COMMENTARY

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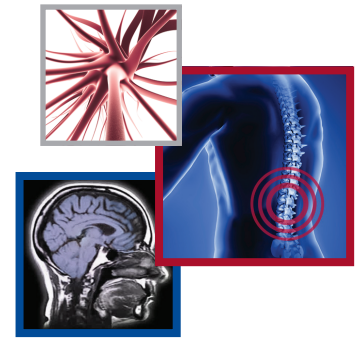
Pain Management



Using omics in chronic pain conditions to delineate mechanisms and provide new therapeutic strategies

"...the bench-to-bedside route in omics studies of chronic pain is not entirely straightforward and intensive functional and clinical research will be required to achieve a breakthrough in chronic pain management."

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• chronic pain • exome • genome-wide association studies • global methylation • metabolome • omics • PAIN-OMICS • quantitative sensory testing

Time for omics to challenge pain

The concept of omics refers to the large-scale structural and functional investigation of multiple measured biological variables and has become firmly rooted in contemporary biomedical studies. This reflects both the advances in the development of high-throughput techniques for simultaneous analysis of biological processes and entities and a trend toward employing a more holistic view on the control and regulation of biological systems and its implication for medicine. A web-based omics encyclopedia lists hundreds of 'omes' and 'omics' including customary ones, such as genomics or transcriptomics, and quite extravagant ones such as killifishomics or opiniomics. Attempts are being made to integrate different omics into a single pipeline for better understanding of biological pathways and to boost translational studies [1,2].

The advancement in omics technologies has facilitated the study of groups of chronic pain phenotypes allowing novel groupings of clinical manifestations previously considered separately. The phenotypes include both clinical syndromes of chronic pain, such as chronic low back pain (LBP), and chronic widespread pain (CWP)/fibromyalgia. In addition, objective measures of pain sensitivity known as quantitative sensory testing (QST), such as heat pain sensitivity, may provide a proxy for capturing the biological variation in the manifestation of pain. Whether QST relates to the mechanisms of the development of pain diseases and syndromes remains to be seen. Chronic pain, either as an individual chronic pain syndrome

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(LBP) or as a comorbidity (such as in inflammatory arthritis), is a recognized major healthcare issue and a leading cause of disability and work absence worldwide [3]. In Europe, the prevalence of chronic pain in one form or another is estimated to be 20% of all adults [4], and the financial costs of its management exceed €200 billion per annum in Europe and US$150 billion per annum in the USA. Some authors are urging a reclassification of chronic pain from 'symptom' to 'condition' [5]. The main risk factors for chronic pain are well-described and include female gender, age, lower social class, cultural background, lifestyle, employment status and occupational factors [6]. However, the biological component of chronic pain development and its maintenance is currently very poorly understood, and thus requires considerable epidemiological pathophysiological, immunological and genetic studies to delineate the mechanisms underlying chronic pain. On this basis, an understanding of the biological pathways involved will facilitate the development of effective biomarkers and novel therapeutic approaches.

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Genome-wide association studies

Genome-wide association studies (GWAS) utilizing hundreds of thousands of genetic single nucleotide polymorphisms (SNPs) have been proven to be advantageous over linkage and candidate gene studies of complex diseases and traits, especially in terms of their ability to reveal new – and sometimes unanticipated – pathways in disease [7]. This approach has been applied to a number of pain phenotypes. For example, a GWAS meta-analysis of Europeans coupled with follow-up functional studies identified *CCT5* and *FAM173B* genes associated with joint-specific chronic CWP [8]. A small GWAS study accompanied by a comparative genomic hybridization identified *MYT1L* and *NRXN3* as new candidates for the development of fibromyalgia [9]. Interestingly, *CCT5, MYT1L* and *NRXN3* participate in nervous system development or function, suggesting the impact of neuronal component in CWP predisposition or manifestation. Similarly, a GWAS identified *GFRA2* encoding receptor for a factor playing a key role in the control of neurone survival and differentiation as a novel candidate gene for diabetic neuropathic pain [10]. The largest GWAS meta-analysis so far comprised 29 studies of migraine coupled with gene expression profiling in brain and identified 12 loci, some of which directly or indirectly participate in nerve development and function [11], though our work on chronic pain syndromes suggests that phenotypically migraine is not part of the chronic pain spectrum [12].

Postsurgery pain phenotypes offer the advantages of a relatively standardized painful stimulus and may be assessed using a variety of techniques, for example, visual analog scales, and by the amount of strong analgesics to control postsurgery pain. They provide a useful QST-like model to measure nervous system input–response relations, providing insight into the mechanisms of nociceptive neuroplasticity [13]; however, analgesic responsiveness is also part of the latter model. Two GWAS of postsurgical pain measured by visual analog scale and morphine dosage respectively identified *ZNF429* and *TAOK3* genes not known to be specifically involved in nervous system activity and formation [14,15]. This means that neuronal components may not account for the overall genetic background of chronic pain. Also, this may reflect the difference in mechanisms of complex clinical pain syndromes and instrumentally measured pain sensitivity.

GWAS have also been carried out for musculoskeletal causes of chronic pain such as osteoarthritis [16] and lumbar intervertebral disc degeneration, which is associated with LBP [17,18]. Two genes were identified, *PARK2*, whose mutations cause some forms of Parkinson's disease [17], and *CHST3*, whose mutations are associated with musculoskeletal conditions, such as spondyloepiphyseal dysplasia and humerospinal dysostosis [18]. Interestingly, in both cases epigenetic factors appeared to be important – differential methylation for *PARK2* and interaction with a miRNA for *CHST3*.

Epigenomics

The role of epigenetic factors in the development of pain syndromes attracts much attention and at least one study has been carried out on an omic scale. The study was focused on QST phenotypes such as heat pain threshold in sensitivity discordant identical twins, and utilized a novel high-throughput technique for global analysis of methylation – methylated DNA immunoprecipitation (MeDIP) sequencing [19]. A number of epigenetic regions were revealed associated with heat pain threshold with the strongest signal established and further supported by an expression analysis for the *TRPA1* gene encoding an

ion channel likely involved in thermosensitivity [20]. Other plausible candidate genes were identified including *ST6GALNAC3*, *MICAL2*, *MICAL2*, *KCNE4*, *CDH11*, *COL18A1*, *DTNA*, *FHIT*, *KEL*, *PCDH7*, *PLG* and *SLC8A1*, all of which are involved in pain pathways [19].

Exome sequencing

While GWAS usually look at common genetic variants, rare variants and mutations are also of significant interest in the studies of complex diseases and phenotypes [21]. Nowadays, this sort of genetic variability can be routinely assessed thanks to the development of powerful methods of next generation sequencing. The exome sequencing analysis aiming to establish associations between rare variants and heat pain threshold has recently been carried out [22] revealing associations between pain and rare variants in 14 genes, with the strongest evidence seen for *GZMM* encoding granzyme M. This gene has not been related to pain sensitivity before, but may be involved in apoptosis and inflammation. Network analysis of genes associated with differential pain sensitivity revealed a significant enrichment of rare variants in genes of the angiotensin II pathway known to be involved in central pain sensitivity. Other genes identified in the study were noted to be indirectly involved in the reception and transduction of pain signals [22]. Thus, the combination of exome sequencing and sophisticated statistical analysis proved to be fruitful in the identification of new candidate genes, and may shed light onto mechanisms of pain sensitivity.

Metabolomics

Joint analysis of different omics is expected to provide more insight into pathophysiology of chronic pain syndromes, as exemplified by a recent genome-wide study of 324 plasma metabolites, CWP and anthropometry in adult female twins [23]. This is because intermediate phenotypes such as metabolites lie 'nearer' to the action of the gene than the trait of interest, so signals are stronger. This study identified several metabolites associated with CWP with the strongest association seen for epiandrosterone sulfate (EAS). Subsequent GWAS showed an association between EAS and variants at the 7q22.1 chromosome, suggesting *CYP3A5* gene involvement. Finally, a Mendelian randomization approach revealed that the association between CWP and EAS is likely driven by CWP in a genotype-dependent manner. Thus, this combined study of metabolomics and genomics carried out in an agnostic fashion confirmed the importance of steroid hormones in CWP pathophysiology, suggested EAS as a biomarker of CWP and confirmed *CYP3A5* as a CWP candidate gene. Other metabolomic studies, though not accompanied by a GWAS, have discovered multiple alterations in sphingomyelin-ceramide metabolism in a rat model of chronic neuropathic pain [24]. The study identified an endogenous metabolite N,N-dimethylsphingosine never before implicated in nociception, which authors found was able to induce pain and inflammation. The inhibition of this metabolite may offer a novel therapy approach for neuropathic pain.

Joint efforts

Thus, the use of omic techniques and approaches provides a wealth of additional knowledge into the mechanisms of pain and may result in new biomarkers to facilitate diagnosis and more accurate categorization of chronic pain subphenotypes, revealing new pathways of pain development, design of new therapeutics and stratification of patients for personalized treatment. These perspectives underlie an EU FP7-funded project PAIN-OMICS: 'Multi-dimensional omics approach to stratification of patients with low back pain'. The project set out to discover and validate new 'omics biomarkers' for the stratification of patients with chronic LBP, to help assess the risk of progression of acute to chronic LBP, and for predicting the response to therapy. Also the project aims at the discovering of molecular mechanisms of the genesis and maintenance of LBP. This is going to be achieved through the complementary exploitation of four omics: genomics, epigenomics, glycomics (the entire composition of glycans) and activomics (combined data about enzymatic activity of multiple proteins). The latter two are quite new omics with significant biomarker potential. Certainly, the integration of these multidimensional data is inconceivable without extensive and sophisticated statistical analysis, which is based on a combination of approaches including those being developed and validated in framework of another FP7 project MIMOmics: 'Methods for Integrated analysis of Multiple Omics data'.

Clinical applications

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new agents introduced in recent years, and an increasing burden of chronic pain for reasons that are unclear [25]. Classical descriptions of the nature of pain have revealed underlying pathogenic mechanisms to some extent, but the omics technologies offer a way to recategorize and phenotype chronic pain in a more meaningful way. By understanding better the pathways involved, new therapeutic targets may be revealed.

Among the examples of success using high-throughput methods for identification of new promising strategies for targeting pain are the discovery of N,N-dimethylsphingosine in neuropathic pain via metabolomics [24] and the KCNS1 potassium channel via a combination of global gene-expression profiling, network analysis and association study (reviewed from clinical perspectives in [26]). More similar findings can be anticipated by the extending omics scale researches. Also, being focused on the building of the whole mechanistic pathway from genotypic variation through to phenotypic expression, they also provide an avenue to stratification of patients into mechanism-based subgroups of disease subtypes and, therefore, point to personalized treatment. However, it must be noted that the bench-to-bedside route in omics studies of chronic pain is not entirely straightforward and intensive functional and clinical research will be required to achieve a breakthrough in chronic pain management.

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