A unique angle to discover novel mechanisms in cardiovascular protection

Oxford supervisors: Associate Professor Mathilda Mommersteeg[1,2], Professor Robin Choudhury[3]

Departments:
1. Institute of Developmental and Regenerative Medicine
2. Department of Physiology, Anatomy and Genetics
3. Division of Cardiovascular Medicine, RDM

Project outline:

Background This proposal uses a unique fish model, the Mexican cavefish (*Astyanax Mexicanus*), combined with mouse and patient data to uncover key novel mechanisms underlying protection from cardiovascular disease caused by diabetes mellitus, promoting identification of innovative therapeutic approaches. *Astyanax mexicanus* is a single fish species comprising cave-dwelling and surface populations. Thousands to millions of years ago, surface fish living in rivers became trapped in caves. During their independent evolution in the caves, the fish lost their eyes and pigment, redundant in the absence of light.[1,2] In addition, to be able to survive food scarcity, the fish adapted their metabolism. They developed an increased appetite and increased fat accumulation when food is abundant, such as in the lab.[3,4] This leads to increased body fat, including a fatty liver.[3,5] Alongside these fat-based adaptions, cavefish also display hyperglycaemia and insulin resistance – all phenotypes associated with human obesity and diabetes mellitus. Indeed, cavefish possess a mutation in the insulin receptor (SNP p211L) that has been associated with Rabson-Mendenhall syndrome, a condition of severe insulin resistance.[6] From our own work, we also know that the hearts show features of diabetic cardiomyopathy ([7,8] and unpublished data). Intriguingly, these same cavefish (lab) populations exhibit robust health and longevity, living much longer than their surface fish counterparts,[4–6] without features of pathologies typically associated with obesity and diabetes such as accumulation of advanced glycation end products (AGEs), chronic tissue inflammation, impaired growth due to insulin dysregulation, and low survivability due to arterial disease.[5,6] While they have increased body fat, this does not accumulate on the heart and vessels,[8] suggesting a mechanism protecting these organs, which could be an essential factor for their longer lifespan. From published work and our own unpublished scRNAseq data, we know that the immune system of cavefish is altered with a reduced pro-inflammatory response.[9] In particular, il1b upregulation (a feature of human diabetes) during inflammation is much less strong in cavefish compared to surface fish. Additionally, their fat deposits contain fewer immune cells.[9] This suggests that cavefish are able to live healthily even while obese and diabetic, potentially due to adaptations of their immune system and these changes could be crucial for their longevity. This project will use the *Astyanax* model as a discovery model, directly comparing the cavefish with not only the surface fish, but also mouse and humans, and specifically, will tease out which genomic adaptation and pathways reduce the pathological response to obesity and diabetes.

Hypothesis Cavefish are protected from the pathological effects of hyperglycaemia and related cardiovascular disease due to evolutionary adaptations to their immune system. Understanding the mechanisms underlying this protection will provide novel inroads into therapies.

Aims

1. To identify the key regulatory genes underlying the cavefish protective phenotype
2. To identify similarities/differences in inflammatory gene profile between cavefish and diabetic mice and patients
3. To identify inhibitors/activators inducing the protective mechanisms.

Description of the work to be undertaken For Aim 1 we will take full advantage of the *Astyanax* model: As the fish are still one species, this allows for forward genetic screening methods including QTL analysis. We will generate 200 second generation (F2) offspring from a cavefish-surface fish cross. The adult fish will be tested for hyperglycaemia and hypercholesterolemia. The spleens and hearts will be isolated for bulk RNAseq and the body will be embedded for sectioning to analyse for athero-sclerosis in the aorta and crown-like structures in the fat. DNA will be collected from all F2 fish to perform RADseq to be able to perform QTL analysis on traits such as il1b and other pro-inflammatory gene levels in the spleen, level of cardiomyopathy, and the amount of atherosclerosis in the aorta. This will identify the key regions in the genome regulating the protective response. Aim2: To identify the unique 'protective' inflammatory gene signature and look for conservation, we will also perform scRNAseq on cavefish and surface fish spleen and circulating leukocytes. This data will directly be compared and integrated with existing scRNAseq data from spleens of diabetic STZ mice and controls as well as human diabetes versus control leukocytes (PBMC). Aim3: We will test inhibitors/activators of the identified 'protective' genes/pathways in fish and mice with the aim to test conservation and find novels ways to induce protection from hyperglycaemia and hypercholesterolemia.

References

1. Gross, J. B. The complex origin of Astyanax cavefish. BMC Evol Biol 12, 105 (2012).
2. Gross, J. B., Meyer, B. & Perkins, M. The rise of Astyanax cavefish. Developmental Dynamics n/a-n/a (2015) doi:10.1002/dvdy.24253.
3. Aspirasa, A. C. et al. Melanocortin 4 receptor mutations contribute to the adaptation of cavefish to nutrient-poor conditions. Proceedings of the National Academy of Sciences 201510802 (2015) doi:10.1073/pnas.1510802112.
4. Xiong, S., Krishnan, J., Peuß, R. & Rohner, N. Early adipogenesis contributes to excess fat accumulation in cave populations of Astyanax mexicanus. Dev Biol 441, 297–304 (2018).
5. Medley, J. K. et al. Resilience in a Natural Model of Metabolic Dysfunction Through Changes in Longevity and Ageing-Related Metabolites. bioRxiv 2020.10.27.358077 (2021).
6. Riddle, M. R. et al. Insulin resistance in cavefish as an adaptation to a nutrient-limited environment. Nature 555, 647–651 (2018).
7. Stockdale, W. T. et al. Heart Regeneration in the Mexican Cavefish. Cell Rep 25, 1997-2007.e7 (2018).
8. Tang, J. L. Y. et al. The developmental origin of heart size and shape differences in Astyanax mexicanus populations. Dev Biol 441, 272–284 (2018).
9. Peuß, R. et al. Adaptation to low parasite abundance affects immune investment and immunopathological responses of cavefish. Nat Ecol Evol 4, 1416–1430 (2020).

Supervisor's recent relevant publications:

Mathilda Mommersteeg

1. Potts HG*, Stockdale WT*, **Mommersteeg MTM**. Unlocking the Secrets of the Regenerating Fish Heart: Comparing Regenerative Models to Shed Light on Successful Regeneration. *J Cardiovasc Dev Dis. 2021 Jan 16;8(1):4.* doi: 10.3390/jcdd8010004
2. Warren WC, Boggs TE, Borowsky R, Carlson BM, Ferrufino E, Gross jB, Hillier L, Hu Z, Keene AC, Kenzior A, Kowalko JE, Tomlinson C, Kremitzki M, Lemieux ME, Graves-Lindsay T, McGaugh SE, Miller JT, **Mommersteeg MTM**, Moran RL, Peuß R, Rice E, Riddle MR, Sifuentes-Romero I, Stanhope BA, Tabin CJ, Thakur S, Yamamoto Y, Rohner N. A chromosome level genome of Astyanax mexicanus surface fish for comparing population-specific genetic differences contributing to trait evolution. *Nat Commun. 2021 Mar 4;12(1):1447.* doi: 10.1038/s41467-021-21733-z
3. Koth J*, Wang X*, Killen AC*, Stockdale WT, Potts H, Jefferson A, Bonkhofer F, Riley PR, Patient R, Göttgens B, **Mommersteeg MTM**. Runx1 promotes scar deposition and inhibits myocardial proliferation and survival during zebrafish heart regeneration. Development 2020, 147: dev186569.
4. Stockdale WT, Lemieux ME, Killen AC, Zhao J, Hu Z, Riepsaame J, Hamilton N, Kudoh T, Riley PR, van Aerle R, Yamamoto Y* and **Mommersteeg MTM***. Heart regeneration in the Mexican cavefish. *Cell Reports 2018, 25, 1997–2007*
5. Tang JLY, Guo Y, Stockdale WT, Rana K, Killen A, **Mommersteeg MTM*** and Yamamoto Y*. The Developmental Origin of Heart Size and Shape Differences in Astyanax mexicanus populations. *Dev Biol. 2018 Sep 15;441(2):272-284* *corresponding authors

Robin Choudhury

1. Laurienne Edgar, Naveed Akbar,[…], **Robin P. Choudhury.** Hyperglycemia Induces Trained Immunity in Macrophages and Their Precursors and Promotes Atherosclerosis. Circulation. 2021;144:961–982.
2. **Robin P. Choudhury**, Laurienne Edgar, Mikael Rydén, Edward A. Fisher. Diabetes and Metabolic Drivers of Trained Immunity New Therapeutic Targets Beyond Glucose. Arteriosclerosis, Thrombosis, and Vascular Biology. 2021;41:1284–1290
3. Joshua T. Chai, Neil Ruparelia, Anuj Goel, Theodosios Kyriakou, Luca Biasiolli, Laurienne Edgar, Ashok Handa, Martin Farrall, Hugh Watkins, and **Robin P. Choudhury**. Differential Gene Expression in Macrophages From Human Atherosclerotic Plaques Shows Convergence on Pathways Implicated by Genome-Wide Association Study Risk Variants. Arteriosclerosis, Thrombosis, and Vascular Biology. 2018;38:2718–2730
4. Mohammad Alkhalil, Evan Edmond, Laurienne Edgar, Janet E Digby, Omar Omar, Matthew D Robson, **Robin P Choudhury**. The relationship of perivascular adipose tissue and atherosclerosis in the aorta and carotid arteries, determined by magnetic resonance imaging. Diabetes and Vascular Disease Research 2018 vol 15(4) 286-293