Impact Objectives

- Use the next generation liquid chromatography-based mass spectrometry (LCMS) to profile and quantify the differential protein changes in the vitreous of developing chick eyes
- Identify novel biomarkers related to the onset and progression of myopia
- Use new biomarkers to prevent, halt or even reverse the effects of myopia, thus aiding the third of the global population predicted to be affected by the disease by 2020

Mapping the pathways to myopia

*Like most of the Hong Kong population, **Dr Thomas Chuen Lam**'s family are affected by myopia. This situation fuelled his desire to map the biochemical pathways behind this disease, which often leads to further sight problems in later life*

What interests you most about myopia?

Myopia or short-sightedness, is typically characterised by excessive growth of the eyeball, resulting in blurred vision when trying to focus on far away objects. It now affects over 80 per cent of the young population in some Asian countries, but the prevalence and severity has also dramatically increased in the West. This refractive error has been conventionally managed by optical corrections using spectacles and contact lenses, but myopia often promotes other ocular complications and diseases that can lead to permanent visual impairment. Despite decades of research, little is known about the exact mechanisms that drive myopic eye growth. My family members are all myopes. Therefore, I have been interested in finding out what biochemical signals trigger aberrant eye growth ever since my undergraduate training in optometry.

Why is the severity of myopia worsening on a global scale?

Myopia is thought to be a multifactorial disease in which both genetic and environmental factors play a role. Clinical studies have shown a greater prevalence of myopia in those of Asian ethnicity, regardless of location. Therefore, it is possible that Asians are genetically more susceptible to myopia. Also, if one or both parents have myopia, there is clear evidence that their children are more likely to develop the condition. Apart from hereditary factors, high levels of educational demand and less outdoor exposure have been proposed as key factors contributing to myopia in children. These factors, combined with extensive use of electronic devices at a young age, are thought to adversely affect eye development.

What are the key objectives of your research and what do you hope to ultimately achieve?

Proteomics has contributed greatly to our understanding of gene function in the post-genomic era. Identification of the unique biomarkers specifically related to the onset and subsequent progression of myopia will provide new insights that should eventually lead to therapies. By manipulating these biomarkers, we hope to prevent, halt or even reverse the effects of myopia. After a number of years working in the field, we are well versed in different proteomics testing and screening techniques. Using recent data-independent next-generation mass spectrometry, we have shown much improved reproducibility across multiple biological samples – something which has long been a major challenge in conventional discovery-based proteomics. For the first time, we can continuously monitor and quantify the same set of protein panels with full confidence in their reproducibility. This is the first step to identifying key biomarkers and developing therapeutic interventions.

Why do you believe vitreous is key to formulating a better understanding of the progression of myopia?

Vitreous is a clear, gel-like fluid that forms 80 per cent of the total volume of the eyeball. Its main role is to provide structural stability and shock stabilisation to the eyeball. Proteomic studies on the vitreous are important because the vitreous chamber depth is the main contributor to the increased eye axial length and resulting refractive errors seen in myopia. Unlike other biological fluids in the eye, vitreous gel is relatively stagnant, meaning it may retain the biochemical clues to the development of myopia. In the past, it was very difficult to comprehensively study the non-water component of the vitreous. However, as proteomic technology has matured, we are now able to identify and quantify all low abundant proteins inside the vitreous, not just the most plentiful. Taken together with our established proteomics work in the retina, we can thus build a more complete profile of the proteome changes and regulations in an elongated eyeball. ●

The myopia epidemic

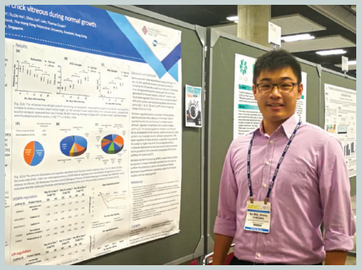
***The Hong Kong Polytechnic University** is undertaking comprehensive and quantitative proteomic profiling of normal and lens-induced myopic eye vitreous, using next generation gel-free mass spectrometry, with a view to stemming the rising myopia epidemic*

Dr Thomas Chuen Lam warns: 'Around one third of the global population will be affected by myopia by 2020 and it is expected that half of the world's population, a total of nearly five billion, will become myopic by 2050, according to more recent projections.' He adds: 'Because the condition can lead to serious conditions including cataracts, posterior vitreous detachment, glaucoma and retinal detachment, it presents a major burden to health systems and affects individuals' productivity and quality of life.' Lam's team has been working for several years on eye diseases, with the objective of mapping the causative biochemical pathways and identifying potential pharmaceutical targets. The current project, which concludes this year, is using highly sensitive technology to study changes in the proteome of vitreous gel in myopic chick eyes, in comparison to that of normal eyes.

LIQUID CHROMATOGRAPHY-BASED MASS SPECTROMETRY

Myopia is characterised by abnormal axial elongation of the eyeball, meaning sufferers cannot focus properly on objects at a distance. Along with environmental interventions, including spending more time outdoors, pharmaceuticals could be developed to target specific biomarkers. However, as Lam says: 'Currently, the roles of different biochemical compounds and their interplay in myopia regulation remain unresolved.'

Lam's ongoing project hopes to reverse this situation by using new generation liquid chromatography-based mass spectrometry (LCMS) to profile and quantify the proteomics in the vitreous of chick eyes. LCMS technology combines highly sensitive protein separation with the ability to quantify and identify all proteins present in a sample. Proteins are expressed by RNA and may also be subject to post-translational modifications, the most important of which is phosphorylation. Lam describes proteomic profiles as 'snap-shots', which must be compared over time and against disease-free control systems. He adds: 'Even five years ago, we did not have the technology to undertake unbiased and detailed quantitative proteomic profiling on this scale.'



Mr Jimmy Cheung, PhD student, presenting the chick vitreous study at the 2018 ARVO Annual Meeting in Hawaii, US

BIOINFORMATICS AND INSIGHTS

Lam's team has previously undertaken proteomic screening of retinas in chick and guinea-pig myopes, but feels: 'the importance of vitreous has often been overlooked and could hold the key to identifying the biochemical pathways in the development of myopia'. The current project uses a lens to induce myopia in one eye of new-born chicks and compares the proteomes in the vitreous of the diseased and normal eyes and how they change over time. The chick model is a well-established technique for the study of myopia and is directly relevant to the condition in humans. One of the biggest challenges has been preparing reproducible samples of the minute protein content in chick vitreous and this has taken over a year to perfect.

Several thousands of proteins have been identified in different proportions in the vitreous and Lam's team is seeking to pinpoint the handful of proteins that are being up or down-regulated in myopia. The project has generated a vast amount of data, which according to Lam: 'requires interpretation by joint collaborative efforts from molecular scientists, biologists, clinicians and bioinformaticians'. He adds: 'In the beginning, the different disciplines lacked a common language, but by storing, analysing our data and sharing results on a cloud-based platform, we have largely addressed these communication challenges.'

As the project draws to a close, Lam is pleased the team has: 'developed a proteome library and found novel vitreous proteins that are related to eye growth', and adds: 'We are currently correlating these to known myopia signalling pathways and testing new molecules in manipulating specific ones.' Lam is hopeful his team's work, combined with: 'precise cellular-level measurements of molecular changes', will lead to: 'new targeted formulations or drugs that can slow down or stop human myopia in the coming five to 10 years'. ●

Project Insights

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