TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Leveraging the "omnigenic model" and zebrafish for identifying and testing core genes in Osteoarthritis

Lead PhD supervisor (please provide name, affiliation and email)
Dr Erika Kague, Centre for Genomic and Experimental Medicine (CGEM), University of Edinburgh, ekague@ed.ac.uk

Second PhD supervisor (please provide name, affiliation and email)
Professor Paul McKeigue, Usher Institute, Institute of Genetic and Cancer, University of Edinburgh, paul.mckeigue@ed.ac.uk

Background

Osteoarthritis (OA) is one of the leading causes of disability and pain worldwide, affecting over 500 million people. OA is a chronic disease that affects the whole joint, characterised by cartilage degradation, subchondral bone thickening, osteophyte formation and other joint structural changes. Unfortunately, there are no current treatments available that reverse disease progression, and management strategies focus on pain relief and surgical procedure. Identifying therapeutic targets for OA is a top priority worldwide.

It is estimated that a drug with genetic support has more than double the chances of success during clinical development, particularly for target genes associated with Mendelian disorders when compared to complex traits. Since Genome-Wide Association Studies (GWAS) started in 2010, there has been an unprecedented identification of genomic loci associated with complex traits and a considerable leap in understanding the biology behind these diseases. However, GWAS have not resulted in the development of therapeutics. One of the reasons for this is that associated genetic variation is spread over many variants of low effect distributed widely across the genome. However, a recent idea called the **"omnigenic hypothesis"** could significantly change how we approach the genetics of complex diseases to discover efficient therapeutic targets. The omnigenic model postulates that the genetic effects on a complex trait coalesce on a relatively sparse set of effector "core" genes, which the gene product has a direct effect on the disease. Recently, the McKeigue group developed statistical tools to apply the omnigenic model in diabetes, and highlighted core genes that contribute to mendelian forms autoimmune diabetes and therefore potential therapeutic targets. We have used the same approach to identify core genes in OA. Our current analyses uncovered nine core genes in OA, in which three genes passed our prioritization baseline for further functional experiments.

We use **zebrafish** as animal models to study the genetics of osteoarthritis. Zebrafish are freshwater bony fish used to model human bone diseases. They offer rapid development, transparency, regenerative capacity, and the availability of an arsenal of genetic tools to study bone cells. Aged zebrafish have OA like in humans. Very often zebrafish knockouts for genes important in OA present cartilage changes starting early in development when zebrafish are still transparent. This offers

unique advantageous opportunities to image and follow disease process dynamically in a live organism.

Aims

The aim of this PhD project is to test the three core genes identified using the "Omnigenic hypothesis" using zebrafish as model organisms.

AIM1: Further analysis of the three top genes will be undertaken to identify which have the strongest evidence of causality. This will include Mendelian randomization analyses that allow for pleiotropic trans-effects, and investigating effects of rare variants in the UK Biobank exome sequence data.

AIM2: Answer which of the three genes are the most relevant for detailed experimental studies. The CRISPR/Cas9 system is extremely efficient in zebrafish, allowing to analyse knockout phenotype in days whether than months. Here, the student will perform cartilage and bone analysis of G0s during early larvae (less than 5 days post-fertilization). This will include targeting the genes with CRISPR, performing cartilage and bone staining, immunostaining for different markers, 3D imaging and analyses.

AIM3: Deep dive into the core gene function in OA. The will generate a zebrafish knockout line of the gene selected from Aim2. We will analyse the knockout from larval to adult stages looking for changes in cartilage and bone. The adult fish will be histologically sectioned, graded for OA. OA markers will be quantified using molecular and immunohistochemistry (cartilage degradation), and will be imaged using micro-Computed tomography for volumetric analysis). Knockouts will be crossed with transgenic lines to analyse osteoblast activity, bone growth rates and bone and cartilage repair processes. Knockout phenotype will be modulated using available drugs.

Training and experience provided

This project will provide multidisciplinary training that includes:

- A preclinical in vivo model organism (Zebrafish), including genetic modification, molecular biology, and phenotypic analysis of skeletal changes using diverse methodologies (Kague Lab)
- Imaging techniques: stereo and compound microscopes, confocal microscopy, micro-computed tomography (Kague Lab)
- Understanding of bone diseases (Kague Lab)
- Epidemiology and statistical analyses including big data analyses (McKeigue Lab)
- Omnigenic hypothesis test (McKeigue Lab)

Expected outcomes

We will answer whether the putative core genes are causal in Osteoarthirtis and shed mechanisms of their function in OA.

We will demonstrate the relevance of the "Omnigenic model" for identification of core causal genes in disease and potential therapeutics.

References

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