leading to loss of joint function and pain. Whilst the exact aetiology of the disease remains unclear, age, gender, obesity, previous joint trauma, menopausal status, and genetic variations are known risk factors associated with OA [1].

A wide range of biological processes play important roles in the pathogenesis of OA. Pro- and anti- inflammatory, angiogenic, and growth signalling pathways are central aspects of disease progression. Likewise in other multifactorial disorders, each aspect of the disease is subject to genetic variations in the genes encoding and regulating individual proteins in each pathway resulting in alterations in patient's susceptibility to OA, prognosis, and the efficacy of intervention. Due to the large range of genetic diversity involved, high-throughput sequencing techniques are valuable tools for the continued research in such diseases.

Next generation sequencing (NGS) is a technique that has already begun to revolutionise research and clinical genomics. With the ability to sequence target transcriptomes, exomes or whole genomes in a relatively quick and economically viable way, the understanding of genetic factors in diseases is improving, leading to improved therapeutics and tailored treatment plans in clinical practice. Figure 1 displays a summary of techniques being used to analyse genetic variations at various levels (reviewed in more details by Soon et al. [2] and Zhou et al. [3]). Additionally, developments in computational analysis and the growth of bioinformatic databases, such as HapMap and HuGE, have supported the increased use of large scale genetic studies. Genomic variations in individuals include single nucleotide polymorphisms (SNPs), insertions, and deletions which can lead to the heritability of diseases. NGS offers a genome-wide view of a patient which can prove to be an indispensable tool in multifactorial disease like OA. Here, we briefly describe how NGS is being utilised in OA and how future applications will likely improve our understanding of the pathophysiology and ultimatelypatient care.

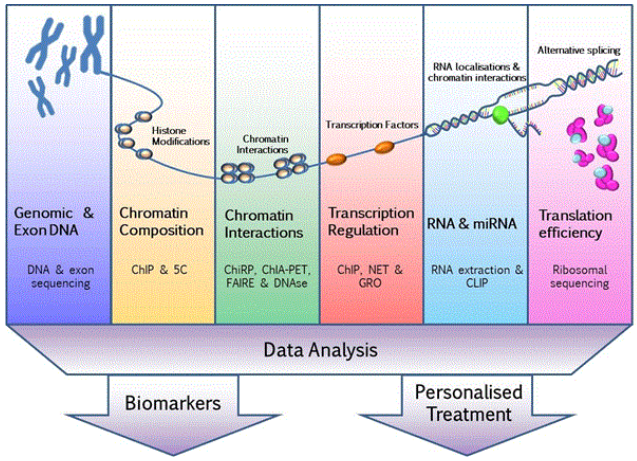

Figure 1: The levels at which genetic variations can occur and example techniques for their analysis. ChIP: Chromatin Immunoprecipitation; 5C: ; ChiRP: Chromatin isolation by RNA purification; ChIA-PET: Chromatin Interaction Analysis with Paired-End Tag Sequencing; FAIRE: ormaldehyde-ssisted solation of egulatory lements; DNAse: DNase I hypersensitive site sequencing; NET: Native elongating transcript sequencing; GRO: Global run-on sequencing; CLIP: Cross-linking immunoprecipitation.

Candidate gene association studies

A large number of studies in SNPs and their association with OA have provided greater understanding of its pathophysiology in recent years. The range of genes investigated reflects the complex nature of OA. Table 1 demonstrates the range of the 88 genes associated with knee OA to date. Due to differences in ethnic, age, sex, and other variables, numerous studies remain to be replicated and often have limited statistical power from small sample sizes. However, they have offered an inside into a range of genetic variations and their effects in OA. Frequently genes have multiple possible SNP locations in the exons, introns, or promotor and regulatory regions which can complicate the roles possible mutations may play. The narrow field of view of such studies limits their ability to efficiently identify disease-associated variations but do allow future studies build on their findings.

ACE	DIO2	IL1RN	PRKAR2B*
ADAM12	DIO3	IL6	PTGS2*
AGER	DUS4L*	KL	RHOB
ANP32A	EDG2	LEP	SERPINA3
AR	EPAS1	LEPR	SLC26A2
ASPN	ESR1	LOC344875	SMAD3
BCAP29*	ESR2	LPAR1	SOST
BDKRB2	FRZB	LRCH1*	TLR2
BMP2	GDF5	LRP5	TLR3
BTNL2*	GHRHR	LRP6	TLR4
CALCA	GPR22**	MCF2L	TLR7
CALM1	HFE	MMP1	TLR8
CCL2	HLA-DQB1*	MMP13	TLR9
CD36	HLA-DRB1	MMP2	TNF
CILP	HPBP*	MMP3	TNFAIP6
CLEC3B	ICAM1	MMP9	TNFRSF11B
COG5**	IL10	NCOR2	TNFRSF1A
COL11A1	IL18	PAPSS2	TNFRSF1B
COL2A1	IL18R1	PCSK6	TRPV1
COL6A4P1	IL1A	PITX1	TXNDC3
COL9A3	IL1B	PLA2G4A*	VDR
COMP	IL1R1	PPARG	VEGFA

* Gene association supported by a GWAS [5-9]

** Gene association supported by more than one GWAS [10, 11]

Table 1: The 88 genes associated with knee osteoarthritis according to HuGE Navigator [4]

Genome wide association studies

By scanning the entire genome of large samples of individuals with or without a disease, variations can be found that may be associated with a disease or condition. Such high-throughput studies rely on large sample sizes to increase the statistical power of their results and are made possible by NGS. Hitherto, 9 genome wide association studies (GWASs) have been conducted with respect to OA [12]. Panoutsopoulou and Zeggini have reviewd GWASs of European and Asian individuals [13]. A total of 15 loci have been identified through the use of GWASs. The authors also noted the importance of phenotype definition. Qualitative techniques are the primary diagnostic tools for OA. The Kellgren-Lawrence scale [14] is used extensively as a measure of disease severity in osteoarthritis but it is subjective and open to interpretation in clinical practice. Such variables in the assessment of osteoarthritis severity introduce uncertainties in the results of GWASs which should be considered when interpreting their conclusions.

The arcOGEN GWAS study revealed 5 novel OA-associated loci and a further three 3 just below the genome-wide association threshold in a sample group of 7410 individuals [15]. The rs6976 SNP in the *GNL3* gene on chromosome 3 showed the strongest association. Guanine nucleotide binding protein-like 3, also known as nucleostemin, is encoded by the *GNL3* gene and is known to regulate the cell cycle in mesenchymal stem cells which ultimately differentiate into cartilage forming chondrocytes [16]. However, its role in OA is not fully understood. Whilst this study was critisised by Pang et al. for having beem restricted to European participants [17], these findings provide the basis of future functional studies.

The use of meta-analysis of multiple cohorts improves the statistical power of such studies. Very recently, Rodriguez-Fontenla et al. performed a meta-analysis of 9 SNP-level GWASs [18]. They found

that of 199 previous suggested candidate genes associated with OA, only 2 showed significant association, *COL11A1* and *VEGF*. Vascular endothelial growth factor (VEGF) levels in plasma synovial fluid have previously been reported to correlate with disease severity in knee osteoarthritis [19].

With thousands of participants, GWASs yield vast amounts of information which presents possible problems with information security and patient privacy. As DNA is the most personal of information types, study participants should be well informed of the risks and all precautions taken to prevent data loss or misuse.

Although the large sample sizes and often complex data analysis can be expensive and time consuming, GWASs are powerful tools at detecting variations on a genome-wide scale which can serve as the bedrock for further researches in cell and molecular biology.

Biomarkers and therapeutics

The search for diagnostic and disease-assessment markers in OA has been primarily focused on investigating biochemical components of cartilage degradation and inflammation related cytokines in serum/plasma and synovial fluid [20]. The potential of biomarkers to assist in diagnosis of OA is obviously an attractive tool for clinicians. However, local and systemic biochemical levels have limited use for predicting the susceptibility of osteoarthritis and other diseases with long onset periods often lasting many years. Genetic variations that can predict the risk of such disease may help patients and clinicians take mitigating or preventative measures. Additionally, the development of effective therapeutics may be supported by improved understanding of the pathophysiology of OA that comes from a greater knowledge of the genetic influences.

Genetic markers from a range of physiological processes have been investigated. The IL-18 rs1946518 SNP has been shown to distinguish between knee OA patients and healthy controls with borderline significance [21]. Interleukin (IL)-18 is a proinflammatory cytokine which has been shown association with disease progression [22]. Moreover, SNPs of the osteopontin gene which is involved in bone formation and remodeling have been associated with an increased susceptibility of OA and radiographic KL grades [23]. Our laboratory recently built on the work of Rodriguez-Lopez et al. and showed that a non-synonymous (ns)SNP at rs4747096 in ADAMTS14 to be associated with knee OA in Thai and Caucasian females [24,25]. However, this study also shows that genetic variations can be heavily gender-dependent and therefore, the sex of individuals should be considered when performing genetic studies.

By understanding the variations in genes and the resultant effects, therapeutic agents can be designed to target these vulnerabilities. In the future, as the understanding of genetic OA continues to develop, gene therapy may be a viable treatment method in OA. Further research in this field is ongoing but studies show promising results, however, therapy may be most effective in early and often asymptomatic stages of the disease which may be a hurdle in the clinical application of such treatments [26].

Sanger sequencing (termination sequencing) and PCR techniques lack a wide field of view when investigating a patient's genetics. Furthermore, shortcomings in the older techniques often result in rarer deletion mutations being missed. As shown by Koboldt et al. [27], such mutations can affect protein structure and function leading to changes in the efficacy of small molecule drugs. Despite the introduction of NGS, PCR and Sanger sequencing still have roles to play in the development of better understanding of the genetic roles in OA. NGS offers a wider perspective which results in less bias and open the possibility to investigate many genes simultaneously and at a lower cost.

Due to OA being a multifactorial disease, the assessment of OA patients will require the ability to access multiple genes for multiple variations in a short time frame. NGS has the power to revolutionise medicine with personalised treatment courses.

Clinical Applications

Medicine will be revolutionised by advances towards so called "bed-side" sequencing. Future patients may have tailored treatment programs selected by their genetic information suggesting the efficacy of different therapeutics; however the risk of false data is always a concern. In OA, antibodies have been used in clinical trials to inhibit proinflammatory agents, such as tumour necrosis factor (TNF)-α, IL-1α and IL-1β, and matrix metalloproteinase (MMP)-13 [28-30]. Other existing treatments include the administration of hyaluronic acid and non-steroidal anti-inflammatory drugs (NSAIDs). However, the efficacy of these treatments may be affected by phenotypic variations on a cellular and tissue level because of genomic variations in an individual. Being able to predict which treatment is best suited to a patient will not only be beneficial to the patient's care but also save money. As the cost of sequencing continues to fall, the money saved from selective administration will inevitably outweigh the cost of analysis making it an economically viable option in the clinical environment.

Conclusion

In conclusion, next generation sequencing is a powerful tool that promises to continue to revolutionise research and clinical genetics. Large scale genome wide association studies offer broad views of individuals with complex disease and enable the detection of associated variations that may otherwise have been missed by older techniques. Additionally, through the relatively quick and cost effective sequencing of patient genomes, tailored medicine has the potential to offer personalised treatment plans and improve patient care.

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