Spinal disc degeneration

Professor Kathryn Cheah, a molecular geneticist working in Hong Kong, discusses how her group's research is challenging widely held assumptions about the role of ageing in lower back pain

To begin, could you introduce the main objectives of the Developmental Genomics and Skeletal Research initiative at The University of Hong Kong (HKU)?

At HKU, one of Hong Kong's Areas of Excellence (AoE) programmes, the Developmental Genomics and Skeletal Research initiative, is one of the few worldwide that is taking large-scale, multidisciplinary, multipronged approaches to address key issues in skeletal biology such as: how is normal longitudinal growth of cartilage and bone regulated? How is skeletal integrity maintained? How do gene mutations cause skeletal disease? And what genetic factors affect predisposition to degenerative skeletal disorders? Some of our discoveries have changed our concepts of fundamental processes in skeletal biology and disease, gaining international recognition and placing Hong Kong research firmly on the world stage.

Why is it necessary to adopt such an all-encompassing approach to the research of genetic skeletal disorders?

The studies being undertaken in the AoE programme aim to understand the control of cartilage and bone formation and apply this knowledge to the underlying molecular pathogenesis of genetic skeletal disorders. Such ambition requires a broad yet highly coherent, integrated and focused programme combining genomics, genetics with clinical studies, *in vivo* and cell models, and biochemistry which is supported by multidisciplinary expertise: skeletal biology, developmental genetics, cell and structural biology, genetics, genomics, bioinformatics and clinical orthopaedics.

What are the current therapeutic methods used to treat intervertebral disc disease (IDD)? How could these be improved upon?

The majority of the time, IDD can be asymptomatic and not painful; in fact, most patients do not need treatment. For advanced cases, when the disc degenerates to the point where it may lead to lower back pain, surgery is performed which may consist of motion preservation or immobilisation strategies. Motion preservation can involve the removal of the disc material and its replacement with a prosthetic disc or device that will recreate the normal motion and function of the spine. Immobilisation strategies entail fusion, whereby the disc is removed and replaced with bone or other material.

For less severe cases, methods may include educational measures to understand proper body posture and biomechanics, lifestyle modifications, the use of medication, multidisciplinary rehabilitation, limited bed rest, exercise therapy, behavioural therapy, steroid injections, braces/corsets, alternative medicines like acupuncture, chiropractic treatments and many more.

To what extent will your research advance knowledge of the biological processes of disc degeneration and the signalling pathways responsible for maintaining a healthy disc?

This knowledge will help us better understand how disc cells cope with mechanical stress and hence their roles in maintaining the extracellular matrix (ECM) and consequently normal disc function. Moreover, if we could define the normal function of disc cells in maintaining the disc ECM under loading stress, we would be able to use this criterion as one of the functional tests for stem cell-derived nucleus pulposus cells (NPC), which are to be developed from other parts of this study. In addition, if we understand the molecular pathways, it may be possible to develop strategies for stimulating endogenous activity of cells and even stimulating a repair or protective mechanism.

How might an understanding of the genetic variation of degenerative skeletal disorders correlate to improving patients' quality of life?

The most significant outcome of understanding genetic variation of degenerative skeletal disorders would be the identification and understanding of disease pathways and causation, and therefore the design of effective and rational treatment strategies for the long term. Such knowledge may ultimately lead to better understanding if an individual is at risk of developing the disorder, and if they may develop more progressive forms of the condition that can significantly impact individual function and quality of life. Understanding the genetic underpinnings of the condition may lead to measures to help prevent its development, as well as understanding its natural history.

In the short term, preventative measures may be developed from risk stratification based on genes and lifestyle factors. Such measures may help prevent the development of pain, diminished function (thereby reducing time off of work), psychological distress and decreased quality of life, ultimately leading to improved productivity on the individual and population levels.

Will this study contribute to the development of effective patient care strategies?

By improving our understanding of which patients may develop certain conditions and perhaps who may respond well with certain treatments, clinicians can improve their clinical decision making and tailor more effective patient-specific strategies that can improve clinical outcomes and be more cost-effective.

Structural integrity

In a highly synergistic team effort at The University of Hong Kong, a research group supported by the Areas of Excellence scheme, a University Grants Council programme, is using state-of-the-art genome sequencing approaches to elucidate the pathogenesis of congenital skeletal disorders

AS THE GLOBAL population of elderly adults increases, societies and cultures must find ways of meeting the growing challenges ageing brings to the preservation of health and quality of life; challenges in which a critical role is likely to be played by advances in genomic and regenerative medicines.

An impediment to individual health that is commonly associated with ageing populations is intervertebral disc disease (IDD), a complex disorder resulting from a cocktail of environmental, lifestyle and genetic factors that, while going unnoticed in many people, can lead to severe pain and even disability in some cases. IDD is very common in older people; by the age of 60 more than 90 per cent of the population will have degeneration of intervertebral discs.

Although it is known that physical problems stem from the intervertebral disc's deteriorating capacity for mechanical strength and shock absorbance, very little is understood about the causal mechanisms of IDD. Back pain caused by the disease is a major public problem which greatly impacts the economy through working days lost.

With this gap in current knowledge, there are no cures for degenerative disc disease (DDD), only invasive and complication-ridden operations. Motion preservation and immobilisation strategies, which routinely employ tried and tested fusion methods for replacing the offending disc, are commonly used for treating IDD, but recent discoveries may change this situation. It is now known that degeneration of the lower spine's lumbar disc is linked to the presence of genes, making individuals 50 per cent more susceptible to the disease if one of their parents has it. Properly characterised, future treatments could see a more personalised approach to care with non-surgical solutions becoming more prominent for a wide range of skeletal diseases.

COVERING EVERY CORNER

Currently Chair Professor of Biochemistry and Jimmy & Emily Tang Professor of Molecular Genetics at The University of Hong Kong (HKU), Kathryn Cheah leads a group that has uncovered several new genetic risk factors for IDD. Founding President of the Hong Kong Society for Developmental Biology (HKSDB), serving from 2004-13, Cheah was elected last year as a Fellow of The World Academy of Science (TWAS). In addition, as Project Coordinator/Director of one of University Grants Council's (UGC's) Areas of Excellence (AoE) programmes – 'Developmental Genomics and Skeletal Research' – her work on the genetics and underlying mechanisms of congenital and degenerative skeletal disorders has already made a significant impact. The follow-on project funded by the Research Grants Council's Theme-based Research Scheme (TRS) – 'Functional analyses of how genomic variation affects personal risk for degenerative skeletal disorders' – has the potential to benefit the lives of millions of people over the world. Cheah continues to lead a group of multidisciplinary, international scientists and clinicians to further understand skeletal disorders.

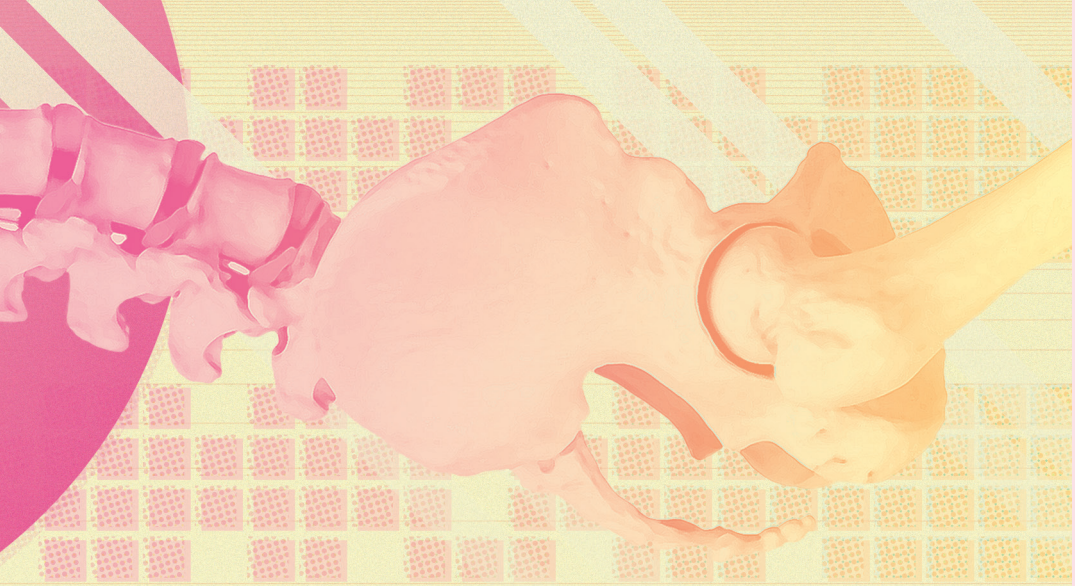
Uncovering the complex molecular mechanisms at the root of genetic skeletal disorders requires a highly collaborative multidisciplinary team. In searching for the factors responsible for proper cartilage and bone formation and how they relate to diseases like IDD, clinicians, epidemiologists, molecular and statistical geneticists, developmental biologists, cell biologists, chemical biologists, experts in proteomics and bioinformaticians combine their skills in a pioneering, synergistic group effort: "A model of scientist-clinician collaboration," adds Cheah.

DIFFERENTIATED DEVELOPMENT

Until recently, a debate existed over a key issue in skeletal development: the fate of growth plate chondrocytes in bone formation. The only cells to be found in healthy cartilage that differentiate, proliferate, mature and become hypertrophic, it has been hotly debated whether terminally differentiated chondrocytes died and were replaced by bone or live on and became bone-synthesising osteoblasts. Having recently discovered that these cells do not, in fact, die but can finally become osteoblasts in all endochondral bones, Cheah's group has resolved this long-standing question. This discovery provides major insights into normal bone formation and the congenital skeletal disorders.

In vivo studies show that the transcription factor SOX9 is responsible for switching the genes in immature growth plate chondrocytes both on and off, indicating that SOX9 is a fundamental regulator of skeletal development. Campomelic dysplasia (CD) is a congenital maldevelopment that causes multiple abnormalities such as skeletal defects, sex reversal and sensorineural deafness. Conventionally, CD is thought to be caused by inadequate amounts of SOX9 (half is not enough), but in mouse models that replicate CD it was discovered that for certain mutations in SOX9 the mechanism has a dominant negative impact on other genes.

Through models of Schmid-type metaphyseal chondrodysplasia (MCDS), Cheah and her group have uncovered evidence that stress induced by abnormal protein is the underlying cause of the disease. Misfolding of the mutant collagen X – 13 del – causes endoplasmic reticulum (ER)



stress to negatively impact this process, but hypertrophic chondrocytes have been shown to survive ER stress by returning to an earlier phase in their differentiation. These insights into their adaptive behaviour and function in congenital skeletal disorders are revealing, as Cheah underlines: "It is critical to maintain the proper differentiation steps for chondrocytes in the formation of the growth plate". Better understanding this complex process could potentially lead to the the development of regenerative medicines and prevent disability.

RISK FACTORS

It has taken more than 10 years to establish, but with a population cohort of 3,500 individuals (age 25 to 55), the world's largest, Cheah's group has been able to perform genetic, clinical and phenotypic studies to identify genetic risk factors for IDD. Using candidate gene, linkage and genome-wide association studies (GWAS), Cheah aims to isolate the genetic factors that predispose and prevent individuals from developing IDD. By comparing subjects with severe disc degeneration to those with average degeneration relative to their age and those with unusually well-preserved discs, it is hoped that by the project's end in 2018 the results will provide a comprehensive representation of common and rare variants of IDD. Using these data, it will be possible to develop predictive models describing how sequence variants affect the onset and progression of IDD.

Although still in the project's early stages, several gene mutations have been identified as contributing factors in IDD development, yielding substantial clues about the disease's pathogenesis. Many of these key risk factors are variations of the intervertebral disc's (IVD) extracellular matrix (ECM), supporting the view that a structurally sound IVD built on normal ECM components, is necessary for it to function properly. The Cheah group's recent discovery of a new risk factor – a variant of the carbohydrate sulotransferase 3 (CHST3) gene – is exciting as it implicated regulation of an enzyme that contributes to the synthesis of ECM proteoglycans.

The mutated genes so far identified as risk factors only represent a moderate threat of developing IDD and cannot account for its estimated 74 per cent heritability. Clearly, many remain to be found, but these findings already make it possible to conceive of new directions in the treatment of degenerative disc disease.

CELLULAR TEMPLATE

Although extensive research is required into the safety and efficacy of such an approach in humans, a move toward stem cell-based regenerative therapies for illnesses like IDD has already seen the proposal of strategies in which stem cells and growth factors are injected directly into the disc. At the centre of the IVD in the nucleus pulposus (NP), there are notochord-like cells (NLCs) and chondrocyte-like NP cells. As IDD progresses, the numbers of these cells decline, suggesting that if they could be induced to persist they would perform a protective role in the NP causing it to retain its functionality. Crucially, all cells present in the NP are derived from a common notochordal precursor: "This raises the possibility that the NLCs may be a progenitor cell that could be used for repair strategies," Cheah muses. If so, these findings could lead to an exciting new phase in spinal care.

Following approaches like these, and with genome wide gene expression studies, *in vivo* animal models, human disc samples and *in vitro* methods planned to help elucidate the differential functions of healthy and degenerative disc cells, the Cheah group's pioneering work is pushing toward a more tailored treatment for individuals with a range of developmental bone conditions and degenerative skeletal disorders. Conventional understanding of lower back pain has already been turned on its head; it may not be long before salvage operations are replaced with cures.

INTELLIGENCE

UNDERSTANDING THE MECHANISMS UNDERLYING THE GENETICS OF CONGENITAL AND DEGENERATIVE SKELETAL DISORDERS

OBJECTIVES

- To deepen knowledge of the systems biology of disc degeneration and the signalling pathways responsible for maintaining a healthy disc
- To assess the functional impact of putative genetic risk factors and gain phenotype-genotype insight with implications for prognosis
- To integrate this knowledge with clinical and environmental factors to predict total personal risk for intervertebral disc disease in order to improve prevention and disease management

KEY TEAM MEMBERS

Co-PIs: **Associate Professor Barbara Chan**; **Professor Danny Chan**; **Professor Kenneth M C Cheung**; **Professor Dong Yan Jin**; **Assistant Professor Qizhou Lian**; **Chair Professor Keith D K Luk**; **Chair Professor Pak Sham**, The University of Hong Kong (HKU) • **Associate Professor Yun Wah Lam**, City University of Hong Kong (CityU) • **Chair Professor Michael Zhang**, HKU and University of Texas Dallas, USA • **Shiro Ikegawa**, Laboratory Head, Center for Genomic Medicine RIKEN, Japan • **Professor Jaro Karpinnen**; **Adjunct Professor Minna Mannikko**, University of Oulu, Finland

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