Conference Venue

Li Ka Shing Knowledge Institute (LKSKI), St. Michael’s Hospital (SMH)
209 Victoria Street, Toronto, Ontario, Canada, M5B 1T8

Acknowledgments

Cover Page Design & Welcome Posters: Koroboshka Brand-Arzamendi (ZCADD, St. Michael's Hospital)
The bulb represents an idea; the reflection depicts a scientist inside the bulb thinking about zebrafish experiments. When an idea pops up, it releases and breaks through the bulb. The yellow background represents the sunrise of new scientific ideas using zebrafish as a model.

Conference Coordinator: Jamie Li
Conference Assistance: Cherry Ng
Conference Booklet: Jamie Li (Special thanks: Robin Ng, Junghwa Yun)

Special Thanks

SMH Scheduling and Events Administrator: Katerina Vonj
SMH Catering Manager: Paula Jack
SMH Research Financial Analyst: Fatima Abrar
SMH Research Administration: Dalton Charters & Dina Coronios
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Dr. Stephen Ekker, Mayo Clinic, Rochester, USA

Session II: Mutagenesis & Disease Modeling

Chair: Stephen Ekker (Mayo Clinic, USA)

Invited Speaker

Dr. Erica Davis, Center for Human Disease Modeling, Duke University

Oral Presentations

Dr. Keith Cheng, Penn State Hershey College of Medicine, USA

Dr. Raman Sood, National Human Genome Research Institute, Bethesda, USA

Han Lee (PhD Candidate), Mayo Clinic Graduate School of Biomedical Sciences, Rochester, USA

Invited Speaker

Dr. Jeffrey Essner, Iowa State University

Oral Presentation

Dr. Weibin Zhou, University of Michigan, USA

Dr. Onofrio Laselva (Postdoc), The Hospital for Sick Children; University of Toronto, Canada

Ivo Eijkenboom (PhD Candidate), Maastricht University Medical Centre, The Netherlands

Session III: Mechanisms of Development, Organ Dysfunction & Disease

Chair: Oliver Bandmann (University of Sheffield, UK)

Invited Speaker

Dr. Marie-Andrée Akimenko, University of Ottawa, Canada

Oral Presentations

Dr. Ela Knapik, Vanderbilt Genetics Institute, VUMC, Nashville, TN, USA

Dr. Peng Huang, University of Calgary, Alberta, Canada

Dr. Tamjid A. Chowdhury (Postdoc), Medical College of Wisconsin, Milwaukee, USA

Invited Speaker

Dr. Catarina Henriques, University of Sheffield, UK

Oral Presentations

Dr. Han Wang, Center for Circadian Clocks, Soochow University, China

Dr. Marika Kapsimali, Institut de Biologie de l’École Normale Supérieure, Paris, France

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About the Zebrafish Centre for Advanced Drug Discovery at St. Michael’s Hospital

With infrastructure funding from the Canada Foundation for Innovation, St. Michael's Hospital launched a Zebrafish Centre for Advanced Drug Discovery (ZCADD) and is home to the first automated high throughput zebrafish drug screening platform in Canada and one of the most advanced zebrafish screening facilities worldwide. This platform performs robotic, fully automated screens from zebrafish embryo sorting, incubation, drug dosing to efficacy readout (by fluorescence and luminescence), controlled by robotic arms and software. Over the past two years, ZCADD has secured major operating funds from Brain Canada and CQDM in high throughput screening system automation and early drug development.

St. Michael’s Hospital is a Catholic teaching and research hospital founded by the Sisters of St. Joseph in 1892 to care for the sick and poor of Toronto’s inner city. Affectionately known as the Urban Angel, St. Michael’s is renowned for providing exceptional patient care. As downtown Toronto’s adult trauma centre, the hospital is a hub for neurosurgery, complex cardiac and cardiovascular care, diabetes and osteoporosis care, minimally invasive surgery and care of the homeless and disadvantaged. St. Michael’s is also one of the province’s major sites of care for critically ill patients.

Fully affiliated with the University of Toronto, St. Michael’s provides outstanding medical education to healthcare professionals in more than 23 academic disciplines. Home to the Li Ka Shing Knowledge Institute, made up of the Keenan Research Centre and the Li Ka Shing International Healthcare Education Centre, the hospital is among the first in the world to bring together researchers, educators and clinicians to take best practices and research discoveries to patient bedsides faster.

The Keenan Research Centre of the Li Ka Shing Knowledge Institute is home to researchers whose areas of expertise cover a wide variety of disciplines and methodologies. Together they generate knowledge about:

- The biological mechanisms underlying health and disease
- The application of fundamental research to improve the understanding and treatment of human disease
- The best methods of preventing disease and providing health care
- The social, economic, and policy determinants of health
- The best methods of partnering with our community to generate policy relevant research and ensuring greater health equity. Researchers at the Keenan Research Centre work closely with educators, clinicians and community members to generate and transfer knowledge that improve the health of our patients and communities

International Organizing Committee Members:

3rd Zebrafish for Personalized/Precision Medicine (ZPPM) Conference

Dr. Randall Peterson (College of Pharmacy, University of Utah, USA)
Dr. Stephen Ekker (Mayo Clinic in Rochester, USA)
Dr. Ian Scott (University of Toronto, Canada)
Dr. Oliver Bandmann (University of Sheffield, UK)
Dr. Xiao-Yan Wen (Chair, ZCADD, St. Michael’s Hospital, Canada)

2nd Z-BRAIN Neuroscience Workshop

Dr. Pierre Drapeau (University of Montréal, Canada)
Dr. Christopher Barden (Treventis Corporation)
Dr. Xiao-Yan Wen (Chair, ZCADD, St. Michael’s Hospital, Canada)
Randall T. Peterson, PhD is a chemical biologist whose research utilizes high-throughput screening technologies to discover new drug candidates for cardiovascular and nervous system disorders. Unlike conventional drug discovery programs that utilize simplified, in vitro assays, the Peterson lab screens using living zebrafish, ensuring that the drug candidates discovered are active in vivo. Several of the compounds discovered by the Peterson laboratory have become widely used research tools or are in clinical development.

Dr. Peterson received his PhD from Harvard University where he studied as a Howard Hughes Medical Institute predoctoral fellow in the laboratory of Stuart Schreiber. He completed a postdoctoral fellowship with Mark Fishman at Massachusetts General Hospital. Dr. Peterson spent 14 years as a faculty member at Harvard University where he was the Charles Addison and Elizabeth Ann Sanders Chair in Basic Science at Harvard Medical School, Scientific Director of the MGH Cardiovascular Research Center, and Senior Associate Member of the Broad Institute of Harvard and MIT. In 2017 he moved to the University of Utah as L.S. Skaggs Presidential Endowed Professor and Dean of the College of Pharmacy.

Stephen C. Ekker, Ph.D. is Professor of Biochemistry and Molecular Biology at the Mayo Clinic and an Adjunct Professor, University of Minnesota. Dr. Ekker has been conducting genome engineering for over 30 years with an emphasis on elucidating the mechanisms underlying health and disease. Ekker is President of the Genome Writers Guild Genome Engineering Society, Editor-in-Chief of the Zebrafish journal and Associate Director of the Clinical and Translational Sciences PhD program. Dr. Ekker is Co-Founder of three biotech companies and one non-profit. Dr. Ekker received bachelor of science degrees (Genetics and Developmental Biology, Electrical Engineering) from the University of Illinois where he conducted genome science work with Dr. Carl Woese. Dr. Ekker earned a PhD in Molecular Biology and Genetics at the Johns Hopkins University and Howard Hughes Medical Institute. Dr. Ekker was the founding Director of the Arnold and Mabel Beckman Center for Transposon Research (now called the Center for Genome Engineering) at the University of Minnesota. The Ekker laboratory has used a zebrafish-first approach to pioneer the use of diverse genome engineering tools include transposons, morpholino antisense oligonucleotides, and targeted genome editing methods including nuclear and mitochondrial programming in diverse organisms and human cells. Dr. Ekker also serves on the advisory boards of several biotechnology and pharmaceutical companies.
**Dr. Ian Scott,** *The Hospital for Sick Children & University of Toronto, Canada*

*3rd ZPPM Conference Organizing Committee*

**Senior Scientist,** Program in Developmental and Stem Cell Biology, The Hospital for Sick Children;
**Associate Professor,** Department of Molecular Genetics, University of Toronto

Research in the Scott lab is focused on how cardiac fate is first established, and how the heart later grows and develops. Using the advantages of the zebrafish embryo, we employ genetic, embryological, live imaging and genomics approaches to study in real time the earliest events of cardiovascular development. Current research topics include: 1) role of Aplnr signalling in migration of cardiac progenitors to the heart-forming region; 2) transcriptional control of early cardiac fate and migration; 3) deciphering the gene regulatory networks that govern vertebrate heart development and regeneration; 4) regulation of second heart field (SHF) development; and 5) role of CCM3 signalling in cranial vasculature development.

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**Dr. Oliver Bandmann,** *University of Sheffield, UK*

*3rd ZPPM Conference Organizing Committee*

Professor Oliver Bandmann is an academic Neurologist at the Sheffield Institute for Translational Neuroscience (SITraN) and the Bateson Centre, both University of Sheffield, UK. He has a particular interest in movement disorders, especially Parkinson’s disease. His group has been working on zebrafish models for Parkinson’s disease for > 10 years. His group implicated a novel pathway, namely TIGAR activation, in the pathogenesis of early onset Parkinson’s due to PINK1 mutations (Flinn et al, 2013). His group also undertook the first ever compound screen in Parkinson’s disease patients’ tissue (Mortiboys et al, 2013 and 2015). His group has now become particularly interested in elucidating the role of genetic susceptibility factors for Parkinson’s disease. He will present the result of gene-gene interaction studies with particular focus on genes interacting with glucocerebrosidase 1 (*gbal*), the most common genetic risk factor for Parkinson’s disease (Keatinge et al, 2015).
Dr. Pierre Drapeau, *University of Montréal, Canada*

2nd Z-BRAIN Neuroscience Workshop Organizing Committee

Dr. Drapeau has been studying the development of the motor network in zebrafish by combining cellular neurophysiology and molecular genetics. He records and images the activity patterns of identified spinal cord and hindbrain neurons in normal and genetically engineered embryos. His work has led to the discovery of a novel mechanism of synaptic transmission at fast neuromuscular junctions and the role of glycine in promoting neural differentiation during development. The long-term goal of his research is to elucidate the molecular choreography of motor network formation, plasticity and function. More recently Dr. Drapeau has expressed human genes in zebrafish, allowing for the validation of mutations and the screening of small chemical libraries in genetic models of neurodegenerative diseases such as ALS and hereditary spastic paraplegia, and developmental disorders such as autism and schizophrenia. He is collaborating on large-scale genomics projects to identify mutations of synaptic genes related to developmental brain diseases that he is validating in zebrafish embryos. In particular they have discovered that de novo mutations (in patients but not in their parents) are a major cause of autism and schizophrenia.

Dr. Christopher Barden, *Treventis Corporation, Canada*

2nd Z-BRAIN Neuroscience Workshop Organizing Committee

Dr. Christopher Barden, Treventis Corporation Zebrafish Neuroscience Workshop Organizing Committee Dr. Barden, a registered patent agent and computational scientist, manages the Company’s Canadian operations and its overall intellectual property portfolio. A ten-year veteran of drug discovery programs in neurodegenerative and anti-infectives therapeutics, he concurrently holds an Associate Scientist position at Toronto Western Research Institute. Dr. Barden developed the Company’s virtual high-throughput screening library (>11 million compounds) and was a key architect of our proprietary “CCM” model of amyloid aggregation. Dr. Barden received his Ph.D. in Physical Chemistry from the University of Georgia.
Dr. Xiao-Yan Wen, Zebrafish Centre for Advanced Drug Discovery, St. Michael’s Hospital, Canada
Chairs, 3rd ZPPM Conference & 2nd Z-BRAIN Neuroscience Workshop Organizing Committees

Dr. Wen’s research focuses on incorporation of zebrafish model system and advanced high throughput screening technologies for drug discovery. He established the Zebrafish Centre for Advanced Drug Discovery (ZCADD) at St. Michael’s Hospital. With a large infrastructure funding from Canada Foundation for Innovation (CFI), ZCADD houses Canada’s first fully automated zebrafish high throughput screening platform for zebrafish. In partnership with many academic labs and industrial partners, the Centre has launched over 10 zebrafish-based drug development projects targeting many neurological, cardiovascular, inflammatory diseases, diabetes and cancer.

http://stmichaelshospitalresearch.ca/labs/zebrafishcentre/

Dr. Wen received his MD at Jiangxi Medical University in China and doctorate at the University of Toronto. He is currently a Staff Scientist in the Keenan Research Centre for Biomedical Science and an Assistant Professor in the Department of Medicine & Physiology at the University of Toronto. He is the founding director of the ZCADD and the Director for the St. Michael’s Hospital Zebrafish Core Facility. He is also a member of the Institute of Medical Science (IMS) at the University of Toronto and directs the graduate course module “Animal Models of Human Diseases”.


Welcome Message

On behalf of the 2017 Conference Organizing Committees and the Zebrafish Centre for Advanced Drug Discovery (ZCADD), I am pleased to welcome you to the 3rd Zebrafish for Personalized & Precision Medicine (ZPPM) Conference and the 2nd Zebrafish Neuroscience Workshop.

Fundamental research using zebrafish has facilitated a technical revolution in our ability to directly edit the genome. We can now make mutations in literally any gene in the zebrafish genome. Coupled with the high throughput capability in genetic and chemical genetic screens, zebrafish research has entered into a new era, which is expected to make a significant impact toward promoting the development of personalized and precision medicine.

The goal of this conference is to bring together scientists from both biological and medical research fields to accelerate the translational process of zebrafish-based knowledge to clinical applications. This will include generation of more predictive “humanized” disease models, dissection of disease mechanisms and discovery of new disease treatment methods.

In 2013 and 2015, we successfully organized the 1st and 2nd ZPPM Conference, which attracted local and international scientists, clinicians, and researchers. With a strong international organizing committee and through your participation, we anticipate that the 3rd ZPPM conference will excel.

In this conference, we assembled a group of expert scientists to give plenary lectures in personalized medicine, cutting-edge technology, and drug discovery. Three main scientific research sessions include: (1) Mutagenesis & Disease Modeling, (2) Mechanisms of Development, Organ Dysfunction & Disease, and (3) Chemical Biology, Pharmacogenomics & Drug Discovery. We also established a very strong workshop program on zebrafish neuroscience.

It is our desire to ensure that this conference provides a stimulating environment for trainee education. As such, we have selected trainee oral presentations in each of scientific sessions. We have also established a number of awards for trainees, including McLaughlin Centre Trainee Awards, Travel Awards, Best Flash Talk and Poster Awards. We applaud our trainees in their dedication and we hope that this conference will pave the way for their scientific discoveries and future success. This year, we also hosted a competition for the Conference Book Cover Page Design with a special cover design award.

Our conference is made possible by the generous support of our partners and sponsors. Special thanks to Ontario Brain Institute and University of Toronto for strong support to this conference, to St. Michael’s Hospital for providing the venue and administrative support, and to the volunteers for their hard work and commitment.

We whole-heartedly welcome you to join us in Toronto for an exciting conference, and please don’t miss the Networking & Dinner social at Ripley’s Aquarium on Sept. 29th, 2017.

Sincerely,

Xiao-Yan Wen, MD, PhD
Chair, 3rd ZPPM Conference Organizing Committee
Chair, 2nd Zebrafish Neuroscience Workshop Organizing Committee
Director, Zebrafish Centre for Advanced Drug Discovery, St. Michael’s Hospital
<table>
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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:30am – 8:50am</td>
<td>Registration &amp; Breakfast (2nd floor, Exhibition Hall)</td>
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</table>
| 9:00am – 9:10am | **Welcome and Opening Remarks** (Allan Waters Family Auditorium, Room 209)  
+ Dr. Xiao-Yan Wen, Director, ZCADD, St. Michael’s Hospital  
+ Dr. Ori Rotstein, Director, Kennan Research Centre for Biomedical Science, SMH  
+ Dr. Tom Mikkelsen, President and Scientific Director, Ontario Brain Institute |
| 9:10am – 10:25am | **Scientific Session A, Chair: Dr. Pierre Drapeau** (CRCHUM & University of Montréal)  
9:10am – 9:35am | **Invited Lecture** (20 min + 5 min Q&A)  
Dr. Anne Dekeyne, Institute of Research Servier, Paris, France  
“Zebrafish as a tool for target validation and drug discovery in proteinopathies of the CNS” |
| 9:35am – 10:25am | **Mini-talk** (8 min + 2 min Q&A)  
Dr. Ted Allison, University of Alberta, Canada  
“Towards stem cell therapy of blindness: Defining gene regulatory networks in the premier genetic model of cone photoreceptors”  
Dr. Lee Ellis, National Research Council of Canada  
“Assessing the uptake kinetics, biological activity and potential interaction of individual cannabinoids using zebrafish larvae”  
Dr. Peng Huang, University of Calgary, Canada  
“PHRESH analysis of cell signaling dynamics during spinal cord patterning”  
Khang Hua (PhD candidate), University of Ottawa, Canada  
“Elucidating Molecular Changes During Dopaminergic Neuron Regeneration in Zebrafish”  
Dr. Justin W. Kenney (Postdoc), Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada  
“Towards the generation of a digital adult zebrafish brain atlas” |
| 10:25am – 10:40am | **Coffee Break** (Back of Auditorium / Room 209)                                                                                           |
| 10:40am – 11:50am | **Scientific Session B, Chair: Dr. Xiao-Yan Wen** (ZCADD, St. Michael’s Hospital)  
10:40am – 11:05am | **Dr. Alexander J. Parker**, University of Montreal, Canada  
“Early drug discovery and development for neurological disorders using C. elegans” |
| 11:05am – 11:30am | **Dr. Karl Clark**, Mayo Clinic at Rochester, Minnesota, USA  
“Targeted mutagenesis and analysis of the vertebrate stress response in Danio rerio” |
| 11:30am – 12:10pm | **Mini-talk** (8 mins + 2 min Q&A)  
**Dr. Tod Thiele**, University of Toronto Scarborough, Canada  
“Investigation of neural circuits controlling action selection in larval zebrafish”  
**Dr. Han Wang**, Center for Circadian Clocks, Soochow University, Suzhou, China  
“A circadian model for major depressive disorder (MDD)”  
**Dr. Éric Samarut** (Postdoc), CRCHUM, Montréal, QC, Canada  
“Light-induced generalized epilepsy in gabral knockout zebrafish: a simple model for unravelling the molecular basis of epilepsy and screening for anti-epileptic drugs”  
**Dr. Brock Schuman** (Postdoc), Zebrafish Centre for Advanced Drug Discovery, Keenan Research Centre of St. Michael’s Hospital, Toronto, Canada  
“Zebrafish Models of Parkinson’s Disease” |
| 12:30pm – 2:00pm | **Session C: Z-BRAIN Platform Discussion** (1st floor, CIBC Hall, Room 136)  
*Co-Chairs: Drs. Pierre Drapeau (CRCHUM) & Xiao-Yan Wen (SMH)*  
*Lunch (Z-BRAIN Platform Members Only)* |

### 3rd Zebrafish for Personalized/Precision Medicine Conference

**Wednesday, September 27th, 2017**

<table>
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<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>3:00pm – 5:00pm</td>
<td>Registration and Refreshments (1st floor, CIBC Hall, Room 136)</td>
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<tr>
<td>5:00pm – 6:00pm</td>
<td>Registration and Refreshments (2nd floor, Exhibition Hall)</td>
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</tbody>
</table>
| 6:00pm – 6:10pm | **Welcome and Opening Remarks** (Allan Waters Family Auditorium, Room 209)  
דו. Xiao-Yan Wen, Director, ZCADD, St. Michael’s Hospital  
דו. Ori Rotstein, Director, Kennan Research Centre for Biomedical Science, SMH  
דו. Vasundara Venkateswaran, Institute of Medical Science, University of Toronto  
דו. Garth Smith, Director, Industry Relations, Ontario Brain Institute  
*Light Dinner provided* |
| 6:10pm – 8:30pm | **Session I: Advances in Zebrafish Research & Precision Medicine**  
*Co-chairs: Dr. Xiao-Yan Wen (ZCADD, St. Michael’s Hospital)  
Dr. Ian Scott (The Hospital for Sick Children & University of Toronto)* |
| 6:10pm – 8:10pm | **Invited Lectures** (25 min + 5 min Q&A)  
**Dr. Michael Wilson**, The Hospital for Sick Children & University of Toronto  
“From mammals to fish and back again: using comparative epigenomics to discover ancient enhancers active in early heart development” |
| 6:40pm – 7:10pm | **Dr. Lila Solnica-Krezel**, Washington University School of Medicine in St. Louis  
“Forward and Reverse Genetic Approaches to Scoliosis in Zebrafish” |
| 7:10pm – 7:40pm | **Dr. Peter Liu**, University of Ottawa Heart Institute, Canada  
“Search for Novel Biomarkers and Biotargets for Precision Medicine in Heart Disease” |
| 7:40pm – 8:10pm | **Dr. Stephen Ekker**, Mayo Clinic, Rochester, USA  
“Engineering the Power House of the Cell” |
### Thursday, September 28th, 2017

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<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:30am – 8:50am</td>
<td>Registration and Breakfast (2nd floor Exhibition Hall, Room 240 &amp; 241)</td>
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| 9:00am – 12:00pm | **Session II: Mutagenesis & Disease Modeling**  
*Chair: Dr. Stephen Ekker (Mayo Clinic, Rochester, USA)*  
**Invited Lecture** (25 min + 5 min Q&A):  
Dr. Erica Davis, Center for Human Disease Modeling, Duke University  
“Functional dissection of pediatric genetic diseases” |
| 9:00am – 9:30am | **Oral Presentations** (12 min + 3 min Q&A)  
Dr. Keith C. Cheng, Penn State College of Medicine, USA  
“From Morphology to Math: Using whole zebrafish to create the engineering and computational infrastructure for Microanatomic Phenomics”  
Dr. Raman Sood, National Human Genome Research Institute, National Institutes of Health, Bethesda, USA  
“Multiplexed CRISPR/Cas9-mediated knockout of nineteen Fanconi anemia pathway genes to understand their roles in disease pathophysiology”  
Han B. Lee (PhD Candidate), Mayo Clinic Graduate School of Biomedical Sciences; Mayo Clinic, Rochester, USA  
“Development of a dual-light assay system to identify genetic or epigenetic modifiers of the hypothalamic-pituitary-adrenal axis” |
| 9:30am – 10:15am | **Coffee Break** (2nd floor, Exhibition Hall) |
| 10:15am – 10:30am | **Invited Lectures** (25 min + 5 min Q&A)  
Dr. Jeff Essner, PhD, Iowa State University  
“Enhanced efficiencies using short regions of homology for precise DNA integration in zebrafish” |
| 10:30am – 11:00am | **Oral Presentations** (12 min + 3 min Q&A)  
Dr. Weibin Zhou, University of Michigan, USA  
“Characterization of a new zebrafish model of finnish-type nephrotic syndrome”  
Dr. Onofrio Laselva (Postdoc), Programme in Molecular Medicine, Hospital for Sick Children, University of Toronto, Canada  
“Studying the distinct sensitivities of CFTR modulators using Zebrafish-CFTR”  
Ivo Eijkenboom (PhD candidate), Maastricht University Medical Centre, Maastricht, The Netherlands  
“A zebrafish model for small-fiber neuropathy” |
| 11:00am – 1:00pm | **Lunch** (2nd floor, Exhibition Hall, Room 240 & 241) |
| 1:30pm – 4:30pm | **Session III: Mechanisms of Development, Organ Dysfunction & Disease**  
*Chair: Dr. Oliver Bandmann (University of Sheffield, UK)* |
<table>
<thead>
<tr>
<th>Time</th>
<th>Session Description</th>
<th>Speakers</th>
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<tr>
<td>1:30pm – 2:00pm</td>
<td><strong>Invited Lecture (25 min + 5 min Q&amp;A)</strong></td>
<td><strong>Dr. Marie-Andrée Akimenko</strong>, University of Ottawa, Canada</td>
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<tr>
<td></td>
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<td>“A regulatory pathway leading to joint formation and maintenance in Zebrafish fin regenerates”</td>
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<tr>
<td>2:00pm – 2:45pm</td>
<td><strong>Oral Presentations (12 min + 3 min Q&amp;A)</strong></td>
<td><strong>Dr. Ela Knapik</strong>, Vanderbilt Genetics Institute, VUMC, Nashville, TN, USA</td>
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<td>“Disease Mechanism Discovery: Iteration among a Biobank, Zebrafish, and Electronic Health Records”</td>
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<td><strong>Dr. Peng Huang</strong>, University of Calgary, Calgary, Alberta, Canada</td>
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<td>“In vivo dynamics of muscle-associated cells during zebrafish muscle regeneration”</td>
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<td><strong>Dr. Tamjid Chowdhury</strong> (Postdoc), Medical College of Wisconsin, Milwaukee, USA</td>
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<td>“Temporal and spatial post-transcriptional regulation of zebrafish tie-1 mRNA by long non-coding RNA”</td>
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<tr>
<td>2:45pm – 3:00pm</td>
<td><strong>Coffee Break</strong></td>
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<tr>
<td>3:00pm – 3:30pm</td>
<td><strong>Invited Lecture (25 min + 5 min Q&amp;A)</strong></td>
<td><strong>Dr. Catarina Henriques</strong>, University of Sheffield, UK</td>
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<tr>
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<td>“Telomeres, telomerase and inflammation in zebrafish ageing”</td>
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<tr>
<td>3:30pm – 4:15pm</td>
<td><strong>Oral Presentations (12 min + 3 min Q&amp;A)</strong></td>
<td><strong>Dr. Han Wang</strong>, Center for Circadian Clocks, Soochow University, China</td>
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<td>“Rhythmically expressed ezh2 promotes clock function and hematopoiesis independent of histone methyltransferase activity in zebrafish”</td>
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<td><strong>Dr. Marika Kapsimali</strong>, INSERM, IBENS, Paris, France</td>
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<td>“The zebrafish taste sensory cells: a screening tool for harmful chemicals and compounds enhancing regeneration”</td>
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<td><strong>Dr. Yahui Lan</strong> (Postdoc), Weill Cornell Medical College, New York, USA</td>
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<tr>
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<td></td>
<td>“Epigenetic Control of Zebrafish Cardiogenesis by TET2/3”</td>
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<tr>
<td>4:30pm – 4:50pm</td>
<td><strong>Flash Talks (1 slide, 1 min/person)</strong></td>
<td><strong>Chair: Dr. Ian Scott (Sick Kids Hospital &amp; UofT)</strong></td>
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<td><strong>John Dawson</strong>, University of Guelph, Canada (P2-8)</td>
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<td><strong>Michèle G. DuVal</strong>, University of Alberta, Canada (P2-9)</td>
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<td><strong>Suzan El-Rass</strong>, ZCADD, St. Michael’s Hospital, U of T, Canada (P2-10)</td>
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<td><strong>Izabella A. Pena</strong>, CHEO Research Institute, Ottawa, Canada (P2-21)</td>
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<td><strong>Jana Pfeiffer</strong>, University of Münster, Germany (P2-22)</td>
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<td><strong>Éric Samarut</strong>, CRCHUM, University of Montréal, Canada (P2-24)</td>
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<td><strong>Heejin Lee</strong>, Weill Cornell medical College, New York, USA (P3-34)</td>
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<td><strong>Ryan Thummel</strong>, Wayne State University School of Medicine, USA (P3-38)</td>
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<td><strong>Daniel Zuppo</strong>, University of Pittsburgh, USA (P3-44)</td>
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<td><strong>Nun Ribeiro Palha</strong>, Institut de Recherches Servier, Paris, France (P5-46)</td>
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<td><strong>Hyunjin Jeong</strong>, University of Toronto, Canada (P5-47)</td>
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<td><strong>Junghwa Yun</strong>, ZCADD, St. Michael’s Hospital, U of T, Canada (P5-55)</td>
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5:00pm – 6:30pm | **Dinner** (2nd floor Exhibition hall, Room 240 & 241) 

| Session IV: **Poster Competition** (1st floor CIBC Hall, Room 136) 
**Chair: Dr. Ian Scott (The Hospital for Sick Children & University of Toronto)** |
| --- | --- |

7:30am – 8:50am | Breakfast (2nd floor Exhibition Hall, Room 240 & 241) 

| **Friday, September 29th, 2017** |
| --- | --- |

| 7:30am – 12:00pm | **Session V: Chemical Biology, Pharmacogenomics, and Drug Discovery** 
**Chair: Randall Peterson (University of Utah, USA)** |

| 9:00am – 9:30am | **Invited Lecture** (25 min + 5 min Q&A): 
**Dr. Pierre Drapeau,** University of Montréal, Canada 
“Neuroleptics for ALS: small compound screen and small clinical trial” |

| 9:30am – 10:15am | **Oral Presentations** (12 min + 3 min Q&A) 
**Dr. Oliver Bandmann,** University of Sheffield, UK 
“Gene-gene interaction studies in a zebrafish model of Gaucher disease” 
**Dr. Martin Distel,** Children’s Cancer Research Institute, Vienna, Austria 
“A zebrafish model mimicking aspects of Costello Syndrome amenable to compound screening” 
**Purnima Manghnani** (PhD Candidate), National University of Singapore 
“Zebrafish as a predictive model for photodynamic therapy” |

| 10:15am – 10:30am | **Coffee Break** (2nd floor, Exhibition Hall) |

| 10:30am – 11:00am | **Invited Lecture** (25 min + 5 min Q&A) 
**Dr. Edward Burton,** University of Pittsburgh, USA 
“Unbiased phenotype-based chemical modifier screens in zebrafish models of neurodegenerative movement disorders” |

| 11:00am – 11:45am | **Oral Presentations** (12 min + 3 min Q&A) 
**Dr. Richard Glenn dela Cruz,** Oklahoma Medical Research Foundation, USA 
“Identification of novel inhibitors of the activated Wnt/β-catenin signaling pathway using the developing zebrafish embryo” 
**Dr. Charles H. Williams** (Postdoc), Vanderbilt University Medical Center, USA 
“ZePASS (Zebrafish Phenotypic Anatomical Similarity System (Guides Target Identification of a Wnt Inhibitor Incaskin, a Novel, Highly Selective CK2α Kinase Inhibitor)” 
**Arman Hassanpour** (MSc candidate), IMS at UofT, ZCADD, St. Michael’s Hospital 
“Preclinical development of drugs for intracerebral hemorrhage (ICH)” |

<p>| 12:00pm – 1:00pm | <strong>Lunch</strong> (2nd floor, Exhibition Hall, Room 240 &amp; 241) |</p>
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<tr>
<th>Time</th>
<th>Session VI: Translational Research &amp; Drug Development</th>
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<tr>
<td>1:30pm – 2:00pm</td>
<td><strong>Invited Lecture</strong> (25 min + 5 min Q&amp;A)</td>
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<td>Dr. Ori Rotstein, Keenan Research Centre for Biomedical Science, SMH</td>
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<td>“Using zebrafish to study mechanisms of inflammation”</td>
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<td>2:00pm – 2:30pm</td>
<td>Dr. Charles Hong, Cell an Developmental Biology, Vanderbilt University, USA</td>
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<td>“Chemical Genetics of Zebrafish Embryonic Development to Drive Therapeutic Target Discovery in Man”</td>
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<td>2:30pm – 3:00pm</td>
<td>Dr. Clara van Karnebeek, University of Amsterdam, Netherlands</td>
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<td>“Zebrafish modelling for Neurometabolic diseases: Identification of disease mechanisms &amp; treatment targets”</td>
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<td>3:00pm – 3:15pm</td>
<td>Coffee Break (2nd floor, Exhibition Hall)</td>
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<td>3:15pm – 3:45pm</td>
<td>Dr. Yibin Feng, School of Chinese Medicine, The University of Hong Kong</td>
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<td>“Drug discovery from Chinese medicines: the past and prospect”</td>
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<td>3:45pm – 4:15pm</td>
<td>Dr. Randall Peterson, College of Pharmacy, University of Utah, USA</td>
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<td>“Modeling human CNS disorders in the zebrafish”</td>
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<td>4:15pm – 4:30pm</td>
<td>Award Presentations and Closing Remarks</td>
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<td>✦ Dr. Randall Peterson (Dean, College of Pharmacy, University of Utah, USA)</td>
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<td>✦ Dalton Charters (Director, Research Operations, St. Michael’s Hospital, Canada)</td>
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<td>5:30pm – 6:00pm</td>
<td>St. Michael’s Hospital (LKSKI Building) to Ripley’s Aquarium of Canada</td>
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<td>1. <strong>Bus pick-up:</strong> 5:30pm at lobby of Li Ka Shing Knowledge Institute</td>
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<td>3. <strong>Subway</strong> (15 mins)</td>
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<td>6:30pm – 10:30pm</td>
<td>Networking Dinner at Ripley’s Aquarium of Canada</td>
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<td>11:00pm</td>
<td>Ripley’s Aquarium of Canada to St. Michael’s Hospital (LKSKI Building)</td>
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<td><strong>Bus pick-up:</strong> 11:00pm</td>
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Welcome and Opening Remarks
✦ Dr. Xiao-Yan Wen, ZCADD, St. Michael’s Hospital
✦ Dr. Ori Rotstein, Director, Kennan Research Centre for Biomedical Science, SMH
✦ Dr. Tom Mikkelsen, President and Scientific Director, Ontario Brain Institute

Scientific Session A
Chair: Dr. Pierre Drapeau (University of Montréal (CRCHUM), Canada)

Invited Lecture:

Zebrafish as a tool for target validation and drug discovery in proteinopathies of the CNS
Dr. Anne Dekeyne, Institute of Research Servier, Paris, France

Mini-talks:

Towards stem cell therapy of blindness: Defining gene regulatory networks in the premier genetic model of cone photoreceptors
Dr. Ted Allison, University of Alberta, Canada

Assessing the uptake kinetics, biological activity and potential interaction of individual cannabinoids using zebrafish larvae
Dr. Lee Ellis, National Research Council of Canada

PHRESH analysis of cell signaling dynamics during spinal cord patterning
Dr. Peng Huang, University of Calgary, Canada

Elucidating Molecular Changes During Dopaminergic Neuron Regeneration in Zebrafish
Khang Hua (PhD candidate), University of Ottawa, Canada

Towards the generation of a digital adult zebrafish brain atlas
Dr. Justin W. Kenney (Postdoc), Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada
**Scientific Session B**

*Chair: Dr. Xiao-Yan Wen (ZCADD, St. Michael’s Hospital & University of Toronto, Canada)*

**Invited Lectures:**

Early drug discovery and development for neurological disorders using *C. elegans*

*Dr. Alexander J. Parker, University of Montreal, Canada*

Targeted mutagenesis and analysis of the vertebrate stress response in *Danio rerio*

*Dr. Karl Clark, Mayo Clinic at Rochester, Minnesota, USA*

**Mini-talks:**

Investigation of neural circuits controlling action selection in larval zebrafish

*Dr. Tod Thiele, University of Toronto Scarborough, Canada*

A circadian model for major depressive disorder (MDD)

*Dr. Han Wang, Center for Circadian Clocks, Soochow University, Suzhou, China*

Light-induced generalized epilepsy in *gabra1* knockout zebrafish: a simple model for unravelling the molecular basis of epilepsy and screening for anti-epileptic drugs

*Dr. Éric Samarut (Postdoc), CRCHUM, Montréal, QC, Canada*

**Zebrafish Models of Parkinson’s Disease**

*Dr. Brock Schuman (Postdoc), Zebrafish Centre for Advanced Drug Discovery, Keenan Research Centre of St. Michael’s Hospital, Toronto, Canada*

**Session C: Z-BRAIN Platform Discussion**

*Co-Chairs: Drs. Pierre Drapeau (CRCHUM) & Xiao-Yan Wen (SMH)*

*(Platform members only)*
Invited Speaker

Anne Dekeyne, PhD.
Neuropsychiatry Research Department, Institute of Research Servier, Paris, France

After my Ph D. (University of Paris XI, France), I worked 7 years in a pharmaceutical company named Roussel UCLAF, acquiring a strong experience in the field of neuropsychopharmacology and drug discovery. I joined Servier in 1997, where I was involved as Head of laboratory and Project Director in assessing drug efficacy in behavioral rodent models of neuropsychiatric diseases, and managing preclinical development of potential antidepressant and antipsychotic agents. Four years ago, I began to develop a zebrafish platform aimed in the set up and validation of misfolded protein-based models (tau, alpha-synuclein and beta-amyloid) of neurodegenerative diseases (Alzheimer’s and Parkinson’s), and readouts (imaging and behavior) suitable to pharmacological screening, e.g. compatible with high throughput approaches. Currently, I’m the Manager of the “In Vivo” team of the Neuropsychiatry Research Department, which is working on proteinopathy models and readouts not only using zebrafish but also transduced or transgenic mice.

Zebrafish as a tool for target validation and drug discovery in proteinopathies of the CNS

Author(s): Anne Dekeyne and Nuno Ribeiro Palha

Affiliation: Neuropsychiatry Research Department, Institut de Recherches Servier, Croissy-sur-Seine, France

Email: anne.dekeyne@servier.com

Zebrafish is becoming an increasingly popular model in drug discovery programs. At Servier, either internally or in collaboration with academics, CROs and biotechs, we are currently establishing zebrafish misfolded protein-based models (tau, alpha-synuclein and beta-amyloid) of neurodegenerative diseases (Alzheimer’s and Parkinson’s), and developing readouts suitable for high throughput pharmacological screening. As an example related to tauopathy models, the transgenic zfTAUP301L line (Paquet et al., 2009) was re-characterized. Behavioral alterations, axonal motoneuron defect, neuronal death and abnormal phosphorylation of TAU were confirmed in larvae and some of these phenotypes were shown to be rescued by reference compounds (LMTX, Surfen). Unfortunately, although Tau aggregation was originally detected in this model by Gallyas silver staining, oligomers were not detected using an in house-validated western blot approach. Therefore, in order to obtain stronger phenotypes, generation of new transgenic fish has been launched using modified Huc:GAL4 and Hb9:GAL4 driver lines to be crossed with UAS:Aβ1-42/TAU wild type/TAUP301L/TAUP301S responder lines. Furthermore, phenotyping focused on potential aggregation and propagation is under progress, using transient expression of wild type or mutated human TAU in motoneurons or retina, α-synuclein in motoneurons, with or without pro-aggregating treatments. Other approaches are also under development, namely non-integrative lentiviral vector injection as a new tool to obtain transient overexpression and Aβ oligomers-induced neurotoxicity in larvae. In all, technological developments in genome editing, imaging and behavior analysis and throughput offer a great opportunity to make zebrafish a key player in proteinopathy drug discovery programs, from target engagement and validation to pharmacological screening.
Ted Allison, PhD., Associate Professor
Department of Biological Sciences, University of Alberta, Canada

Dr. Allison has been directing two research Programs since arriving to the University of Alberta nine years ago. One Program considers the evolution of photoreceptor degeneration and regeneration, including use of zebrafish to model developmental events that occur over the life history of trout and salmon. Applied Aims arising from this address a major hurdle of clinical stem cell therapy for blinding disorders: understanding the cellular and molecular cues that drive retinal stem cells to regenerate and functionally integrate cone photoreceptors to restore daytime vision. The second Program aims to understand Alzheimer and Prion diseases, by assessing the roles of key disease proteins in healthy brains and by engineering zebrafish models of disease. Dr. Allison completed his Doctoral Thesis on trout retinal physiology with Prof. Craig Hawryshyn at the University of Victoria, Canada. He began studying zebrafish genetics of retinal development during an NSERC PDF with Collegiate Prof. Pamela Raymond at the University of Michigan, Ann Arbor. Dr. Allison received Young Investigator Awards from the Alberta Prion Research Institute and the Alzheimer Society of Canada while in his current positions as Associate Professor in the Centre for Prions and Protein Folding Disease, and in the Departments of Biological Science and Medical Genetics at the University of Alberta.

Towards stem cell therapy of blindness: Defining gene regulatory networks in the premier genetic model of cone photoreceptors

Author: W. Ted Allison

Affiliation: University of Alberta, Edmonton, Alberta, Canada

Email: ted.allison@ualberta.ca

Zebrafish are the premier genetic model of cone photoreceptor biology and regeneration. The retina of zebrafish is densely packed with cones of various subtypes, akin to the human macula and distinct from nocturnal rodent models that are dominated by rod photoreceptors. Regeneration and protection of cone photoreceptors is key, as they underpin our daytime high-acuity colour vision. We have engineered fish with cone-specific cell ablations to permit study of regeneration. To improve and control this cone regeneration, we also work to delineate the genes regulating cone development. This has provided insights into the transcriptional control of cone subtypes and the development of rods vs. cones. Comparing the development of mice and zebrafish has permitted insights into the evolution of rods from cones in early evolving mammals during the ‘nocturnal bottleneck’, including the exploitation of nocturnal niches to avoid daytime predators including dinosaurs. Thus consideration of photoreceptor evolution is reiteratively inspiring hypotheses about control of photoreceptor development towards repair of blinding disorders.

Keywords: retina, photoreceptor, regeneration, transcription factor
Lee Ellis, PhD., Research Officer
National Research Council of Canada

Lee Ellis received his PhD from the Center for Research in Neuroscience at McGill University in 2007. His graduate studies primarily focused on the role of potassium channels in sensory processing using the weakly electric fish *Apteronous leptorhynchus* as a model species. He then moved to a post-doctoral position in the Department of Physiology and Biophysics at Dalhousie University in the lab of Dr. Alan Fine. There, Dr. Ellis designed a project to evaluate the behavioural, molecular and physiological changes that occur during learning in zebrafish. In 2009, he became a research associate at the National Research Council of Canada in Halifax, NS where he designed and implemented a research platform centered on the development of models of neurological disease. These models included various forms of neuro-hyperactivity, a model of bi-polar disorder and a model of addiction in conjunction with Health Canada. Since 2015 Dr. Ellis has been a Research Officer with the NRC and the primary investigator leading the zebrafish platform. Since that time they have developed additional models of disease including a model of nociception. The focus of his current research is to use the various disease models at our disposal in order to test the efficacy of novel compounds. Currently there is a large focus on extracts of *Cannabis sativa*, and purified cannabinoids.

Assessing the uptake kinetics, biological activity and potential interaction of individual cannabinoids using zebrafish larvae

**Author(s):** Ellis LD; Achenbach JC; Morash M; Hill J, Hui J; Berrue F

**Affiliation:** National Research Council of Canada

**Email:** lee.ellis@nrc-cnrc.gc.ca

The Cannabis Sativa plant contains numerous cannabinoids and terpenes with known or potential bioactivity. Many plants are bred specifically to contain various ratios of these compounds. As these ratios can vary a high throughput in vivo model that can test the effects of the various cannabinoids is valuable for the assessment of which combinations may have the best therapeutic potential. Measuring changes in the behavioural patterns of zebrafish larvae is an established model with which to test the bioactivity of neuroactive compounds. However, there is currently little information regarding the uptake kinetics and metabolism of compounds by larvae. In this study we chose to compare the uptake kinetics and metabolism of selected cannabinoids, alone or in combination, with their effects on larval behaviour. We measured the changes in larval behaviour following exposure to 3 cannabinoids, tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) alone or in combination. HPLC-MS was used to measure the uptake kinetics and metabolism of the cannabinoids. We have shown that all 3 of the compounds have distinct behavioural concentration response patterns. The uptake kinetics observed for each cannabinoid appears to match the kinetics observed in the larval behaviour assay. In combination exposure experiments there appears to be a shift in both the behavioural activity and the uptake kinetics of each compound compared to when they are tested alone. Finally, the cannabinoid metabolites detected in the larval are similar to the ones found in mammalian systems.

**Keywords:** zebrafish larvae cannabinoid THC CBD
Peng Huang, PhD., Assistant Professor
Department of Biochemistry and Molecular Biology, University of Calgary, Canada

Peng Huang is an Assistant Professor in the Department of Biochemistry and Molecular Biology at the University of Calgary. He received his BSc degree in Biochemistry and Molecular Biology from Beijing University, China. He then obtained his PhD in Genetics with Dr. Michael Stern at Yale University studying FGF signalling in C. elegans. After PhD, he joined Dr. Alexander Schier’s laboratory as a postdoctoral fellow at Harvard University studying primary cilia and Hedgehog signalling in spinal cord patterning in zebrafish.

The Huang lab continues to use zebrafish as a model system to address two main questions: 1) how interactions of different cell signaling pathways drive precise pattern formation in the spinal cord; and 2) how muscle-associated cells regulate muscle regeneration and degeneration.

Dr. Huang’s research is generously supported by grants from CIHR, NSERC and CFI.

PHRESH analysis of cell signaling dynamics during spinal cord patterning

Author(s): Craig Jacobs and Peng Huang

Affiliation: Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada

Email: huangp@ucalgary.ca

Throughout development, tissue patterning is governed by interactions of many different cell signaling pathways. During spinal cord patterning, neural progenitors are maintained by Notch signaling, whereas neural fates are specified by Hedgehog (Hh) signaling. However, how dynamic interactions between Notch and Hh signaling drive this precise pattern formation is still unknown. Using the zebrafish spinal cord as a model, we applied the novel PHRESH (PHotoconvertible Reporter of Signaling History) technique to analyze cell signaling dynamics. This technique takes advantage of the photo-convertible property of Kaede reporters to distinguish early and late signaling response. We generated sensitive transgenic reporters for Notch signaling (her12:Kaede) and Hh signaling (ptc2:Kaede). Using photo-conversion and 3D image reconstruction, we generated high-resolution spatiotemporal maps of Notch and Hh signaling response in the developing spinal cord. Initially, Notch signaling is active along the entire D-V axis of the spinal cord, while active Hh signaling constitutes the ventral portion of the Notch response domain. Gradually, active Notch and Hh response become restricted to the medial domain of the spinal cord while largely absent in the lateral and the most ventral region. Using small molecule inhibitors, we showed that inhibition of Notch signaling results in loss of Hh response but not vice versa, suggesting that active Notch signaling is required for maintaining cellular responsiveness to Hh.

Combining PHRESH analysis with genetic mutants and gain-of-function transgenic tools, we can analyse both global signaling response and single cell interactions to understand how interactions of Notch and Hh signaling achieve precise pattern formation.

Keywords: Spinal cord patterning, Notch signaling, Hedgehog signaling, Signaling dynamics, Pattern formation
Khang Hua, *(PhD Candidate)*
*University of Ottawa, Canada*

Khang Hua received his Bachelor of Science degree with specialization in Biochemistry from the University of Ottawa. He then went on to Simon Fraser University where he received his Masters of Science degree in Molecular Biology and Biochemistry. During his Master's degree, he worked in the laboratory of Dr. Nancy Hawkins where he investigated the structure and function of HAM-1, an intrinsic factor which regulates asymmetric division of neuroblasts during *Caenorhabditis elegans* embryonic development. After completing his Master’s degree, Khang continued his research on HAM-1 with the goal of identifying biochemical interacting partners of HAM-1 as a research technician. Course work that he took on neuroscience and neurodegenerative diseases throughout his Master’s degree sparked his interest in neuroscience which led him to pursue a PhD in neuroscience with a focus on Parkinson’s Disease. Khang is currently a PhD candidate in the laboratory of Dr. Marc Ekker at the University of Ottawa where he is investigating aspects of neuronal regeneration in adult zebrafish. The goal of his project is to elucidate molecular changes that occur during dopaminergic neuron regeneration in adult zebrafish brain in hopes of identifying potential therapeutic targets for the treatment of Parkinson’s Disease.

**Elucidating Molecular Changes During Dopaminergic Neuron Regeneration in Zebrafish**

**Author:** Khang Hua, Rafael Godoy, Sandra Noble, Marc Ekker

**Affiliation:** University of Ottawa, Ottawa, Ontario, Canada

**Email:** khua103@uottawa.ca

Parkinson’s Disease (PD) is a progressive neurodegenerative disease in which patients suffer from motor impairments. Pathologically, motor symptoms arise due to the progressive loss of dopaminergic neurons within the substantia nigra. Current treatments for PD alleviates the motor symptoms associated with the disease; however, the progression of neurodegeneration cannot be halted or reversed. The ability to regenerate lost dopaminergic neurons could provide an alternative therapeutic approach to stall or reverse the progression of the disease. To study the process of neuronal regeneration, we utilized zebrafish due to their widespread neural stem cell activity in the adult brain and regenerative ability following brain injury. To specifically ablate dopaminergic neurons in the zebrafish brain, we used a chemogenetic approach. Tg(dat:CFP-NTR) transgenic zebrafish show restricted expression of nitroreductase in dopaminergic neurons and can be used to specially ablate most dopaminergic neuron clusters following metronidazole (MTZ) treatment. Following ablation of dopaminergic neurons in larvae, there is an increase in cell proliferation, and in the expression of genes involved in neurogenesis and tissue repair. Adult transgenic zebrafish treated with MTZ show an increase in the production of new dopaminergic neurons in the olfactory bulb, and recovery of dopaminergic neurons 45 days after neuronal ablation. In addition, treated fish show an increase in cell proliferation as well as in the expression of radial glia and neural stem cell markers in response to dopaminergic neuron ablation. Altogether, these data will help elucidate the molecular changes that take place during dopamine neuron regeneration in zebrafish.

**Keywords:** neuronal regeneration, dopamine neurons
Justin W. Kenney, (PhD Candidate)  
Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, Canada

Dr. Justin Kenney is a Human Frontiers Science Program long-term fellow at the Hospital for Sick Children working in the lab of Dr. Paul Frankland. After growing up in New Jersey, Justin earned undergraduate degrees in Physics and Philosophy from Case Western Reserve University in Cleveland, OH. After spending a year doing volunteer work with AmeriCorps* NCCC (National Civilian Community Corps), Justin went on to graduate work at Temple University in Philadelphia, PA. At Temple, Justin worked with Dr. Thomas Gould on identifying novel molecular mechanisms necessary for the memory enhancing effects of nicotine in mice. Following his doctoral work, he spent 3.5 years at the University of Southampton in the UK working with Dr. Christopher Proud on the regulation of protein synthesis. In Southampton, he developed techniques for studying rapid changes in protein synthesis and studied the signaling mechanisms involved in the regulation of translation elongation in murine primary neurons. In his current position at the Hospital for Sick Children, Justin is working on applying network analysis to neuronal activity data in order to derive insights into brain-behavior relationships. He has also developed protocols for studying long-term learning and memory in adult zebrafish. To facilitate the study of whole-brain function in adult zebrafish, he is currently working on generating an adult zebrafish digital brain atlas using a combination of tissue clearing techniques and light-sheet microscopy.

Towards the generation of a digital adult zebrafish brain atlas

Author(s): Justin W. Kenney, Patrick S. Steadman, Sheena A. Josselyn, Paul W. Frankland

Affiliation: Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada

Email: jkenney9a@gmail.com

Adult zebrafish are an increasingly popular and important animal model in neuroscience research. As a genetically tractable vertebrate with a sophisticated behavioral repertoire and high genetic similarity to humans (70%), zebrafish are an ideal system for the study of neurobiology and behavior in both health and disease. However, although a comprehensive brain atlas for adult zebrafish was published over 20 years ago in book form, the field is lacking a digital brain atlas necessary for automated whole brain studies. Here, we report on our progress in creating such an atlas by combining tissue clearing techniques, fluorescent labelling, light sheet microscopy, automated image registration, and manual parcellation. We anticipate that the generation of such an atlas will significantly increase the utility and sophistication of adult zebrafish as an animal model in neuroscience research.

Keywords: adult, brain, tissue clearing, light sheet microscopy
Invited Speaker

Alexander J. Parker, PhD.
Principal Investigator, CRCHUM
Assistant Professor, Department of Neuroscience, University of Montréal, Canada

Dr. Alex Parker obtained his PhD at the University of British Columbia in Medical Genetics and did postdoctoral training at INSERM (Paris, France). He is currently a principal investigator at the CRCHUM, Assistant Professor, Department of Neuroscience, University of Montréal.

Dr. Parker models and studies neurodegeneration using the model organism Caenorhabditis elegans. His team focuses on amyotrophic lateral sclerosis, dementia as well as the DNA repeat expansion diseases. Dr. Parker’s team has developed high throughput, in vivo screening approaches for use in unbiased small molecule screening for drug discovery and development. With this chemical-genetic approach Dr. Parker and colleagues have discovered several small molecules that may be clinically relevant for human neurodegenerative diseases.

Early drug discovery and development for neurological disorders using C. elegans

Author(s): Kathrin Schmeisser, Yasmin Fardghassemi, James Doyle, Claudia Maios, Alex Parker

Affiliation: CRCHUM, Department of Neuroscience, University of Montreal

Email: ja.parker@umontreal.ca

Our research program is based on modeling human neurological disorders to discover pathogenic mechanisms and new therapeutic approaches. Major breakthroughs in the genetics of many neurological conditions has led to the identification of numerous potentially causative genes. With these discoveries comes the need to understand the normal biological roles of these genes, as well as their function in the pathogenic state. Central to this approach is the development of animal models to investigate molecular mechanisms of neuronal dysfunction, neurodegeneration as well as the identification and validation of novel therapeutic targets. To better understand the links between development, aging and neuronal function we use the model organism C. elegans, mainly focusing on amyotrophic lateral sclerosis (ALS), but we have expanded our approach to study frontotemporal dementia, Parkinson’s disease, polyglutamine-expansion disease, hereditary spastic paraplegia, as well as autism spectrum disorders. The molecular mode of action through which genetic variations cause neuronal phenotypes in humans are often unclear. Thus, it is seldom possible to perform target-directed chemical screens for many of these newly discovered genes using molecular assays, which in any case may lack in vivo relevance. On the other hand, our in vivo C. elegans models are useful for phenotype-directed chemical screens since they mirror several aspects of the neuronal dysfunction and degeneration observed in patients, opening new avenues for future screens at a molecular level. An overview of our C. elegans chemical-genetic approach for drug discovery and development will be presented.

Keywords: drug screening, C. elegans, neurodegeneration
Invited Speaker

Karl Clark, PhD. Assistant Professor
Mayo Clinic in Rochester, Minnesota, USA

Dr. Karl Clark is an Assistant Professor at the Mayo Clinic in Rochester, MN. Dr. Clark’s research laboratory uses genome engineering and zebrafish to study the vertebrate stress response system, a diverse suite of neuronal, endocrine and autonomic response mechanisms that play key roles in environmental interactions. Dr. Clark’s research goals are to contribute to better understanding of the development of the stress response system, how it impacts patient health, and how it can be modulated for the benefit of patients. Dr. Clark is also the director of the Functional Validation Lab, a component of the Individualized Medicine Investigative and Functional Genomic Research Program. Dr. Clark earned his Bachelor of Science from the University of Wisconsin- Eau Claire in Biochemistry and Molecular Biology. He attended graduate school at the University of Minnesota, where he trained with Dr. Perry Hackett and helped develop the Sleeping Beauty transposon system, earning his Ph.D. from the Molecular, Cellular, Developmental Biology, and Genetics program. Dr. Clark worked at Discovery Genomics, Inc., a gene therapy startup in Minneapolis, MN, before returning to the University of Minnesota for post-doctoral training in Dr. Scott Fahrenkrug’s laboratory. There he helped improve genome engineering applications in livestock that contributed to starting Recombinetics, Inc., a livestock genome-editing company. He moved to Mayo Clinic to work and train with Dr. Stephen Ekker before starting his own laboratory.

Targeted mutagenesis and analysis of the vertebrate stress response in Danio rerio

Author(s): Han B. Lee, Tanya L. Schwab, Randall G. Krug II, Jennifer L. Gauerke, louis Y. El Khoury, Ashley N. Sigafoos, and Karl J. Clark

Affiliation(s): Mayo Clinic, Rochester, Minnesota, USA

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The vertebrate neuroendocrine stress response system (SRS) consists of neuronal, endocrine, and behavioral response mechanisms that react to perceived or real threats. Progression and use of zebrafish (Danio rerio) as a behavioral genetic vertebrate model makes it ideal for discovery of genetic and environmental modifiers of the vertebrate-conserved SRS. We have used genome engineering tools to further develop the utility of the zebrafish model for the study of the SRS and ultimately our understanding of the vertebrate SRS. First, we have developed a transgenic zebrafish line, SR4G, that produces a short half-life GFP in response to glucocorticoid signaling. Second, to better understand whether or not glucocorticoid signaling is required for the rapid locomotor response following a stressor, we used custom nucleases to mutate both mc2r and nr3c1—two key receptors in the hypothalamus-pituitary-adrenal (HPA) signaling cascade. We have tested these mutations in the two larval locomotor assays that utilize two distinct stressors—hyposmotic challenge and rapid exposure to light. In both assays, mc2r and nr3c1 frame-shift mutants block or blunt locomotor activation following the stressor. We also created frame-shift mutation in several genes involved in the metabolism and signaling of the endocannabinoid (eCB) anandamide signaling pathway, which appears to be an important regulator of the rapid, non-genomic stress response. Of the eCB related genes we have tested cnr1 and faah2a mutants potentiate and block the locomotor response following a stressor, respectively.

Keywords: stress, behavior, glucocorticoid, genome engineering
Investigation of neural circuits controlling action selection in larval zebrafish

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Neural circuits in the basal ganglia play a central role in determining what actions we perform, however, much remains unknown concerning the circuit mechanisms within these structures that underlie behavioral decisions. Remarkably, it has recently been shown that the majority of the components and connections of the mammalian basal ganglia are present in the ancient jawless lamprey, indicating that they are also likely to be present in teleosts. If these circuits exist, the reduction in complexity and unparalleled optical access in juvenile zebrafish, should make relationships between basal ganglia activity and behavioral control more salient. To dissect basal ganglia circuits in zebrafish, we are utilizing Gal4 lines that target the fish’s putative direct and indirect pathways which, in mammals, promote or inhibit movement respectively. To gain genetic control of the indirect pathway, we are using a BAC transgenic line which stably expresses Gal4 in neurons that express the dopamine receptor gene drd2a (generated in a Baier Lab BAC screen). For the direct pathway, we recently generated a CRISPR/Cas9 integration of Gal4 into the drd1a locus. Following anatomical characterization of the drd2a and drd1a Gal4 lines, we will examine the function of labeled circuits using a combination of population calcium imaging, pharmacology, optogenetics and behavioral analyses. By taking this multifaceted approach, we should be able to identify the logic of cellular activity patterns throughout the direct and indirect pathways of young zebrafish and hopefully provide new insights into general circuit mechanisms controlling action selection across species.

Keywords: Basal ganglia, calcium imaging, behaviour
Han Wang, PhD, Professor and Director of Soochow University Center for Circadian Clock (SUCCC), Suzhou, Jiangsu, China. Han received PhD degree from Wayne State University, Detroit, Michigan, did postdoctoral trainings with Shuo Lin at Medical College of Georgia, Augusta, Georgia and John Postlethwait at University of Oregon, Eugene, Oregon. His group has been working on molecular genetic mechanisms underlying circadian clocks and sleep as well as establishing zebrafish models for blood diseases and psychiatric disorders.

A circadian model for major depressive disorder (MDD)

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The circadian clock as an endogenous time-keeping mechanism modulates fundamental life processes from molecular, biochemical, cellular, physiological to behavioral with a period of approximately 24 hours. Previous studies showed that circadian misalignment often lead to human psychiatric disorders such as seasonal affective disease (SAD). The underlying mechanisms, however, are far from certain. Here we show that zebrafish per2 mutant fish display lower locomotor activities, more time staying in the center region of the tank, less time for social interaction, reduced sleep time, and disrupted sleep continuity in comparison with wild types, indicating a clear depression phenotype of the per2 mutant zebrafish. Further, cortisol, and expression of pomc (proopiomelanocortin) and crh (corticotropin releasing hormone) are significantly up-regulated in the per2 mutant male fish, reminiscent of human depression patients with disruptive activities of the hypothalamic-pituitary-adrenal (HPA) axis. Quantitative RT-PCR shows that glucocorticoid receptor (gr) is significantly down-regulated in both the per2 mutant fish. Luciferase reporter assays show that Per2 can enhance Rorα-mediated expression of gr, and ChIP assays show that Per2 binds to the three RORE elements in the gr promoter region, suggesting that Per2 positively regulates gr in zebrafish. Our findings demonstrate that Per2 acts through glucocorticoid receptor signaling to impact functions of the hypothalamic-pituitary-adrenal (HPA) axis and to contribute to depression pathogenesis. Taken together, our analyses of zebrafish circadian mutants shed light on new roles of the circadian clock in psychiatric disorders.

Keywords: circadian clock, per2, ADHD, depression, zebrafish
Light-induced generalized epilepsy in zebrafish gabra1-/- knockout associated with a reduction of GABAergic connections in the brain

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Mutations of the GABA receptor, such as in the alpha 1 subunit (GABRA1), have been reported as predisposing for idiopathic generalized epilepsy. However, to date no functional studies of in vivo models have reported tonic-clonic generalized seizures associated with mutations of GABRA1, which has severely limited studies of the epilepsy phenotype. Here we show that gabra1 knockout in zebrafish leads to light-induced generalized tonic-clonic seizures in 2 week-old larvae and Sudden Unexpected Death in Epilepsy (SUDEP) related events at later stages. Remarkably, this epileptic phenotype resembles the severe generalized tonic-clonic seizures observed in patients. Moreover, we show that gabra1-/- epileptic seizures can be differentially rescued by known anti-epileptics, thus validating our model for further drug screens. Finally, by RNA-sequencing of whole brains from gabra1-/- larvae, we found many misregulated genes in pathways that are essential for correct brain development, indicating that pathogenicity of GABRA1 mutations may at least be partially due to defects in early neurodevelopment. More specifically, we showed a reduction of GABAergic synaptic connections as well as a reduction in the complexity of GABAergic projections throughout the brain of gabra1 -/- larvae.

Keywords: Epilepsy, GABA, Transcriptomics
Brock Schuman, PhD., (Postdoc)
Zebrafish Centre for Advanced Drug Discovery, St. Michael’s Hospital, Toronto, Canada

Brock Schuman received his PhD performing structural/kinetic analysis of carbohydrate acting enzymes at the University of Victoria in 2012. He has been a postdoctoral fellow working with zebrafish for drug discovery and validation since, in both industry and academia. Works presented at this conference include models of Parkinson's disease and major depression disorder: *In vivo* quantification of neurodegeneration and neuroprotective effects of candidate disease-modifying Parkinson's disease therapeutics; pharmaco-behavioral design and analysis; Zebrafish brain miRNA quantification (ddPCR); and biophysical characterization of α-synuclein pathological conformers.

Zebrafish Models of Parkinson's Disease

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The Parkinson's disease research community is in need of high throughput screens capable of identifying disease-modifying compounds that slow progression of the disease. Parkinson's disease neurodegeneration is afflicted by the protein α-Synuclein, misfolded multimers of which are prion-like, neurotoxic, bioaccumulate at presynaptic terminals and progressively transmit to surrounding neurons from their point of origin in the substantia nigra. These neurotoxic α-synuclein structures are distinct from, but related to the structures of protein fibrils found in Lewy bodies, both of which are unknown to atomic resolution. To quantify neurodegeneration in vivo zebrafish with labeled dopaminergic neurons have been produced by expressing GFP under transcriptional control of the dopamine transporter. Using these, chemically induced (MPP+) neuronal cell death in the ventral diencephalon can be quantified in live embryos. Several strategies are being employed to induce increasingly PD-like neurodegeneration in these fish, including overexpression of human pathogenic mutant α-synucleinA53T and LRRK2G2033S.

**Keywords:** Parkinson's Disease, Neurodegeneration
Welcome and Opening Remarks

- Dr. Xiao-Yan Wen, ZCADD, St. Michael’s Hospital
- Dr. Ori Rotstein, Director, Kennan Research Centre for Biomedical Science, SMH
- Dr. Vasundara Venkateswaran, Institute of Medical Science, University of Toronto
- Dr. Garth Smith, Director, Industry Relations, Ontario Brain Institute

Session I: Advances in Zebrafish Research & Precision Medicine
Co-chairs: Dr. Xiao-Yan Wen (ZCADD, St. Michael’s Hospital)
          Dr. Ian Scott (The Hospital for Sick Children & University of Toronto)

Invited Lectures

From mammals to fish and back again: using comparative epigenomics to discover ancient enhancers active in early heart development
Dr. Michael Wilson, The Hospital for Sick Children & University of Toronto

Forward and Reverse Genetic Approaches to Scoliosis in Zebrafish
Dr. Lila Solnica-Krezel, Washington University School of Medicine in St. Louis

Search for Novel Biomarkers and Biotargets for Precision Medicine in Heart Disease
Dr. Peter Liu, University of Ottawa Heart Institute, Canada

Engineering the Power House of the Cell
Dr. Stephen Ekker, Mayo Clinic, Rochester, USA
Invited Speaker

Michael D. Wilson, PhD., Assistant Professor

Department of Molecular Genetics, University of Toronto; The Hospital for Sick Children, Toronto, Canada

Michael D. Wilson is a Scientist at the Hospital for Sick Children and an Assistant Professor in the department of Molecular Genetics at the University of Toronto (05/2012-present). He is a Canada Research Chair in Comparative Genomics and a Member of the Heart and Stroke/Richard Lewar Centre of Excellence. He leads a research group that uses genomic technologies, multi-species comparisons, bioinformatics and molecular biology to uncover gene and genome regulatory mechanisms that are relevant to developmental and disease processes. By comparing epigenetic regulation between species, with a focus on the cardiovascular system, his team is uncovering fundamental mechanisms of genome regulation. Wilson trained as a postdoctoral fellow in Duncan Odom’s lab at Cancer Research UK – Cambridge Institute/ University of Cambridge. There he studied the evolution of transcription factor binding. He did his PhD in the molecular evolution and immunology group of Ben Koop (University of Victoria).

From mammals to fish and back again: using comparative epigenomics to discover ancient enhancers active in early heart development

Author(s): Xuefei Yuan(1,2,3), Mengyi Song(1,2,3), Patrick Devine(4,5,6), Benoit G. Bruneau(4,5,6), Ian C. Scott*,(2,3), and Michael D. Wilson*(1,2)

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Conserved transcription factors (TFs) control heart development in diverse species. However little is known about the cis-regulatory elements (CREs) that specify the early cardiac lineages. To discover CREs active before the expression of canonical cardiac TFs, we used a mouse Smarcd3 enhancer to isolate cardiac progenitors from gastrular zebrafish embryos. We found that the Smarcd3-labelled cells were enriched for cardiac lineages and identified ~4000 open chromatin regions specific to the Smarcd3-labelled cells. Using direct and indirect DNA sequence alignments, we mapped more than 150 open chromatin regions to human or mouse non-coding open chromatin regions. These conserved open chromatin regions were largely novel and distinct from the classic ultra-conserved non-coding elements. Around 80% of the conserved open chromatin regions tested drove cardiac expression in zebrafish embryos. In summary, we discovered a set of anciently conserved CREs active during heart development and gained insights into functional conservation in the absence of striking DNA sequence similarity.
Invited Speaker

Lilianna (Lila) Solnica-Krezel, Ph.D, Professor and Head

Department of Developmental Biology, Washington University School of Medicine, St. Louis, Missouri, USA

Lilianna (Lila) Solnica-Krezel, was raised in Poland and completed her undergraduate education and M.S. in molecular biology at the University of Warsaw. She obtained her Ph.D. in Oncology at the University of Wisconsin in Madison, WI. She carried out her postdoctoral work at Harvard Medical School in Boston, MA. In 1996, she established her independent laboratory focusing on zebrafish embryogenesis at Vanderbilt University in Nashville, TN, where she became the Martha Rivers Ingram Professor of Developmental Genetics and University Professor. Since 2010, Solnica-Krezel has been the Professor and Head of the Department of Developmental Biology at Washington University School of Medicine in St. Louis, MO and in 2014 was named the Alan A. and Edith L. Wolff Distinguished Professor of Developmental Biology. In 2017, she was named the president-elect of the Society for Developmental Biology. Solnica-Krezel’s research group is employing zebrafish and embryonic stem cells to study the genetic mechanisms underlying early embryonic development. She established methods of chemical mutagenesis and coordinated the first large-scale genetic screens that netted thousands of mutations affecting various aspects of zebrafish development. Her laboratory has been focused on dissecting the roles of Wnt/PCP, GPCR, and BMP pathways and epigenetic control of the process of gastrulation during which the animal body plan is established. Dr. Solnica-Krezel has a long-standing interest and commitment to training biomedical scientists at all stages of their education and scientific career.

Forward and Reverse Genetic Approaches to Understand Scoliosis in Zebrafish

Author(s): Lila Solnica-Krezel¹, Yinzi Liu¹, Diane Sepich¹, Ryan Gray²

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Adolescent idiopathic scoliosis (AIS) or late-onset scoliosis affects ~3% of the pediatric population, presenting with body curvature without overt structural defects of vertebral units. In some cases, AIS progressively worsens requiring long-term braces or surgery to mitigate the deformity. Despite this significant burden to the society, there is limited understanding of the genetic basis of AIS. We are aiming to identify genes involved in AIS by forward and reverse genetic approaches in zebrafish in collaboration with human genetic and genomic approaches. Having a well-annotated genome and plentiful, transparent progeny, zebrafish afford a powerful vertebrate model to study AIS by employing forward and reverse genetic approaches. Thus far, we analyzed 314 ENU-mutagenized genomes by screening over 36,000 individual fish, yielding 31 adult recessive scoliosis mutants, exhibiting whole body or more regionalized scoliotic curves. Complementation analysis of 17 mutations leading to whole-body scoliosis revealed at least 10 complementation groups. Using massively parallel sequencing to analyze the genome of one scoliotic mutant, we identified a non-synonymous mutation in the kinesin family member 6 (kif6) and adamts9 genes. We have recently initiated a continuation of this productive screen to define genes required for normal spine development in zebrafish. The scoliotic zebrafish mutant loci will become candidates in human genetic and genomic analyses. Towards validating candidate loci identified in human AIS patients and genomic studies, we generated indel mutations in the stat3 gene, as dominant autosomal STAT3 mutations account for numerous symptoms in Hyper-IgE syndrome patients such as misregulated TNFα levels and scoliosis. We found that neither maternal nor zygotic stat3 functions are essential for the completion of embryogenesis in zebrafish, but identified a transient requirement for stat3 in axis extension by promoting cell proliferation. However, stat3 mutants die during juvenile stages exhibiting scoliosis and excessive inflammation. As the scoliotic curves appear without any clear notochord or vertebral malformation, stat3 zebrafish mutants warrant evaluation as a model for AIS.
Search for Novel Biomarkers and Biotargets for Precision Medicine in Heart Disease

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Heart disease is still the #1 killer worldwide, and heart failure now kills 1 in 5 adults in Canada. There are multiple phenotypes of heart failure with distinct pathophysiology, yet we have few treatment options. There is a critical need for validated novel biomarkers to inform pathophysiology and tools for diagnosis and prognosis, and novel treatment targets for personalized therapy. Using the new Phenotypic Target Discovery approach, efficient functional analysis such as Zebrafish platform is critical. Taking advantage of high-throughput systems biology techniques such as proteomics with tandem mass spectrometry or aptamer chip analysis, we have a number of novel biomarker candidates for unique diagnoses and prognosis (Circ 2016). The data have yielded novel potential processes of (1) senescence, (2) matrix-remodeling cross talk, (3) innate immune activation (4) cell stress and injury and (5) metabolic dysregulation, etc.

For example, a novel heart failure target HACE1 is highly expressed in advanced heart failure, is a dual function E3 ubiquitin ligase, that is critically required for protein quality control in acute cardiac stress (Nature Comm 2015). Similarly, Tmem65 is a gap junction protein that regulates connexin 43 function during development and disease, using Zebrafish analysis (Nature Comm 2015). Finally, a recent discovery is IGFBP7, which regulates both IGF-1 and insulin receptor signaling. IGFBP7 is exquisitely increased in patients with heart failure with preserved ejection fraction, correlates with diastolic stiffness and senescence (Circ Heart Failure 2017). All of these are also potential targets for therapy, opening opportunities to improve the outcomes of heart failure.
Invited Speaker

Stephen Ekker, PhD.
Mayo Clinic, Rochester, USA

Stephen C. Ekker, Ph.D. is Professor of Biochemistry and Molecular Biology at the Mayo Clinic and an Adjunct Professor, University of Minnesota. Dr. Ekker has been conducting genome engineering for over 30 years with an emphasis on elucidating the mechanisms underlying health and disease. Ekker is President of the Genome Writers Guild Genome Engineering Society, Editor-in-Chief of the Zebrafish journal and Associate Director of the Clinical and Translational Sciences PhD program. Dr. Ekker is Co-Founder of three biotech companies and one non-profit. Dr. Ekker received bachelor of science degrees (Genetics and Developmental Biology, Electrical Engineering) from the University of Illinois where he conducted genome science work with Dr. Carl Woese. Dr. Ekker earned a PhD in Molecular Biology and Genetics at the Johns Hopkins University and Howard Hughes Medical Institute. Dr. Ekker was the founding Director of the Arnold and Mabel Beckman Center for Transposon Research (now called the Center for Genome Engineering) at the University of Minnesota. The Ekker laboratory has used a zebrafish-first approach to pioneer the use of diverse genome engineering tools including transposons, morpholino antisense oligonucleotides, and targeted genome editing methods including nuclear and mitochondrial programming in diverse organisms and human cells. Dr. Ekker also serves on the advisory boards of several biotechnology and pharmaceutical companies.

Engineering the Power House of the Cell

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We are in the midst of a scientific and technical revolution in genome engineering with regard to our ability to manipulate DNA. Gene editing is greatly enhancing the functionality and accessibility of genomic programming. This ‘overnight’ success has at its origins novel pioneering work from 1994 with the advent of the first artificial restriction enzyme. With new tools such as CRISPR/Cas9, gene editing reagents are cheaper than ever, 1000x lower today than they were as recently as 2011. This rapid pace of biotechnological innovation is empowering scientists and entrepreneurs as never before, enabling targeted knockouts in the nuclear genome as well as cellular programming with new and creative applications. In addition, new gene editing approaches are now showing promise in tackling the mitochondrial genome, our intracellular microbiome. Mitochondria, often known as the ‘powerhouse of the cell’, play many other critical roles that change with cell type, organ, disease and age. We have unlocked the potential for direct editing of mtDNA, making targeted deletions in single mtDNA genes and in contiguous genes that model common human variants. Using both zebrafish as an in vivo model and human cells for in vitro work, this new toolkit is enabling new mtDNA-based molecular modeling and setting the stage for targeted disease modeling in cells and animals. Knowledge of these tools, core exemplar applications, and opportunities for engineering health in addition to modeling disease will be essential for life scientists in the 21st century.
Session II: Mutagenesis & Disease Modeling
Chair: Dr. Stephen Ekker (Mayo Clinic, Rochester, USA)

Invited Lectures:

Functional dissection of pediatric genetic diseases
Dr. Erica Davis, Center for Human Disease Modeling, Duke University

Oral Presentations:

From Morphology to Math: Using whole zebrafish to create the engineering and computational infrastructure for Microanatomic Phenomics
Dr. Keith C. Cheng, Penn State College of Medicine, USA

Multiplexed CRISPR/Cas9-mediated knockout of nineteen Fanconi anemia pathway genes to understand their roles in disease pathophysiology
Dr. Raman Sood, National Human Genome Research Institute, National Institutes of Health, Bethesda, USA

Development of a dual-light assay system to identify genetic or epigenetic modifiers of the hypothalamic-pituitary-adrenal axis
Han B. Lee (PhD Candidate), Mayo Clinic Graduate School of Biomedical Sciences; Mayo Clinic, Rochester, USA

Invited Lectures:

Enhanced efficiencies using short regions of homology for precise DNA integration in zebrafish
Dr. Jeff Essner, PhD, Iowa State University

Oral Presentations:

Characterization of a new zebrafish model of finnish-type nephrotic syndrome
Dr. Weibin Zhou, University of Michigan, USA

Studying the distinct sensitivities of CFTR modulators using Zebrafish-CFTR
Dr. Onofrio Laselva (Postdoc), Programme in Molecular Medicine, Hospital for Sick Children, University of Toronto, Canada

A zebrafish model for small-fiber neuropathy
Ivo Eijkenboom (PhD candidate), Maastricht University Medical Centre, Maastricht, The Netherlands
Erica Davis is a human genetics and functional genomics researcher at Duke University Medical Center. Although she has a primary academic appointment as an Assistant Professor in the Department of Pediatrics-Division of Neonatology and a secondary appointment in the Department of Cell Biology, her intellectual home is the Duke University Center for Human Disease Modeling. Dr. Davis’ research interests include rare pediatric disorders such as the ciliopathies, craniofacial dysmorphisms, and complex congenital anomalies. She is interested in the interface between whole exome/genome sequencing and the functional annotation of genetic variation to answer two key questions: how can variation at the DNA level be functionally interpreted beyond the resolution of genetics arguments alone, and once empowered with such functional information, how can pathogenic alleles be mapped back to disease phenotypes to inform disease architecture and inform mechanism and therapeutic development. Her research in the Center involves the functionalization of human mutations using manipulation of physiologically relevant zebrafish models to determine allele pathogenicity and genetic epistasis. Along with colleagues both within the US and worldwide, she has collaborated in the publication of >70 original research papers in journals targeting the human genetics community. Dr. Davis is a fellow of the Belgian-American Educational Foundation, and a Ruth L. Kirschstein National Research Service Award recipient. She is a member of the American Society of Human Genetics, an ad hoc reviewer for several peer-reviewed journals, and she is funded by the U. S. National Institutes of Health.

Functional dissection of pediatric genetic disease

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The current ability to sequence whole exomes and genomes has reached an unprecedented pace. Variant data have been cataloged for over one million humans, representative of Mendelian disease cohorts, complex trait consortia, and healthy populations. This flood of information, expected to grow hyper-exponentially in the coming years, has already fueled the development of animal models to assign physiological relevance of genotype to phenotype; to inform variant pathogenicity; and to dissect multi-locus interactions. Here, we discuss our experience with the zebrafish model and its refined molecular toolkit to conduct scalable throughput analyses of novel disease genes implicated in rare pediatric disease in humans. We have placed particular emphasis on congenital anomalies in children impacting the structure of the brain, face, and kidney due to their direct anatomical surrogates in the developing zebrafish. Recent exemplars that we will highlight include: (1) the use of zebrafish to provide supporting data for rare human cases in which genetic evidence from population datasets is insufficient to support causality of novel disease genes; (2) application of zebrafish models to determine direction of allele effect for genes impacted by either copy number variants or missense mutations; and (3) validation of functional networks contributing to overlapping human phenotypes. In addition to the application of our work to inform human health, we will discuss our implementation of automated image acquisition platforms, as well as comparisons between transient and CRISPR/Cas9 zebrafish mutant models.

Key words: neurological, craniofacial, renal, human mutation
Keith Cheng, M.D., Ph.D., Penn State Distinguished Professor of Pathology, is a geneticist and pathologist serving as Director of Experimental Pathology at the Penn State Hershey College of Medicine, working in the Jake Gittlen Laboratories for Cancer Research. His interests include cancer, human skin color genetics, and phenomics. He received his B.A. in Biochemical Sciences from Harvard, M.D. from New York University School of Medicine, anatomic pathology residency training at Brigham & Women’s Hospital in Boston and University of Washington in Seattle, Ph.D. in molecular genetics, and postdoctoral fellowship at University of Washington until he joined Penn State in 1992. His 2005 cover story in Science describes how a zebrafish mutant led to the discovery of a coding mutation that played a key role in the evolution of European skin color. Current work involves finding of the equivalent gene in East Asians, the use of zebrafish in personalized medicine, high-throughput imaging for phenomics, and the building of web-based resources for scientists and the public. He spends his free time as a classical pianist.

From Morphology to Math: Using whole zebrafish to create the engineering and computational infrastructure for Microanatomic Phenomics

Author(s): Keith C. Cheng1,2, Yifu Ding1, Spencer R. Katz1, Alex Lin, Damian B. van Rossum1, and Daniel Vanselow1

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Medicine was brought into the modern era through histological studies of diseased tissue that revealed a profound insight: every major class of human disease can be qualitatively characterized by specific micron-scale changes in cellular and tissue architecture. The principles of cellular pathology need now to be applied to modern biomedicine. Unfortunately, the descriptive terms used in pathology are poorly suited for computational analysis, digital scans of glass sides are unable to result in accurate digital models of tissue, and mm-scale structures such as skeleton, vessels and nerve tracts require 3D scans of tissue. We have developed an isotropic, 3-dimensional approach that, like histology, includes all cell types and can be used to create accurate digital tissue models. Pancellular Tissue Tomography (PANCETTO) is based on the use of rotational X-ray projections of fixed and stained tissue to compute 3D volumes. To enable applications of PANCETTO, we are developing higher-throughput implementations of the imaging hardware, and have begun to develop computational tools for comprehensively defining tissue microanatomy and tissue change. Zebrafish’s dimensions and full range of tissues have allowed us to generate whole-organism 3D images for digital histopathological analysis. Due to the large file sizes (~100GB/fish) associated with these images, we have also created a web-based foundation for data sharing, and begun to compute analytical models of tissue that allow undistorted whole-animal visualizations that take up less digital space. We see success in this grand analytical challenge to yield an achievable foundation for computational, high-throughput and comprehensive morphometric organismal and tissue phenotyping.

Key words: Phenomics, microCT, Computational Microanatomy
**Raman Sood, MD., PhD., Director, Zebrafish Core Facility**

National Human Genome Research Institute, National Institutes of Health, Bethesda, USA

Dr. Sood is a staff scientist and Director of the Zebrafish Core at National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda. Dr. Sood received her MSc (Hons) in Biology from Guru Nanak Dev University in India, PhD from Queen’s University in Kingston, Canada, and postdoctoral training in human genetics and molecular biology from Hospital for sick children in Toronto and National Institutes of Health in Bethesda. Dr. Sood’s research is aimed at understanding the molecular basis of human genetic diseases. In 2005, Dr. Sood started the NHGRI Zebrafish Core to provide its investigators with resources and services to perform functional analysis of genes and disease modeling in zebrafish. Dr. Sood’s laboratory has developed several protocols for robust, high throughput and inexpensive methods to generate loss of function mutations in zebrafish using CRISPR/Cas9. Currently, Dr. Sood is combining her expertise in genetics, genomics and the use of zebrafish model to understand the genetic regulation of hematopoiesis and how its mis-regulation causes leukemia.

**Multiplexed CRISPR/Cas9-mediated knockout of nineteen Fanconi anemia pathway genes to understand their roles in disease pathophysiology**

**Author(s):** Raman Sood¹, Blake Carrington¹, Ramanagouda Ramanagoudr Bhojappa², Gabrielle Robbins¹, Danielle Kimble², MaryPat Jones², Settara Chandrasekharappa²

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Fanconi Anemia (FA) is a rare genetic disorder characterized by developmental abnormalities, an increased incidence of bone marrow failure and predisposition to hematological and other cancers. To date, mutations in 21 genes are known to cause FA. Homologs of all genes, except FANCS/BRCA1 have been identified in zebrafish, thus providing a vertebrate model to explore their roles in FA pathophysiology. We generated 36 frameshift mutant lines for 19 FA pathway genes by multiplexed CRISPR/Cas9 mutagenesis. First, we performed RT-PCR to confirm that all selected indel mutations cause a loss of function as predicted. Two mutations led to splicing defects that were not predicted from the genomic sequences, emphasizing the importance of RT-PCR to interpret the nature of the mutant protein. Next, we performed phenotypic analyses of all knockout fish. Our data showed that homozygous null fish for the FA pathway genes undergo normal embryonic development and survive to adulthood. fancp⁻/⁻ fish exhibited delayed growth during the first three months. As gametogenesis defects have been noted in animal models of some FA genes, we performed detailed analysis of sex ratios and fertility of the surviving homozygous nulls. In several nulls, female-to-male sex reversal was noted. Interestingly, we observed different phenotypes in two lines obtained from the same sgRNA for fancj. We are in the process of determining the underlying reason for this discrepancy. An off-target closely linked mutation maybe the cause for one of the phenotypes. Thus, our data provide a couple of cautionary tales in using CRISPR/Cas9 mutagenesis.

**Key words:** CRISPR/Cas9, Fanconi anemia, Disease modeling, multiplexing
Han B. Lee, (PhD candidate)
Neurobiology of Disease program, Mayo Clinic Graduate School of Biomedical Sciences, Rochester, USA

Han is a 5th-year graduate student in Dr. Karl Clark’s laboratory. Under Dr. Clark’s guidance, Han’s thesis research has focused on the involvement of the hypothalamic-pituitary-adrenal (HPA) axis in rapid behavioral response to various stressors. Han has developed an efficient assay system that enables the characterization of rapid locomotor response to hyperosmotic stress and abrupt light change stress. Using this assay system, Han demonstrated that adrenocorticotropic hormone (ACTH) receptor and glucocorticoid receptor are required for the rapid systemic stress response in larval zebrafish. This sensitive assay system may be used to test patients’ variants in zebrafish models for behavioral phenotypes, as well as to screen for genetic or epigenetic modifiers of the HPA axis.

Han has been productive, publishing 2 research papers as the first author, 6 research papers as a co-author, and 2 review papers as the first author during his Ph.D. study. He just submitted a paper and is defending his thesis in November this year. Han is interested in behavioral neurogenetics and looking for a post-doctoral position in academia or industry to start sometime in 2018.

Development of a dual-light assay system to identify genetic or epigenetic modifiers of the hypothalamic-pituitary-adrenal axis

Author(s): Han B. Lee, Tanya L. Schwab, Ashley N. Sigafoos, Jennifer L. Gauerke, Randall G. Krug, II, MaKayla R. Serres, Dakota C. Jacobs, Biswadeep Das, Morgan O. Petersen, Camden L. Daby, Rhianna M. Urban, Bethany C. Berry, Karl J. Clark

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The hypothalamic-pituitary-adrenal (HPA) axis enables rapid adaptive behavioral changes by secreting cortisol in response to stressors. Alterations in HPA axis activity leading to hyper- or hypo-cortisolemia are one of the most common pathophysiological changes in people with depression. Due to complex gene-environment interactions, identifying pathways/molecules that modulate HPA axis activity remains challenging. Zebrafish respond to acute stressors with rapidly increased locomotion within 2-3 minutes. In our dual-light assay, larval zebrafish are acclimated in infrared light, in which zebrafish cannot see. After a brief exposure to white light for 45 seconds, locomotion of the fish are recorded for 30 min in infrared. We hypothesized that abrupt light changes are a stressor and increased locomotion is a HPA-axis-dependent stress response. We aimed at developing a sensitive assay to screen for genetic or epigenetic modifiers of the HPA axis. We have confirmed our hypothesis through biochemical, genetic, and pharmacological experiments. Increased whole-body cortisol levels are correlated with increased locomotor response. We generated 6 frameshift mutants that have blocked HPA axis activity: Two frameshift mutants in exon 2 of mc2r (adrenocorticotropic hormone receptor) and two in each of exon 2 and exon 5 of nr3c1 (glucocorticoid receptor). Homozygous mutant fish in mc2r or nr3c1 loci show a significantly decreased locomotor response to light changes. When we treated wild-type fish with anxiolytic drugs, the locomotor response is significantly attenuated. These experiments demonstrate that locomotor response to light changes is HPA-axis dependent.

Key words: Stress response, HPA axis, glucocorticoids, glucocorticoid receptor, psychiatric disorders
Invited Speaker

Jeffrey J. Essner, PhD.
Department of Genetics, Development and Cell Biology, Iowa State University, USA

Dr. Essner received his Ph.D. from the University of Minnesota with Dr. Perry Hackett, where he began using the zebrafish model system to understand the cellular roles of proto-oncogenes. He did postdoctoral training at the Scripps Research Institute in La Jolla with Dr. Gerald Edelman followed by a research faculty appointment at the Huntsman Cancer Institute at the University of Utah with Dr. Joseph Yost. Prior to joining the faculty of the Genetics, Development and Cell Biology Department at Iowa State University in 2005, Dr. Essner was the Scientific Director at Discovery Genomics, Inc., a biotech company that uses zebrafish for high-throughput analysis of gene function. At Iowa State University, Dr. Essner has developed methods for targeted mutagenesis using TALENs and CRISPR/Cas9 for modeling human disease in zebrafish with a focus on angiogenesis and cancer progression.

Enhanced efficiencies using short regions of homology for precise DNA integration in zebrafish

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Several methods have been published that utilize homology directed repair for creating knock-ins in zebrafish at sites of directed double-strand breaks. However, the frequencies of precise integrations using these methods are relatively low, which makes recovery of desired events difficult. Here, we extend the method first published by Hisano et al., 2015 and present a homology arm design web interface that provides a streamlined approach to creating precise knock-ins using short-homologies and two associated vector suites: pGTAG, plasmids for precise Gene TAGging for vector-free, multicolor tagging; and pPRISM, plasmids for PRecise Integration with Secondary Markers to drive precise integration with multiple options for secondary fluorescent reporters at the tagged site. We generated an optimal stability universal gRNA (UgRNA), with no predicted off targets in zebrafish and other vertebrates, that efficiently targets our plasmid donor vectors at sites flanking cargo DNA, thus exposing homology engineered to match the sequence flanking a genomic double-strand DNA break. Our work demonstrates that in vivo targeting of a genomic locus of interest with CRISPR/Cas9 and a donor vector containing as little as 48 base pairs of homology directs precise knock-in with little mosaicism in up to 81% of injected animals. Up to 64% of F0 animals transmit tagged alleles, and Southern Blot and junction fragment analysis on F1s demonstrates precise, vector backbone free integration. We expect our results and the accompanying vectors, protocols and web interface will serve to streamline experimental design and broaden the use of designer nucleases for homology-based gene editing.
Weibin Zhou, PhD., Assistant Professor
Department of Pediatric Nephrology, University of Michigan Medical School, USA

Dr. Zhou obtained his Master of Science degree in Biochemistry & Molecular Biology from Fudan University, China and his PhD in Molecular, Cellular & Developmental Biology from the University of Michigan. Dr. Zhou is currently an Assistant Professor in Pediatric Nephrology at the University of Michigan Medical School. His research focuses on the zebrafish model of pediatric kidney diseases, including nephrotic syndrome and nephronophthisis.

Characterization of a New Zebrafish Model of Finnish-type Nephrotic Syndrome

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Nephrotic syndrome (NS) is a chronic kidney disease characterized by proteinuria due to defective glomerular filtration barrier (GFB). The human NPHS1 gene is mutated in nephrotic syndrome (Finnish-type) and encodes NEPHRIN, a protein of the interpodocyte-spanning slit diaphragm essential for the normal GFB. We have generated a new zebrafish model of nphs1 using CRISPR and established two deletion mutant lines that lack nphs1 protein in the kidney. Different from a previous report that zebrafish morphants of nphs1 displayed pericardial edema at 4 days post fertilization (dpf), the homozygous mutants showed no morphological abnormality until 6 dpf, when periorbital edema (POE) (mimicking the symptom in children with NS) emerged. The edema became progressively severe, concurrent with the deterioration of the GFB, and led to whole-body edema and lethality within two weeks of age. Although the GFB matures at approximate 3.5 dpf, transmission electron microscopy showed slit diaphragm was not defective in the nphs1 mutants until after 5 dpf. Our study has revealed novel nephrotic phenotypes in zebrafish and argues that nephrin is not essential for the initial assembly of the slit diaphragm but required for the long-term maintenance of the glomerular filtration barrier. The zebrafish nphs1 mutants avoid the possible off-target effect by morpholino-mediated knockdown and thus produce a more reliable and convincing nephrotic phenotype consistent with that of the zebrafish model of inducible podocyte injury previously published by us. Our new zebrafish model is potentially a useful platform for personalized medicine for individual pathogenic alleles of NEPHRIN.

Key words: nephrin, podocyte, proteinuria, kidney, CRISPR
Dr. Onofrio Laselva is a postdoctoral fellow in Dr. Christine Bear’s group at the Hospital for Sick Children, Toronto. He received his B.Sc and M.Sc in Biomedical Science from the University of Bari, Italy. Laselva completed his PhD in Physiology at the University of Bari (Italy) under the supervision of Dr. Valeria Casavola, where he specialized in physiology and pharmacology. His research program combines physiology, biochemistry and molecular biology approaches to screening CFTR modulators to identify novel therapeutic compounds for Cystic Fibrosis (CF) patients. Recently, his research interests include zebrafish modelling, for use in high-throughput CFTR modulators screens, to discover new compounds that can potentially be used as future CF therapies, and to understand the mechanism of action for these novel compounds. To this end, he has generated Wt-zeCFTR and the most common CF mutation (F507del-zeCFTR) cDNA and transfected in HEK-293 cells, which is now being used to screen and identify small molecules that potentiate and/or correct CFTR-dependent chloride efflux.

Studying the distinct sensitivities of CFTR modulators using Zebrafish-CFTR

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Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is an anion channel in the ATP-Binding Cassette (ABC) transporter family. Recently, the structure of zebrafish CFTR was determined and demonstrated that Human and Zebrafish CFTR share 55% sequence homology (Zhang, Cell, 2016). The loss of CFTR function in zebrafish leads to pancreatic destruction, a pathology similar to pancreatic insufficient cystic fibrosis in humans (Navis., Developmental Biology, 2015). We were prompted to study the regulation of zCFTR channel activity and the consequences of the major CF disease causing mutation. We expressed Wt-zeCFTR and the most common CF mutation (ΔF508), which is ΔF507 in zebrafish. We found that ΔF507-zeCFTR shares similar protein misfolding characteristics as ΔF508-hCFTR, although it exhibits partial processing to the mature, band C form of the protein when expressed in HEK-293 cells. The cAMP-dependent conductance conferred by ΔF507-zeCFTR was severely impaired relative to Wt-zeCFTR. Surprisingly, the approved therapeutic combination (corrector VX-809 plus VX-770) is less effective in Zebrafish than in Human. The new structural information will permit a molecular understanding of differential sensitivities to therapies. As Zebrafish bearing mutant CFTR exhibit a pancreatic phenotype, we plan to develop novel drug screens for new compounds that will form templates for novel CF therapies.

Key words: Cystic Fibrosis, ΔF507-zebrafish-CFTR, CFTR, correctors screening, potentiation screening
Ivo Eijkenboom received his MSc degree in Biomedical Sciences from the University of Maastricht and his BASc from Zuyd University in Heerlen. He started in 2014 as a PhD student in the department of Clinical Genetics at Maastricht University. His PhD project is funded by the European Union Seventh Framework Program (FP7) and is part of an international consortium (Propane study) that is unravelling the genetic architecture of small-fiber neuropathy. The main focus of his research is to model small-fiber neuropathy (SFN) in zebrafish. This allows him to study the pathogenicity of novel genetic variants identified in patients with SFN and test the efficacy of novel sodium channel blockers. In order to do this he set up a read-out panel, consisting of a customized Zebabox system, mimicking important clinical hallmarks of SFN. Next to his appointment as a PhD student he is also working as an assistant Zebrafish Facility Manager in the lab of dr. Jo Vanoeveren, where they model metabolic, cardiovascular and neurological diseases in the zebrafish.

**A zebrafish model for small-fiber neuropathy**

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Small-fiber neuropathy (SFN) patients experience a spectrum of painful sensations; they have aberrant temperature responses and in nerve biopsies the density of the small-fibers is decreased. Gain-of-function mutations in SCN9A and SCN10A, have been identified as an underlying genetic cause. To further unravel the genetic aspects of SFN, members of our Consortium (Propane) apply unbiased sequencing approaches to identify genetic variants in a large patient population with SFN. Our aim is to develop a zebrafish model of SFN which allows us to test the pathogenicity of these identified variants; in order to do this we set up and validated a panel of read-out parameters reflecting SFN in zebrafish. Our read-out panel is based on clinical-diagnostic tests and exists of behavioral tests and morphological characteristics. For the behavioral experiments a customized ZebraBox has been established which allows us to quantify the temperature response. A zebrafish line called; sensory:GFP is being used as morphological read-out. Validation of this panel has been performed using various methods (overexpression, morpholino-mediated knockdown and a knockout line). By applying this panel we have demonstrated that expressing the human pathogenic SCN9A p.I228M mutation in zebrafish results in an aberrant temperature sensitivity. Furthermore, these zebrafish have a decreased density of the small nerve fibers, meaning that we have created a zebrafish model of SFN. Our next step is to test the pathogenicity of variants that are identified within our SFN patient population. Furthermore, this panel will be used to test the analgesic properties of novel compounds.

**Key words:** zebrafish, small-fiber neuropathy model, read-out panel, SCN9A, pathogenic mutations
3rd Zebrafish for Personalized and Precision Medicine Conference

Thursday, September 28th, 2017 (1:30pm – 4:50pm)

Session III: Mechanisms of Development, Organ Dysfunction & Disease
Chair: Dr. Oliver Bandmann (University of Sheffield, UK)

Invited Lecture:
A regulatory pathway leading to joint formation and maintenance in Zebrafish fin regenerates
Dr. Marie-Andrée Akimenko, University of Ottawa, Canada

Oral Presentations:
Disease Mechanism Discovery:Iteration among a Biobank, Zebrafish, and Electronic Health Records
Dr. Ela Knapik, Vanderbilt Genetics Institute, VUMC, Nashville, TN, USA

In vivo dynamics of muscle-associated cells during zebrafish muscle regeneration
Dr. Peng Huang, University of Calgary, Calgary, Alberta, Canada

Temporal and spatial post-transcriptional regulation of zebrafish tie-1 mRNA by long non-coding RNA
Dr. Tamjid Chowdhury (Postdoc), Medical College of Wisconsin, Milwaukee, USA

Invited Lecture:
Telomeres, telomerase and inflammation in zebrafish ageing
Dr. Catarina Henriques, University of Sheffield, UK

Oral Presentations:
Rhythmically expressed ezh2 promotes clock function and hematopoiesis independent of histone methyltransferase activityin zebrafish
Dr. Han Wang, Center for Circadian Clocks, Soochow University, China

The zebrafish taste sensory cells: a screening tool for harmful chemicals and compounds enhancing regeneration
Dr. Marika Kapsimali, INSERM, IBENS, Paris, France

Epigenetic Control of Zebrafish Cardiogenesis by TET2/3
Dr. Yahui Lan (Postdoc), Weill Cornell Medical College, New York, USA

Flash Talks (1 slide, 1 min/person) Chair: Dr. Ian Scott (The Hospital for Sick Children & UofT)
- John Dawson, University of Guelph, Canada (P2-8)
- Michèle G. DuVal, University of Alberta, Canada (P2-9)
- Suzan El-Rass, ZCADD, St. Michael’s Hospital, U of T, Canada (P2-10)
- Izabella A. Pena, Children’s Hospital of Eastern Ontario Research Institute, Ottawa, Canada (P2-21)
- Jana Pfeiffer, University of Münster, Germany (P2-22)
- Éric Samarut, CRCHUM, University of Montréal, Canada (P2-24)
- Heejin Lee, Weill Cornell medical College, New York, USA (P3-34)
- Ryan Thummel, Wayne State University School of Medicine, USA (P3-38)
- Daniel Zuppo, University of Pittsburgh, USA (P3-44)
- Nun Ribeiro Palha, Institut de Recherches Servier, Paris, France (P5-46)
- Hyunjin Jeong, University of Toronto, Canada (P5-47)
- Junghwa Yun, ZCADD, St. Michael’s Hospital, U of T, Canada (P5-55)
Dr. Marie-Andrée Akimenko’s research focuses on the genetic control of fin development and regeneration in zebrafish. More specifically, her research program involves the comparative analysis of fin and embryonic limb development and the molecular basis of the evolutionary fin-to-limb transition. The regeneration aspect of her research program is focused on the analysis of the molecular mechanisms of patterning of intramembranous bone that make up the fin rays. In collaboration with Dr. K. Boycott at the Children Hospital of Eastern Ontario, Ottawa, her laboratory is also generating zebrafish models for the analysis of rare genetic diseases.

A regulatory pathway leading to joint formation and maintenance in zebrafish fin regenerates

Author(s): Stephanie McMillan, Jing Zhang, Eileen-Hue Phan, Derek Sheppard, Shirine Jeradi, Matthias Hammerschmidt and Marie-Andrée Akimenko

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Zebrafish fin rays are dermal bones formed by the deposition of bone matrix secreted by osteoblasts. They are composed of bone segments separated by joints. During growth or regeneration, new bone segments are periodically added to the end of the rays. How osteoblasts are spatially and temporally organized to generate the segment-joint pattern remains unclear. We characterized joint cell origin and differentiation during fin regeneration. During regeneration, a morphologically distinct cell cluster emerges from cells of the osteoblast lineage at joint forming positions. Gene expression analysis of various osteoblast differentiation markers suggests that osteoblasts and joint cells originate from a common cell lineage, but are subsequently committed to different fates. The presumptive joint cells are specified at regular intervals in the distal domain of expression of early osteoblast differentiation markers (runx2a/2b). They progressively differentiate into mature joint cells. This specification correlates with the sequential activation of hoxa13a, evx1, and pthlha in these presumptive joint cells. Using evx1 null mutant analysis and pharmacological treatments, we could define a regulatory pathway, involving these three joint markers, essential for joint formation and maintenance in regenerating fins. A potential co-option of the original mechanisms, that pattern the fin ray bone segments/joints, in the patterning of joints in tetrapod digits will be discussed.

Key words: fin; regeneration; bone; joint; patterning
**Invited Speaker**

**Ela Knapik, MD., Associate Professor**  
*Medicine and Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, USA*

Dr. Ela Knapik is an Associate Professor of Medicine and Cell and Developmental Biology at Vanderbilt University Medical Center and a founding Member of the Vanderbilt Genetics Institute where she uses molecular genetics and cell biology approaches to study pathophysiology of common and rare human syndromes. She received her M.D. degree from the Jagiellonian University, Cracow, Poland, and trained at Max Planck Institute for Biophysical Chemistry, Goettingen, Germany with Dr. Gruss. As a postdoctoral fellow at MGH, Harvard Medical School, Boston, MA she worked to develop genomics resources for the zebrafish genome including the first complete genetic linkage map. In her own laboratory at the National Research Center for Health and Environment in Munich, Germany and the University of Freiburg, and since 2004, at Vanderbilt University and the VU Medical Center, Dr. Knapik has focused on transcriptional regulation of neural crest migration and craniofacial differentiation. Her laboratory’s work on genetic variants in protein transport and secretion machinery helped explain the pathophysiology of human syndromic Osteogenesis Imperfecta and Craniolenticular-sutural dysplasia. Current studies include pathophysiology of adult onset ocular diseases and comorbidities associated with neuropsychiatric disorders. The laboratory has been supported by NIDCR, NIMH, AHA and institutional funds.

**Disease Mechanism Discovery: Iteration among a Biobank, Zebrafish, and Electronic Health Records**

**Author(s):** Gokhan Unlu, Eric Gamazon, Ela Knapik, Nancy J. Cox  
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Recent years have seen development of new methods in both gene editing and genome data integration, including integration of transcript levels across human tissues (e.g. PrediXcan) with genome variation, and the increasing use of electronic health records (EHR) linked to biobanks for discovery research. We illustrate how these new methods can facilitate development of a hypothesis for a primary disease mechanism by rapid iteration from initial discovery of a gene (GRIK5) implicated in diverse eye diseases in BioVU, (the biobank at Vanderbilt University with > 240,000 DNA samples) to validation of the finding in zebrafish, revealing early vascular deficiency. Continued iteration of biobank analysis in larger sample sizes provides support for the vascular mechanism (associations to congenital anomalies of the vascular system and to other vascular phenotypes), and iteration to the Synthetic Derivative (de-identified image of the EHR for 2.6 million VUMC subjects) confirmed that subjects with congenital anomalies of the vascular system have a 2-3 fold increased risk of the eye diseases driving the initial discovery of this gene. Simultaneously, fluorescein angiography in grik5 zebrafish model showed increased vascular permeability. Thus, our study models a novel approach for discovery of gene to phenotype relationships that allows a rapid iteration to the primary genetic mechanisms underlying the pathophysiology of human disease.

**Key words:** Electronic Health Records, vascular disease
Peng Huang is an Assistant Professor in the Department of Biochemistry and Molecular Biology at the University of Calgary. He received his BSc degree in Biochemistry and Molecular Biology from Beijing University, China. He then obtained his PhD in Genetics with Dr. Michael Stern at Yale University studying FGF signalling in *C. elegans*. After PhD, he joined Dr. Alexander Schier’s laboratory as a postdoctoral fellow at Harvard University studying primary cilia and Hedgehog signalling in spinal cord patterning in zebrafish. The Huang lab continues to use zebrafish as a model system to address two main questions: 1) how interactions of different cell signaling pathways drive precise pattern formation in the spinal cord; and 2) how muscle-associated cells regulate muscle regeneration and degeneration. Dr. Huang’s research is generously supported by grants from CIHR, NSERC and CFI.

**In vivo dynamics of muscle-associated cells during zebrafish muscle regeneration**

**Author(s):** Priyanka Sharma and Peng Huang

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Skeletal muscles control many essential functions that our bodies constantly perform such as walking, eating and breathing. Defects in muscle function, for instance muscular dystrophy, have profound consequences. Despite extensive studies of muscles, relatively little is known about how muscle-associated cells modulate muscle function. Recently, several types of non-muscle cells have been discovered to interact closely with muscle fibers and play important roles in muscle regeneration and degeneration. We utilize zebrafish as a model system to study the in vivo dynamics and functions of muscle-associated cells. First, we identified several extracellular matrix (ECM) genes as key markers of muscle-associated cells. Using one of the markers (col1a2), we developed a transgenic line that allows us to perform single cell tracing and time-lapse imaging. In particular, we focused on a population of myogenic progenitor cells localized in the superficial layer external to muscle fibers. With genetic lineage tracing, we showed that col1a2+ external cell layer gives rise to new muscle fibers during normal larval development. In the condition of muscle injury, col1a2+ cells quickly change from the resting ramified morphology to a characteristic polarized and elongated morphology, and contribute to generation of new muscle fibers within 2 days. We are currently investigating the requirement of col1a2+ cells in muscle regeneration by genetic ablation experiments. Altogether, our study will provide important insights into the complex process of muscle regeneration.

**Key words:** Skeletal muscle, regeneration, muscle-associated cells, muscle progenitor cells
I am an RNA biologist who is fascinated with zebrafish as a model system. I obtained a Ph.D. in Molecular, Cellular and Organismal Biology from the University of Massachusetts – Boston in 2014 studying translational control in spermatogenesis. Presently, I am a post-doctoral fellow in the laboratory of Dr. Ramani Ramchandran at the Medical College of Wisconsin where I study the role of long non-coding RNA (lncRNA) in blood vessel development. Dr. Ramchandran’s laboratory was one of the first to identify a functional lncRNA in vascular biology. We identified lncRNAs from the Tyrosine kinase containing Immunoglobulin and Epidermal growth factor homology-1 (tie1) locus in zebrafish, humans and mice. Further, two of the lncRNAs originating from the tie1 locus, tie1 antisense (tie1AS), were shown to regulate tie1 mRNA in zebrafish embryo and human endothelial cells. However, the mechanism of the lncRNA mediated regulation of tie1 mRNA was not identified. My present research uses biochemical and genetic approaches to demonstrate that the zebrafish tie1 mRNA is regulated at post-transcriptional level in a temporal and spatial manner through an RNA - protein complex formed between the tie1AS lncRNA and a well conserved RNA binding protein called Embryonic Lethal and Abnormal Vision Drosophila like-1 (Elav11). We hypothesize that this RNA - protein complex recruits additional proteins to facilitate the regulation of tie1 mRNA.

Temporal and spatial post-transcriptional regulation of zebrafish tie-1 mRNA by long non-coding RNA

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Tyrosine kinase containing Immunoglobulin and Epidermal growth factor homology-1 (Tie1), an endothelial and hematopoietic cell specific receptor tyrosine kinase, is an important regulator of angiogenesis. Tie1 is differentially expressed across vascular beds and shows context-dependent functions. The mechanism(s) leading to differential expression of Tie1 is unknown. Our lab has previously reported the identification of natural antisense transcripts from the tie1 locus, tie1 antisense (tie1AS), that regulate tie1 mRNA levels in zebrafish and human endothelial cells. These natural antisense transcripts are considered to be long non-coding RNA (lncRNA). We have previously shown that an 804 nucleotides long zebrafish tie1AS lncRNA forms hybrid duplex with tie1 mRNA that causes the degradation of tie1 mRNA. Here, we elucidate on the molecular mechanism used by zebrafish tie1AS to regulate tie1 mRNA levels in the cytoplasm. We identified that the zebrafish tie1AS lncRNA interacts with the protein Embryonic Lethal and Abnormal Vision Drosophila like-1 (Elav11) in-vitro and in-vivo. We identified a 10 nt AU rich region in tie1AS responsible for binding Elav1. Disrupting the interaction between tie1AS and Elav11 via constitutively active anti-sense morpholino oligonucleotides (MOs) or photoactivatable MOs resulted in increased tie1 mRNA levels, along with distended and irregularly shaped blood vessels and an increase in endothelial cell numbers, all effects observed primarily in the head during a small window of time. This phenotype was phenocopied by CRISPRi-mediated knockdown of tie1AS. Our results suggest a novel mode of lncRNA mediated spatial and temporal post-transcriptional regulation of tie1 mRNA which is critical for development.

Key words: long non-coding RNA, tie1, elav11, vascular
I started my independent research group at the University of Sheffield, UK, in 2015, investigating tissue repair and immunity in ageing, using zebrafish as a model. Ageing and, in particular, the role of telomeres in ageing, has been a long-term research passion. However, I did my PhD in cancer immunology, in particular studying the role of IL-7 and its receptor regulation in human leukaemia [1, 2]

My PhD exposed me to the wider field of ageing and cancer, and the controversies surrounding the role of telomeres. While human studies show a clear link between telomere shortening and ageing, including immune ageing, inbred mouse strains show no clear phenotypes upon telomerase deletion. It was therefore critical for the ageing field to identify complementary vertebrate models that, like humans, depend on telomerase for health- and life-span. This motivated my post-doc when I spearheaded the setting up of zebrafish as a model organism in Dr. Miguel Ferreira’s lab at the Gulbenkian Institute, PT. We were the first in the world to establish the telomerase mutant zebrafish as a new telomerase-dependent vertebrate ageing model[3, 4].


Telomeres, telomerase and inflammation in zebrafish ageing

Author(s): Pam S. Ellis1,2, Raquel R. Martins1,2, Asma Farhat1,2, Emily J. Thomson1,2, Bernard Corfe1, Ilaria Bellantuono1,4, Stephen A. Renshaw2,3 and Catarina M. Henriques1

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Telomerase, the enzyme capable of elongating telomeres, is mostly restricted in somatic cells, leading to telomere shortening with each cell division. Adaptive immune cells are exceptions and can up-regulate telomerase, which is generally thought to serve the proliferation requirements of these cells. We now show that gut-associated leukocytes have much longer telomeres than surrounding somatic cells, independently of their proliferative status, and that this is telomerase-dependent. In particular, hyper-long telomere cells include both adaptive and innate leukocytes, with most of them being phagocytic macrophages and B-cells. Importantly, gut-associated leukocytes accumulate DNA damage and die prematurely in the absence of telomerase, which is accompanied by a decrease in phagocytic function. Accordingly, we show that that these cells maintain telomerase expression, but telomerase expression insufficient to prevent telomere shortening in gut leukocytes with natural ageing. This is likely to impact not only on clearance of pathogens but also of debris and senescent cells, likely contributing to chronic inflammation in ageing. Our work therefore highlights a new role for telomerase in regulating gut immunity, with potential implications for tissue homeostasis in ageing. Finally, we found that human gut-associated leukocytes also have hyper-long telomeres, highlighting this as a potentially conserved mechanism.

Key words: Telomerase, telomeres, senescence, gut, immunesurveillance
Han Wang, PhD, Professor and Director of Soochow University Center for Circadian Clock (SUCC), Suzhou, China. Han received PhD degree from Wayne State University, Detroit, Michigan, did postdoctoral trainings with Shuo Lin at Medical College of Georgia, Augusta, Georgia and John Postlethwait at University of Oregon, Eugene, Oregon. His group has been working on molecular genetic mechanisms underlying circadian clocks and sleep as well as establishing zebrafish models for blood diseases and psychiatric disorders.

Rhythmically expressed ezh2 promotes clock function and hematopoiesis independent of histone methyltransferase activity in zebrafish

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Histone methyltransferase Enhancer of zeste homolog 2 (EZH2) was shown to be essential for mammalian liver circadian regulation and hematopoiesis. Its roles in circadian regulation in live animals or during hematopoiesis, however, are far from certain due to embryonic lethality of Ezh2 knockout mouse. Here we show that zebrafish ezh2 is regulated directly by the circadian clock. We observed down-regulation of core circadian clock genes in ezh2 mutant zebrafish and either knock down or overexpression of Ezh2 alters locomotor rhythms of zebrafish larvae. We revealed that Ezh2 plays critical roles in circadian regulation by directly binding to key circadian clock proteins occupying the E-box containing promoter region and Ezh2 enhances clock function independent of its histone methyltransferase activity. The defects in primitive and definitive hematopoiesis in ezh2 mutant fish are resulted from deregulation of hematopoietic genes, such as cmyb and lck. Our findings demonstrate that zebrafish Ezh2 is essential for circadian regulation and acts as a nexus for circadian regulation of hematopoiesis.

Key words: Ezh2, hematopoiesis, circadian clock, locomotor activity, zebrafish
During my PhD, I did a phylogenetic, comparative neuroanatomical analysis of the dopaminergic systems in the brain of Vertebrates (mentor Philippe Vernier, at the CNRS campus in Gif-sur-Yvette, France). My 1st postdoc focused on the zebrafish forebrain patterning and microRNA expression in the zebrafish CNS in Steve Wilson’s lab at UCL, London. Then I started working on the development of the taste system, initially as a postdoc in Frederic Rosa’s lab and then as an independent INSERM researcher at the Institute of Biology of the Ecole Normale Superieure (IBENS), in Paris, France.

Our work focuses on understanding the functional mechanisms underlying the formation of the taste sensory organ circuit. We have identified Fgf, Notch and miR-200 as key players in the differentiation process of taste bud cells (Development 2011). We have also shown that forming taste bud cells show diversity in their motility profiles within the epithelium, being displaced in a random, confined or directed mode (Development 2016). The study of these rather simple, small sensory organs may contribute in understanding how larger neuronal circuits are formed. Currently we examine how these organs regenerate and we aim at using them as environmental sensors.

The zebrafish taste sensory cells: a screening tool for harmful chemicals and compounds enhancing regeneration

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The taste buds are sensory organs, located superficially in the zebrafish oropharynx, composed of a variety of cell types including those detecting bitter, sour and potentially harmful compounds. These cells are remarkably frequently renewed in mammals including humans, yet the sense of taste is maintained stable in healthy individuals. They also consist targets of chemotherapeutic agents resulting in dysfunctional taste sensitivity and abnormal nutritional behaviour in cancer patients following chemotherapy. We have recently analysed genetic interactions required for the formation of distinct cell types (1) and cell motility behaviour (2) that underlies cell assembly into taste sensory organs. In particular, we have shown that timely co-ordinated signaling of Fgf, mir-200 and Notch, and Ascl1a, are required for the formation of taste receptor (bitter/umami sensing) and sour sensing cells, respectively. In addition, via in vivo imaging time-lapse and 2-photon laser cell ablation, we have shown that differentiating taste cells are displaced through random, confined or directed motility to each other, the latter being a new type of cell displacement, termed 'slithering'. We aim at using these organs as screening tools a) that alert for potentially harmful chemicals in the human environment b) for compounds that potentiate or accelerate cell regeneration after chemotherapy. We have started genetically modifying these sensory cells in zebrafish and our project and work progress will be discussed in the meeting.


Key words: sensory organ, cell behaviour, migration, chemotherapy, regeneration
Yahui Lan is a postdoc in the Department of Surgery of Weill Cornell Medical College. She received a B.S. degree in Biological Science from the University of Science and Technology of China and Ph.D in Life Science Department from the Hong Kong University of Science and Technology, working on hematopoietic stem cell development and immune-neuro interaction in zebrafish.

In 2014, she joined Dr. Todd Evans laboratory in Weill Cornell Medical College to explore epigenetic control in normal development as well as human congenital and acquired disease, using two primary model systems, human pluripotent stem cells and the zebrafish. In particular, Lan studies the function of TET enzymes, which convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and promote DNA demethylation, during organ development. Collaborating with Dr. Mary Goll’s group, she has described the overlapping requirement for Tet2 and Tet3 in zebrafish hematopoietic stem cell emergence. Combined zebrafish model with TET mutant hESC lines, she is working on the function of TETs in cardiac progenitors specification and epicardium migration.

**Epigenetic Control of Zebrafish Cardiogenesis by TET2/3**

**Author(s):** Yahui Lan¹, Cheng Li²,³, Kelly Madigan Banks¹, Mary Goll², Todd Evans¹*  

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The targeted addition and removal of DNA methylation marks are major epigenetic regulatory mechanisms essential for normal development. Ten-eleven translocation (TET) enzymes (TET1/2/3) mediate methylcytosine (5mC) hydroxylation, which can facilitate DNA demethylation and altered gene expression. Functions for TET enzymes during development of complex organs including the heart have not been explored. Using zebrafish strains with targeted mutations in TET genes, we identified Tet2 and Tet3 as the major 5mC dioxygenases during zebrafish embryogenesis and observed specific defects in cardiogenesis in tet2/3-/- double mutant larvae. Morphological, molecular, and reporter strain analyses indicate defects in both epicardium migration and atrioventricular canal (AVC) development in tet2/3-/- double mutants. Co-culture experiments suggest that the epicardial defect may be indirectly caused by an altered myocardial program. To investigate the molecular mechanism, we compared transcript profiles of embryonic hearts isolated from wild type or double mutant embryos, and identified several candidate pathways that could impact epicardial and AVC development. Genome-wide MeDIP-seq and hMeDIP-seq experiments were performed to correlate DNA methylation and gene expression changes between mutant and wild types. This analysis should elucidate for the first time essential DNA epigenetic modifications that govern gene expression changes during cardiac development.

**Key words:** cardiogenesis, DNA demethylation, TETs, epicardium
Session V: Chemical Biology, Pharmacogenomics, and Drug Discovery
Chair: Randall Peterson (University of Utah, USA)

Invited Lecture:

Neuroleptics for ALS: small compound screen and small clinical trial
Dr. Pierre Drapeau, University of Montréal, Canada

Oral Presentations:

Gene-gene interaction studies in a zebrafish model of Gaucher disease
Dr. Oliver Bandmann, University of Sheffield, UK

A zebrafish model mimicking aspects of Costello Syndrome amenable to compound screening
Dr. Martin Distel, Children’s Cancer Research Institute, Vienna, Austria

Zebrafish as a predictive model for photodynamic therapy
Purnima Manghnani (PhD Candidate), National University of Singapore

Invited Lecture:

Unbiased phenotype-based chemical modifier screens in zebrafish models of neurodegenerative movement disorders
Dr. Edward Burton, University of Pittsburgh, USA

Oral Presentations:

Identification of novel inhibitors of the activated Wnt/β-catenin signaling pathway using the developing zebrafish embryo
Dr. Richard Glenn dela Cruz, Oklahoma Medical Research Foundation, USA

ZePASS (Zebrafish Phenotypic Anatomical Similarity System (Guides Target Identification of a Wnt Inhibitor Incaskin, a Novel, Highly Selective CK2α Kinase Inhibitor
Dr. Charles H. Williams (Postdoc), Vanderbilt University Medical Center, USA

Preclinical development of drugs for intracerebral hemorrhage (ICH)
Arman Hassanpour (MSc candidate), IMS at UofT, ZCADD, St. Michael’s Hospital
Invited Speaker

Pierre Drapeau, PhD., Professor and Director

Department of Pathology and Cell Biology, Faculty of Medicine, University of Montréal (CRCHUM), Canada

Dr. Drapeau has been studying the development of the motor network in zebrafish by combining cellular neurophysiology and molecular genetics. He records and images the activity patterns of identified spinal cord and hindbrain neurons in normal and genetically engineered embryos. His work has led to the discovery of a novel mechanism of synaptic transmission at fast neuromuscular junctions and the role of glycine in promoting neural differentiation during development. The long-term goal of his research is to elucidate the molecular choreography of motor network formation, plasticity and function. More recently Dr. Drapeau has expressed human genes in zebrafish, allowing for the validation of mutations and the screening of small chemical libraries in genetic models of neurodegenerative diseases such as ALS and hereditary spastic paraplegia, and developmental disorders such as autism and schizophrenia. He is collaborating on large-scale genomics projects to identify mutations of synaptic genes related to developmental brain diseases that he is validating in zebrafish embryos. In particular they have discovered that de novo mutations (in patients but not in their parents) are a major cause of autism and schizophrenia.

Neuroleptics for ALS: small chemical screen and small clinical trial

Author: Pierre Drapeau

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressing and fatal disorder with no effective treatment or biomarker. In order to discover new therapeutics, we used simple genetic model organisms to screen phenotypically for compounds that could be used to treat ALS patients. We screened libraries of 3,850 compounds, many approved by the FDA, in C. elegans expressing mutations of human TARDBP causing ALS, validated hits in zebrafish and tested the most potent molecule in mice and in a small clinical trial with sporadic ALS patients. We identified a class of 13 neuroleptics that restored motility in C. elegans and in zebrafish expressing mutant human TARDBP. The most potent was pimozide, which was also effective in SOD1 and FUS models. Pimozide prevented the reduction in neuromuscular transmission upon repetitive stimulation in TARDBP G348C zebrafish and enhanced transmission in SOD1 G37R mice. Finally, treatment of 25 randomized ALS patients over a 6-week period at low doses (2 or 4 mg) of pimozide resulted in a lack of progression of weakness of motor power (MRCSumScore) and a significant reduction in the decremental response to repetitive nerve stimulation of the right thumb abductor pollicis brevis, the only muscle where the placebo group demonstrated worsening. Simple genetic models are thus useful in identifying compounds that hold promise for the treatment of ALS such as neuroleptics, which may be a useful therapeutic approach to stabilize neuromuscular transmission in this disease.

Key words: TDP-43, FUS, ALS, pimozide, sporadic ALS
Gene-gene interaction studies in a zebrafish model of Gaucher disease

**Author(s):** Marcus Keatinge, Lisa Trollope, Hai Bui, Qing Bai, Matthew Gegg, Anthony Futerman, Robin Highley, Anthony Schapira, Ed Burton, Catarina Henriques, Oliver Bandmann

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Background: Homozygous glucocerebrosidase (GBA1) mutations cause Gaucher disease (GD), heterozygous GBA1 mutations are the most common genetic risk factor for Parkinson’s disease (PD). The mechanisms regulating the age at onset of GD or the disease penetrance for heterozygous GBA1 mutations in PD are largely unknown. Zebrafish are ideally suited to undertake gene-gene interaction studies. Aim and objectives: To determine the interaction of GBA1 deficiency with plausible disease-modifying genes, namely the pro-inflammatory microRNA mi-R155, the lysosomal sphingomyelinase SMPD1, and the senescence genes Klotho and telomerase (tert) in vivo. Methods: mi-R155, smpd1 and klotho stable mutant zebrafish lines were generated using CRISPR/Cas, the tert and gba1 mutant zebrafish lines had been described before. mi-R155, smpd1, klotho and tert mutants were crossed onto gba1/- background to generate double homozygous mutants. Survival and other disease-relevant readouts were determined. Results: Unexpectedly, smpd1 deficiency rescued gba1/- behavioural defects and increased lifespan whilst synergistically exacerbating gba1/- morphology and biochemical defects. mi-R155 upregulation was confirmed in Gba1/- mice and microglial cells with chemical inhibition of enzymatic glucocerebrosidase (Gcase) activity. However, mi-R155 deficiency neither prevented inflammation nor did it improve survival or ameliorate the phenotype in mi-R155_gba1 double mutant zebrafish. Klotho and telomerase deficiency did not accelerate the gba1-associated phenotype. Discussion: Our study demonstrates the power of zebrafish to undertake gene-gene interaction studies in an easily tractable vertebrate model system. We provide in vivo evidence for an additive biochemical effect of Gcase and smpd1 enzymatic deficiency suggesting that they contribute to the risk of PD by influencing related pathways.

**Key words:** Parkinson's disease, Gaucher disease, glucocerebrosidase 1, smpd1, mi-R155
Martin Distel, PhD.
*St. Anna Children’s Cancer Research Institute (CCRI), Vienna, Austria*

Martin Distel studied molecular biotechnology at the Technical University Munich, Germany and at Lund’s University, Sweden. During his PhD in the laboratory of Reinhard Köster at the Helmholtz Center Munich he worked on establishing genetic tools to visualize and analyze zebrafish hindbrain development. For his postdoctoral studies he joined the laboratory of David Traver at the University of California, San Diego. Since 2014, Martin Distel is group leader at the St. Anna Children’s Cancer Research Institute (CCRI) in Vienna, Austria. His group is modeling pediatric cancer and cancer predisposition syndromes in zebrafish to study tumor onset and progression. In addition, zebrafish disease models are used in his lab to identify therapeutic strategies through small compound screening. A recently started project, Danio4Can, aims at establishing an automated zebrafish small compound screening platform at the CCRI.

A zebrafish model mimicking aspects of Costello Syndrome amenable to compound screening

**Author(s):** Caterina Sturtzel\(^1\), Cara Bickers\(^2\), David Traver\(^2\), and Martin Distel\(^1\)

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Zebrafish is a well-suited model organism to study rare disease mechanisms. Here we present a zebrafish model mimicking aspects of Costello Syndrome. Costello Syndrome belongs to a group of developmental disorders commonly termed RASopathies as these diseases are caused by aberrations in the RAS/MAPK pathway. A mutation in HRAS underlies Costello Syndrome. In our zebrafish model overexpression of mutated human HRAS leads to several developmental malformations resembling scoliosis, nasal papillomata and an enlarged forebrain observed in Costello Syndrome patients. Due to the early phenotypes our model is well suited for compound screening. In a pilot drug screen inhibition of Mek1/2 and of farnesyltransferase ameliorated developmental abnormalities. This suggests that inhibition of MEK1/2 and the farnesylation of HRAS will be beneficial for Costello Syndrome patients.

**Key words:** zebrafish disease modeling, Costello Syndrome, H-RAS, compound screening
Purnima Manghnani, *(PhD candidate)*

*National University of Singapore, Singapore*

Purnima is a Chemical Engineer pursuing her PhD at National University of Singapore in the department of Chemical and Biomolecular Engineering. She works with the zebrafish model to evaluate efficiency of novel nano-drugs and bio-imaging probes. She has demonstrated optimized photodynamic therapy in transgenic liver hyperplasia zebrafish model using fluorescent photosensitizer nanoparticles. Her current research focus is on using zebrafish xenograft metastatic model for drug screening using fluorescent aggregation induced emission (AIE) cell trackers.

**Zebralish as a predictive model for photodynamic therapy**

**Author(s):** Purnima Manghnani, Shidang Xu, Bin Liu, Cathleen The

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Alternative cancer nano-theranostics is aiming to treat cancer while addressing the severe side effects and poor selectivity associated with chemo and radiation therapy. Photodynamic therapy employs selective accumulation of the non-toxic drug in the tumor followed by triggering the ROS generation of the drug with visible light. The time dependent uptake of the drug in the tumor determines the time at which the therapy can be effectively triggered. We have used the zebrafish transgenic model bearing a liver tumor to study the accumulation dependent photodynamic therapy of efficient AIE (Aggregation Induced Emission) photosensitizer nanoparticles. This theranostic study aims to demonstrate the importance of an ideal time frame for accumulation of polymeric nanoparticles in the hyperplastic liver of zebrafish larvae and initiating effective therapy.

**Key words:** AIE, photodynamic therapy, transgenic
Invited Speaker

Edward A. Burton, MD., PhD., FRCP,
Associate Professor and UPMC Endowed Chair in Movement Disorders
Department of Neurology, Molecular Genetics, and Biochemistry, University of Pittsburgh, USA

Dr. Burton graduated from medical school at the University of Birmingham, UK, and completed postgraduate training in internal medicine at Oxford and neurology at Birmingham. He became a research fellow in molecular biology at the University of Oxford, where he completed his Doctor of Philosophy degree. Following his postdoctoral fellowship in virology and gene transfer at the University of Pittsburgh, he completed higher specialist training in neurology as clinical lecturer at Oxford University, and a clinical fellowship in movement disorders at the National Hospital for Neurology and Neurosurgery, Queen Square, London. He joined the faculty of the University of Pittsburgh in 2004 and currently divides his time between carrying out research into neurological diseases, providing clinical care to patients with neurological diseases, and teaching neurology to medical students. His current research focuses on the role of the proteins tau and α-synuclein in the pathogenesis of progressive supranuclear palsy and Parkinson’s disease. In addition to employing conventional approaches using patient samples, cultured cells and murine models, the Burton Lab has a longstanding interest in exploiting the potential of zebrafish models for phenotype-based chemical rescue screens and other applications to accelerate the development of novel therapies. Dr. Burton is a member of the editorial board of the Journal of Biological Chemistry, and a member of the scientific advisory board of CurePSP.

Unbiased phenotype-based chemical rescue screen in a novel zebrafish model of progressive supranuclear palsy

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The long-term goal of our work is to develop effective treatments for progressive supranuclear palsy (PSP), a common neurodegenerative disease that is characterized by neuronal accumulation of misfolded tau protein. Development of effective pharmacological therapies for PSP and other tauopathies has been limited by lack of animal models that allow high-throughput testing of chemical modifiers against disease-relevant phenotypes in vivo. The zebrafish is a genetically- and chemically-tractable vertebrate model that is particularly suited to drug discovery and comparative efficacy testing and is being used increasingly for studying human neurological diseases. We generated a novel transgenic zebrafish model that expresses human tau in the CNS, resulting in a number of phenotypic abnormalities relevant to PSP. We tested over 200 bioactive compounds, identifying several that rescued the neurological phenotypes in this model. These data provide new insights into pathogenic mechanisms and provide a starting point for the development of effective drugs for PSP and other tauopathies.

Key words: PSP, tau, zebrafish
Identification of novel inhibitors of the activated Wnt/β-catenin signaling pathway using the developing zebrafish embryo

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The Wnt/β-catenin signaling (Wnt) pathway is a highly conserved and essential regulator of normal embryonic development and adult stem cell homeostasis. Inherited or spontaneous Wnt pathway dysregulation is linked to numerous diseases including cancer, where Wnt activity is upregulated. Since mutations in the tumor suppressor adenomatous polyposis coli (APC) gene promote colon adenoma to adenocarcinoma progression due to over-activated Wnt signaling, there is clinical interest in finding novel colon cancer therapeutics specific against APC mutation and Wnt over-activation. We screened for potential Wnt inhibitors using a previously reported chemically-induced Wnt activation assay that results in reproducible “eyeless” phenotype in zebrafish embryos. After confirming that the “eyeless” phenotype induced by 6-bromoindirubin-3-oxime (BIO), a GSK3 inhibitor, in developing zebrafish embryos is due to Wnt activation, we screened 253 chemically diverse terrestrial fungal metabolites for suppression of Wnt activation as evidenced by restoration of eye development. We observed robust rescue of eye development by three metabolites from terrestrial fungi – Dioschin (a sulfated sulochrin dimer) and Xanthoquinodines (Xant) A1 and B2. A compound from the Microsource spectrum compound library, cryptotanshinone (CT), also suppressed the BIO-induced “eyeless” phenotype and intriguingly is specifically lethal to homozygous apc mutant (apcmcr) zebrafish embryos. Additional confirmatory experiments performed corroborated that Dioschin, Xant A1 and B2, and CT inhibit the Wnt pathway. Our work again demonstrates the utility of the zebrafish as a pathway-specific in vivo drug discovery platform for novel and known small bioactive compounds.

Key words: Wnt, APC, colon cancer, fungal metabolite

Richard Glenn dela Cruz, PhD.
Oklahoma Medical Research Foundation, Oklahoma City, USA

Dr. Richard Glenn dela Cruz received his Bachelors of Science degree in Chemistry from the University of the Philippines in 1997. He then worked for four years isolating and characterizing novel neurotoxic peptides from marine snails, called conotoxins, in the lab of Dr. Baldomero Olivera at the University of Utah. In 2001, he shifted from working with conopeptides to proteins and his thesis focused on engineering a cofactor-independent mutant of the anticoagulant serine protease – Antithrombin III - under the supervision of Dr. Susan Bock. For his postdoctoral work, he co-authored a paper on the folding dynamics of another serine protease, alpha1-antitrypsin, but then shifted gears to learn yeast genetics in Dr. Dennis Winge’s Lab where he found an epistatic link between the copper transport protein Cox23 and the mitochondrial protein Cox1. In 2013, he finally saw the light and now works with the zebrafish model system in Dr. David Jones’ Lab in the Functional and Chemical Genomics Department at the Oklahoma Medical Research Foundation. He recently co-authored a paper examining the genetic relationship between mitochondrial pyruvate carrier 1 (MPC1) and the Wnt pathway protein Adenomatous Polyposis Coli (APC). One of the projects he is currently working on is using a library of terrestrial fungal metabolites as a tool to identify and characterize novel small molecules and targets in an activated Wnt pathway zebrafish model.
Dr. Williams is a post-doctoral research fellow at Vanderbilt University in Dr. Charles Hong’s Laboratory, interested in using chemical genetics to probe interesting biological problems. He received his BS degree from Vanderbilt University, during which, he studied under Professor Bruce Appel where he investigated cell fate specification of olig2+ progenitor cells in zebrafish. After graduating he worked with Dr. Tao Zhong to elucidate the role of hedgehog signaling in the cell fate decision between arterial and venous cells in the lateral plate mesoderm of zebrafish. During his graduate studies with Dr. Charles Hong he identified a novel inhibitor of Phosphodiesterase 4, which led to the characterization of PDE4 as a possible target for inhibiting hedgehog signaling in smoothened resistant cancers. After receiving his PhD in 2017 he stayed with Dr. Charles Hong, focusing his work on developing computational and paradigmatic tools to facilitate discovery and development of chemical probes using zebrafish development as a phenotypic platform. He is particularly interested in the biological role of environmental cues on proliferative and migratory cell populations during development and disease.

ZePASS (Zebrafish Phenotypic Anatomical Similarity System) Guides Target Identification of a Wnt Inhibitor Incaskin, a Novel, Highly Selective CK2α Kinase Inhibitor

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As a result of ever increasing coast of drug development, the past decade has seen an explosive growth in phenotypic chemical screens conducted in both academia and industry. Yet the complexity and unbiased nature of phenotypic screens make the crucial follow-up task of identifying the biologically relevant target of each hit very challenging, creating a scientific and translational bottleneck. In zebrafish embryos, an extensive cataloging of phenotype-genotype relationships offer a potential strategy for target identification solely based on a small molecule’s impact on embryonic development. Here, we describe the use of ZePASS (Zebrafish Phenotypic Anatomical Similarity System), similarity ranking algorithm based on publically available genotype-phenotype data to guide target identification. We show that ZEPASS correctly identified FGF as a candidate pathway for SU5402 and VEGF as a candidate for DMH4 based on a holistic assessment of 26 anatomical features. We further use this phenotype-genotype similarity approach to identify a novel 2-(2-phenylethenyl)quinoline as a novel inhibitor of Wnt signaling. Furthermore, we show that this compound, named incaskin, is an exquisitely selective kinase inhibitor of CK2α, which reduces growth of numerous Wnt-dependent cancer cell lines. While the current iteration of ZePASS is subject to observational bias of the screener, we are developing an AI based system using Tensorflow’s (Google) Inception model to identify abnormal anatomical features to reduce bias and increase consistency.

Key words: Phenotypic screen, target prediction, wnt signaling
Arman Hassanpour (MSc. Candidate)
Institute of Medical Science, University of Toronto
Zebrafish Centre for Advanced Drug Discovery, St. Michael’s Hospital, Canada

Arman Hassanpour graduated Summa Cum Laude from York University with a BSc in Health science. During his undergraduate studies, he was involved in pediatric physical activity and health research as well as muscle protease research on a rat model. However, during the last year of university, he developed an avid interest for brain research and decided to take various advanced classes in this topic. This interest led him to explore this topic through graduate studies at the university of Toronto. Following his acceptance to the Institute of Medical Science, he was lucky to be able to conduct his graduate project in the laboratories of Drs Xiao-Yan Wen and Loch Macdonald. He has been investigating the potential of repurposing a class of drugs to act on a specific molecular pathway that is involved in endothelial junction’s stability with the primary aim of preventing intracerebral hemorrhage. These studies were made possible from extensive preliminary research via high throughput screening of drugs in zebrafish models. Outside of research, Arman is a Liverpool fan and enjoys playing soccer and music.

Preclinical development of drugs for intracerebral hemorrhage (ICH)

Author(s): Arman Hassanpour1,3, Farhad Karbassi1,2, Jinglu Ai1,3, Youdong Wang2, Issaka Yougbare2, Heyu Ni2, R. Loch Macdonald1,3, and Xiao-Yan Wen1,2

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Spontaneous Intracerebral Hemorrhage is a form of non-ischemic hemorrhagic stroke with no effective medical therapies. It is a heterogeneous entity that most commonly emerges from instability at the level of brain interendothelial junction proteins due to various genetic and environmental factors. Here, utilizing an atorvastatin-induced brain hemorrhage model in zebrafish larvae, targeting HMGCR-CDC42-Cadherin pathway, we screened 727 compounds from NIH Clinical Collection Libraries and identified a small molecule drug EZF-100 with potent anti-hemorrhagic properties. Additionally, we found that EZF-100 effectively rescued hemorrhage after morpholino knockdown of the pathway proteins downstream of HMGCR. Our qPCR analysis suggests that EZF-100 treatment significantly up-regulates the mRNA levels of junction proteins including β3-integrin and VE-Cadherin. To further validate the efficacy of EZF-100 in a second model organism, we used mouse models of Lipopolysaccharide (LPS)-mediated brain microbleed model as well as a model with blocking antibody against integrin β3 subunit. Using high-resolution magnetic resonance imaging (MRI) and histological examination, we demonstrated that treatment with EZF-100 could significantly attenuate ICH in these mouse models. Our data suggest a role for EZF-100 in conferring vascular stabilization and a novel approach in treating Spontaneous ICH.

Key words: Intracerebral Hemorrhage, Neuroscience, Drug screening, Drug Discovery, vascular stabilization
Session VI: Translational Research & Drug Development
Chair: Dr. Xiao-Yan Wen (St. Michael’s Hospital & University of Toronto, Canada)

Invited Lectures:

Using zebrafish to study mechanisms of inflammation
Dr. Ori Rotstein, Keenan Research Centre for Biomedical Science, SMH

Chemical Genetics of Zebrafish Embryonic Development to Drive Therapeutic Target Discovery in Man
Dr. Charles Hong, Cell an Developmental Biology, Vanderbilt University, USA

Zebrafish modelling for Neurometabolic diseases: Identification of disease mechanisms & treatment targets
Dr. Clara van Karnebeek, University of Amsterdam, Netherlands

Drug discovery from Chinese medicines: the past and prospect
Dr. Yibin Feng, School of Chinese Medicine, The University of Hong Kong

Modeling human CNS disorders in the zebrafish
Dr. Randall Peterson, College of Pharmacy, University of Utah, USA
The use of zebrafish to study the mechanisms of protection exerted by Remote Ischemic Conditioning (RIC)

Author(s): Chungho Leung, Rui Guan, Xiao-Yan Wen, Ori D. Rotstein

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Trauma is a major cause of morbidity and mortality in the civilian population. Hemorrhagic shock followed by resuscitation is a form of global ischemia/reperfusion injury which is known to contribute to poor outcome through the development of multiple organ dysfunction. Remote ischemic conditioning (RIC) is an intervention performed in man, wherein multiple cycles of limb occlusion/release using a blood pressure cuff has been shown to protect against I/R injury. We previously showed that RIC protected against organ injury in a mouse model of shock/resuscitation. We have used zebrafish models of inflammation to study the mechanism. Serum derived from mice following RIC was injected into zebrafish embryos and inflammation was studied using tailfin transection model and also a viability model in response to LPS or hydrogen peroxide. RIC, but not control, serum prevented migration of GFP labeled neutrophils to the site of tailfin transection (Ann Surgery 2015) and also improved animal viability. An antioxidant array study revealed that RIC serum induced multiple antioxidant genes including heme-oxygenase and protection appeared to require the presence of the transcription factor Nrf2. The zebrafish system represents an excellent model to study the mechanism of the protective effects of RIC on inflammation and also may permit high throughput study of anti-inflammatory factors following hemorrhagic shock.
Invited Speaker

Charles C. Hong, MD., PhD.
Department of Pharmacology, Cell, and Developmental Biology, Vanderbilt University, USA

I am a physician-scientist at Vanderbilt University Medical Center (VUMC) with expertise in stem cell biology, drug discovery and genetics. I received my MD-PhD from Yale University and completed my clinical cardiology and chemical biology training (under tutelage of Dr. Randy Peterson) at Harvard Medical School/Massachusetts General Hospital. My research involves chemical biology of vertebrate development, which entails discovery of small molecules that regulate embryonic development using high-throughput, high-content chemical screens. For target identification, we utilize the medicinal chemistry, biochemical and genetic approaches, and are currently developing artificial intelligence algorithms to aid this effort. We have discovered first-in-class molecules that revealed novel biological insights and new therapeutic opportunities. For instance, we discovered the first small molecule inhibitor of bone morphogenetic protein (BMP) signaling. This work has led to drug development programs for treatment of severe devastating diseases. I also serve as the chair of the VUMC Accelerating Drug Repurposing Incubator (ADRI), a multidisciplinary team tasked with leveraging VUMC's DNA database linked to electronic health record to accelerate discovery and validation of new therapeutic targets for important unmet clinical needs. I was elected to the American Society for Clinical Investigation in 2013.

Chemical Genetics of Zebrafish Embryonic Development to Drive Therapeutic Target Discovery in Man

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Chemical genetics involves the discovery, development and use of chemical probes for interrogation of biological processes and for translational discoveries. In a manner analogous to classic forward mutagenesis screens, the Hong lab has conducted an unbiased, high-throughput chemical screen for small molecules that specifically modulate early embryonic development in zebrafish, and has carried out the follow-up task of identifying the pharmacological targets of a number of developmental modulators. Using this target-agnostic, “high content” phenotypic screening platform, we have discovered novel BMP, Wnt and hedgehog inhibitors, as well as first-in-class modulators of cell signaling components. Moreover, since disturbances in developmental pathways play central role in the pathogenesis of many human illnesses, small molecules that selectively target them have significant translational potential. For instance, we have licensed our BMP inhibitor technology for clinical development toward a number of diseases caused by aberrant BMP signaling. In addition, leveraging the available molecular genetic information on early zebrafish embryogenesis, we have developed unbiased computational methods to map the actions of small molecules and accelerate target identification. Finally, we are leveraging Vanderbilt University Medical Center (VUMC) DNA database linked to electronic health record to identify the clinical phenotypes associated with naturally occurring human genetic variations in the target genes, and then utilize these associations to guide therapeutic development for important unmet clinical needs.

Key words: Chemical genetics, phenotypic screen, phenome wide association study, target discovery, drug development
Clara’s research is dedicated to promoting early diagnosis and treatment of neurometabolic diseases in intellectual developmental disorder patients. She holds major research grants and her international TIDEX team integrates genomic and metabolomics technologies to unravel neurometabolic phenotypes, discovering novel genetic conditions and changing management. She focuses on digital apps, pyridoxine-dependent epilepsy and phenotypic modifiers of neurodegenerative disease. She published over 95 peer-reviewed journal articles including in the highest ranking medical journal New England Journal of Medicine, multiple clinical guidelines and several chapters in textbooks. She created digital tools to diagnose and care for patients with inborn errors of metabolism. Clara is regularly invited for keynote lectures at international conferences for professional societies in neurology, genetics and pediatrics. She is a dedicated teacher and mentor for clinical and research trainees at different stages. Her contributions to research and clinical care and commitment to translational science have been recognized by the Canadian Organization for Rare Diseases (Scientific Award 2016), the University of British Columbia Faculty of Medicine (Overall Excellence Early Career Award 2016) and Youth Women Children Association (Woman of Distinction Award nominee 2017).

Zebrafish modelling for Neurometabolic diseases: Delineation of Disease mechanisms and Improved Care

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Zebrafish modelling is increasingly used to understand the genetic basis of neurometabolic diseases, which comprise hundreds of devastating monogenic conditions due enzymatic deficiencies. Although rare, the burden of these diseases is immense given their degenerative nature and severe symptomatology varying from dementia, epilepsy, neuropathy, sensory deficits and systemic organ involvement. The good news is that more than 100 of these diseases is amenable to treatments, varying from medical diets, vitamin supplementation, substrate inhibition / medication, organ transplant, and stem cell transplants. For many more neurometabolic diseases, the genetic basis remains unknown however and even if known, therapy remains elusive. Whole exome sequencing has accelerated the identification of potential disease-causing variants; however, validation of the functional impact and causality remains an enormous challenge, let alone understanding disease mechanism. Zebrafish have proven an efficient model organism to characterize neurometabolic diseases. During my presentation I will illustrate this by describing the discovery of novel inborn errors of sialic acid and vitamin B6 metabolism, as well as the phenotypes, disease mechanisms, biomarkers and treatment targets for each. Finally, I will discuss the barriers to translating zebrafish knowledge into human care, and propose strategies to overcome these.
Invited Speaker

Yibin Feng, PhD., Associate Professor and Associate Director
School of Chinese Medicine, The University of Hong Kong

Dr. Yibin Feng is currently an Associate Professor and Associate Director of the School of Chinese Medicine at the University of Hong Kong. Dr. Feng received his Bachelor degree in Chinese Medicine from Mainland China. He received his PhD degree in molecular medicine from Hokkaido University School of Medicine and finished postdoctoral research in the same University in Japan. Dr. Feng’s research interest focuses on clinical and experimental study for cancer, diabetes, hepatic and renal diseases by using recently developed techniques. He has published over 300 publications in these areas and the citation in total is 6385. He also serves as editor and reviewer for over 60 international journals.

Drug discovery from Chinese medicines: the present and prospect

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Chinese medicine has 5000 thousand years and now is one of the complementary and alternative treatments in the world. Chinese medicine is an important resource for drug discovery. In the past years, drug discovery from Chinese medicines have been acknowledged by worldwide, such as artemisinin and arsenic trioxide. In clinical setting, both Chinese medicine and Western medicine often use combination therapy for diseases treatment, such as cancer, hypertension and AIDS etc. Facing complicated diseases and unmet medical needs, drug discovery from Chinese medicines should have new strategy: not only for new technologies, but also materials. To this goal, it is necessary to continually explore new technologies including high throughput screening approaches and integrated various technologies in identification of natural origin, quality control, gut bacteria metabolite, molecular docking, network pharmacology and randomized clinical trial. On the other hand, from single pure compound drug to multiple components drug, even big molecules are expected. This presentation will talk about drug discovery from Chinese medicines and study on both single compound and multiple compounds as drug candidates by recent developed technologies. The research was financially supported by grants from the RGC GRF, Hong Kong SAR of China (Project codes: 764708, 766211, 17152116), Wong’s Donation for molecular cancer research in Chinese medicines (Project code: 200006276) and some industry grants.

Key words: Drug discovery, Chinese Medicine, single compound, and multiple compounds
Randall T. Peterson, PhD is a chemical biologist whose research utilizes high-throughput screening technologies to discover new drug candidates for cardiovascular and nervous system disorders. Unlike conventional drug discovery programs that utilize simplified, in vitro assays, the Peterson lab screens using living zebrafish, ensuring that the drug candidates discovered are active in vivo. Several of the compounds discovered by the Peterson laboratory have become widely used research tools or are in clinical development.

Dr. Peterson received his PhD from Harvard University where he studied as a Howard Hughes Medical Institute predoctoral fellow in the laboratory of Stuart Schreiber. He completed a postdoctoral fellowship with Mark Fishman at Massachusetts General Hospital. Dr. Peterson spent 14 years as a faculty member at Harvard University where he was the Charles Addison and Elizabeth Ann Sanders Chair in Basic Science at Harvard Medical School, Scientific Director of the MGH Cardiovascular Research Center, and Senior Associate Member of the Broad Institute of Harvard and MIT. In 2017 he moved to the University of Utah as L.S. Skaggs Presidential Endowed Professor and Dean of the College of Pharmacy.

Modeling human CNS disorders in the zebrafish

Author: Randall T. Peterson

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The zebrafish (Danio rerio) has become an excellent tool to study mental health disorders, due to its physiological and genetic similarity to humans, ease of genetic manipulation, and feasibility of small molecule screening. Zebrafish also exhibit a rich repertoire of behaviors that can be tracked and quantified using large-scale automated assays. These behavioral assays can be effective means of characterizing zebrafish models of CNS disorders. They can also be used to screen for drugs or genetic modifications that modify the disease phenotypes. This talk will illustrate examples of diverse CNS disorders modeled in the zebrafish, including a recently developed model of opioid addiction. Using inexpensive electronic, mechanical, and optical components, we developed an automated opioid self-administration assay for zebrafish, enabling us to measure drug seeking and gain insight into the underlying biological pathways. Given the ease and throughput of this assay, it will enable identification of important biological pathways regulating drug seeking and could lead to the development of new therapeutic molecules to treat addiction.
### ZPPM Session II: Mutagenesis & Disease Modeling

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**ZPPM Session III: Mechanisms of Development & Organ Dysfunction**

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SESSION II: Mutagenesis & Disease Modeling

Anatomical characterization of D1 and D2 dopaminergic receptor neurons in the larval zebrafish forebrain

Author(s): Vernie Aguda, Indira Riadi, Helen Chasiotis & Tod Thiele
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The basal ganglia (BG) are a phylogenetically ancient group of subnuclei that control vertebrate movement, and dysfunction in these nuclei is closely associated with the motor deficits seen in Parkinson’s Disease (PD). Remarkably, it has been shown that the majority of the components and connections of the mammalian basal ganglia are present in the ancient jawless lamprey, indicating that they are also likely to be present in teleosts. If these circuits exist, the reduction in complexity and unparalleled optical access in larval zebrafish should make relationships between basal ganglia activity and behavioral control more salient. To address the function of basal ganglia circuits in zebrafish, we are utilizing Gal4 lines that target the fish’s putative direct and indirect pathways which have been shown in mammals to promote or inhibit movement respectively. For the direct pathway, we have already targeted Gal4 to the drd1a locus using CRISPR/Cas9 integration. For the indirect pathway, we are currently developing a drd2a Gal4 line using similar CRISPR/Cas9 genome editing techniques. Stable drd1a Gal4 lines are anatomically characterized using in situ hybridization and immunohistochemistry methods, showing co-localization of GAD67 (a GABAergic neuronal marker), DRD1A, and tac1 (a direct pathway marker). Future examination of the function of labeled circuits in these novel transgenic lines using a combination of calcium imaging, optogenetics and behavioural analyses will hopefully elucidate exactly where and how activity patterns are disrupted following the loss of dopaminergic signaling, and identify potential targets for future therapeutic interventions for PD.

Keywords: Dopamine, Parkinsons, Basal Ganglia, Anatomy
Alzheimer’s Modeling in Zebrafish

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Background: Alzheimer’s disease (AD) is characterized by two neuropathological hallmarks: senile plaques composed of Amyloid beta 1-42 peptides and/or oligomers, which originate from APP processing, and neurofibrillary tangles (NFTs) that consist of abnormally phosphorylated microtubule-assembly tau protein. Various animal models expressing APP with familial AD mutations have been generated. Despite the valuable information these models provided, the cause and mechanisms leading to neurodegeneration are unknown. Thus, there is a need for new models that could provide complementary advantages in terms of investigating the molecular mechanism of the disease as well as screening for potential therapeutics. In this study, our main aim is to work towards creating an AD model in zebrafish using the CRISPR/Cas9 system. Methods & Results: CRISPR was used to target the appb gene, one of the zebrafish’s paralogues of human APP, and was injected into one-cell embryos of appa ua5005/ua5005 knockout fish, along with a template for homology-directed repair to humanize the zebrafish gene. Preliminary evidence suggests germline transmission of mutant appb has been achieved. Additionally, we designed CRISPR targeting mapta and maptb, the zebrafish paralogues of human MAPT, to gain a deeper understanding of the physiological functions of MAPT. Our data shows that most of the CRISPR we designed are working efficiently. Conclusion: Engineering of the humanized appb and mapt knockout model will not only shed light on the molecular mechanisms of AD, it will also provide valuable models for drug screening.

Keywords: Alzheimer's disease, APP, MAPT, knockout, CRISPR

Zebrafish Pigmentation as a Model for Functional Evaluation of Coding Polymorphisms Identified in an Amerindian Population

Author(s): Khai C. Ang¹,², Victor A. Canfield¹, Sarah E. Arnold-Croop¹, Spencer R. Katz¹, Tiffany C. Foster¹ & Keith C. Cheng¹,²
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A major challenge in personalized medicine is evaluating the functional consequences of DNA polymorphisms. Indeed, the growing number of candidate polymorphisms found through SNP chip and whole genome sequencing has created a pressing need for systematic approaches for assessing phenotypic impact. During an ongoing search for skin color alleles in an admixed human population, we collected and genotyped 464 samples from an Amerindian population, the Kalinago of Dominica. Three albino individuals were found by exome sequencing to be homozygous for a coding mutation, R305W, but homozygosity was also found in non-albino individuals, indicating lack of causation. Further analysis yielded a 4 bp inversion resulting in N273K and W274V (NW273KV) in OCA2 that was homozygous in the albinos and heterozygous in 4 obligate carriers. Each NW273KV allele was calculated to cause a reduction in pigmentation of about 8 melanin units, compared to a 5-melanin unit reduction caused by the SLC24A5 A111T polymorphism in Europeans. Haplotype analysis of the albino chromosome indicated that these polymorphisms are African in origin. We are presently working towards validation of these conclusions using CRISPR/Cas-9 knock-in mutagenesis in zebrafish. We expect that similar approaches will enhance our understanding of the functional relationships between DNA sequence variation, human biology, and disease.

Keywords: Zebrafish, functional assessment, genome editing, sequencing
Tet Deficient Myelopoiesis as a Model for 'Pre-autoimmunity'

Author(s): Kelly Banks, Yahui Lan, Cheng Li, Mary Goll, Todd Evans
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The TET are involved in epigenetic regulation through active demethylation. Tet2 and Tet3 have been identified as the primary active 5-mC dioxygenases in zebrafish, and thus Tet2/Tet3 double knock-out fish are essentially devoid of Tet activity. In these Tet2/Tet3 knockout fish, primitive myelopoiesis initiates normally but produces an abnormal population of cells. First, there is a predominance of neutrophils over monocytes indicating a cell fate switch in their common precursor population. Second, the neutrophil morphology indicates a less differentiated phenotype. Finally, in response to sterile injury or bacterial infection of the otic cavity, the neutrophils are hyper-responsive and hyper-activated, releasing Neutrophil Extracellular Traps (NETs), aggregating together, and undergoing more apoptosis. However, despite significantly more cells responding, they are less able to clear infection than sibling embryos. A similar population of cells, dubbed Low Density Granulocytes (LDGs), has been described in studies of neutrophils isolated from patients with Systemic Lupus Erythematosus (SLE). It is thought that these LDGs contribute to the development of autoimmune disease by increasing self-antigen presentation through NET formation and thus the increasing risk of development of auto-antibodies known to be pathogenic in this disease. Further, it appears that altered cytokine production by these cells may play a role in altered immune function. This model allows characterization of LDGs in vivo which has not been possible previously. It also provides an excellent system to understand how these cells develop, especially as it relates to epigenetic dysregulation, which has long been thought to be involved in the process.

Keywords: Autoimmune, Primitive Myelopoiesis, Tet, Epigenetic

Therapeutic potential of neuroleptics against ALS - a small drug screen

Author(s): Christopher J Barden, Xiao-Yan Wen, Pierre Drapeau.
Presenting: Poulomee Bose
Affiliation(s): CRCHUM Universite de Montreal, Montreal, Canada. Treventis Corp, Toronto, Canada. St. Michaels Hospital, University of Toronto, Toronto, Canada. CRCHUM Universite de Montreal, Montreal, Canada.
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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. Although most ALS cases are sporadic (SALS), familial ALS (FALS) comes with a clear Mendelian inheritance. Both FALS and SALS have been linked to mutations in the TARDBP gene (encoding TDP43) that result in abnormal RNA processing, culminating in motor neuron degeneration. We believe that therapeutic strategies could be tailored at the neuromuscular junction (NMJ) of afflicted patients, as shown recently for the recently repurposed neuroleptic pimozide. Previous studies in zebrafish embryos with transient expression of TDP43 mutation indicate altered synaptic activity at the NMJs and aberrant spinal motorneuron outgrowth resulting in reduced motility, effects which are in part rescued by expressing human TDP43. In this study, a small library of 20 compounds from Treventis Inc. (Toronto, Canada; TRV) was screened to identify potential hits that could rescue effects caused by transient expression of mutant TDP43. TRV1441 and TRV1442 were potent in rescuing swimming deficits generated by mRNA injections of mutant TDP43 in 48 hours post fertilization (hpf) zebrafish larvae. To gain further insight into the effects of these drugs on the synaptic physiology at the NMJ, whole cell voltage clamp recordings were done at 48hpf from embryonic fast twitch muscle cells and spontaneous miniature endplate currents were isolated to evaluate synaptic activity. mutant TDP43 mRNA injected zebrafish larvae treated with these drugs were also assessed for motoneuron outgrowth phenotype. We will present the results of these analyses as a potentially rich target with therapeutic implications.

Keywords: TDP43, synaptic activity, drug screen, ALS.
Ndufa7 deficiency plays a critical role in development of hypertrophic cardiomyopathy (HCM)

**Author(s):** Xingjuan Shi\(^1\), Koroboshka Brand-Arzamendi\(^2\), Junghwa Yun\(^2,3\) Xiangdong Liu\(^1,4\), Xiao-Yan Wen\(^2,3,4\)

**Affiliation(s):** \(^1\) Key Laboratory of Developmental Genes and Human Disease, Institute of Life Sciences, Southeast University, Nanjing 210096, China. \(^2\) Zebrafish Centre for Advanced Drug Discovery, Keenan Research Centre for Biomedical Science, St. Michael’s Hospital, Toronto, Ontario, Canada. \(^3\) Department of Medicine, Physiology & Institute of Medical Science, Faculty of Medicine, University of Toronto, Canada.

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Hypertrophic cardiomyopathy (HCM) is a common autosomal dominant inherited cardiovascular disease, mainly caused by mutations in the sarcomere genes. Despite years of study, there is still limited knowledge about the genetic contribution for almost 40% HCM cases, indicating a need to identify new disease genes. In this study, whole exome sequencing analysis on 74 HCM patients has identified that NUDFA7, encoding a subunit of NADH-ubiquinone oxidoreductase (complex I) located in the mitochondrial, is a potential novel causative gene with a candidate mutation c.158A>G, leading to a missense mutation of p.Tyr53Cys. Here we knocked down ndufa7 in developing zebrafish embryos and demonstrated that the morphants had cardiac defect, which can be rescued by wild-type human NDUFA7 mRNA but not NDUFA7 c.158A>G (p.Y53C) mutant mRNA. This data strongly suggest that c.158A>C is a novel pathologic mutation in a newly identified HCM gene. Furthermore, we demonstrated that the expression level of HCM biomarkers nppa and nppb were also increased in ndufa7 morphant. Mechanistic study showed that calcium signaling and cardiac reactive oxygen species (ROS) are involved in ndufa7 knockdown induced cardiac hypertrophy. Together, these results strongly support for the first time that the NDUFA7 is a novel HCM disease gene.

**Keywords:** hypertrophic cardiomyopathy, potential HCM associated gene, heart development, cardiac hypertrophy

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Comparative study of the characteristics of different diets-induced NAFLD models of zebrafish

**Author(s):** Bo Chen, Jing-pu Zhang

**Affiliation(s):** Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, TianTan XiLi No. 1, Dong-cheng District, Beijing 100050, China

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Dietary compositions have important impacts on the development of nonalcoholic fatty liver disease (NAFLD), but the underlining mechanism is still unclear. The purpose of this study was to explore the relationship and differences of NAFLD relevant to main dietary components by different diets-fed zebrafish larvae. Zebrafish larvae fed with HC, HF and EF diets for 10 days all developed steatosis. The incidence and degree of steatosis were more severe in EF diet-fed larvae compared with the other groups. HC diet severely promoted lipid deposited in caudal vein. The triglyceride and glucose contents of zebrafish significantly increased in EF group and decreased in HC diet-fed larvae compared with control group. Moreover, the mRNA expression of oxidative stress gene gpx1a, endoplasmic reticulum (ER) stress gene ddit3, grp78, inflammatory genes tnfα, glucose metabolism genes irs2, glut1, glut2 and lipid metabolism genes cidec, ppara and cpt1a were significantly increased in HF diet-fed larvae. The HC diet was associated with upregulation of grp78, and downregulation of irs2, glut1 and glut2. The mRNA expression of grp78 were significantly up-regulated in the EF diets-fed larvae, whereas the expression of lipogenic molecules and glucose metabolism associated genes lipid1, srebfl1 irs2a, pepck were decreased compared to the control. In addition, the autophagy associated genes atg3, atg5, atg7, atg12 and protein expression of ATG3 and LC3BII were reduced and elevated level of p62 in HC group. We also performed RNA-seq and comparative transcriptome analysis of four groups, and 17 statistically significant pathways were observed in HC, HF and EF groups. This study revealed the relationships between diet ingredients and host factors that contribute to the pathogenesis of hepatic steatosis. Our work may provide a favorable reference in the choice of zebrafish models and new markers as well as mechanisms to prevent and treat NAFLD.

**Keywords:** Zebrafish, NAFLD, Autophagy, RNA-seq
The Ins and Outs of Zebrafish Cardiac Actin

Author(s): John Dawson, Matiyo Ojehomon, Love Sandhu
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Cardiomyopathy is a family of diseases of the heart muscle. Zebrafish is an excellent model organism for studying genetic changes found in human patients with cardiomyopathy. We are using zebrafish to examine the sixteen known mutations in the cardiac actin gene related to the development of hypertrophic and dilated cardiomyopathy (HCM and DCM). Phylogenetic analysis and the limited literature suggests that there are three cardiac-specific actin genes in zebrafish (zfactc genes: zfactc1a, cardiofunk (zfacta1b), and zfactc1c). To verify this prediction, we are knocking out the zfactc genes using CRISPRs and tracking the phenotype trajectories of individual fish with the gene knock outs. Knocking out the cardiofunk gene significantly impacts viability of embryos in the first three days of development and produces cardiac-specific phenotypes. In addition to knocking out the zfactc genes, human cardiac actin is being expressed in zebrafish hearts using transposons to ask if human cardiac actin can partially or wholly rescue zfactc knock out phenotypes. To date, we have generated lines expressing wild type human cardiac actin and the hypercontractile E99K ACTC mutant. In this short talk, our further development and analysis of CRISPR and transposon lines will be described.

Keywords: cardiac actin, cardiomyopathy, CRISPR, transposons

P2-9

Tryptophan residues in TDP43 and SOD1 mediate SOD1 aggregation and toxicity

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Among the growing number of biological hallmarks of amyotrophic lateral sclerosis, SOD1 and TDP43 inclusions are prominent; disease-related mutants of SOD1 and TDP43 are toxic to cells in vitro and to motor neurons in vivo. Determining the mechanisms of aggregation and spread remain key hurdles to understanding ALS. We hypothesize that a cluster of key tryptophan residues on SOD1 and TDP43 are necessary for their interaction, and thus for SOD1 aggregation and neuromuscular deficits in zebrafish embryos. We introduced alternative amino acids at these residues via missense mutations, with the prediction that this would disrupt aggregation and toxicity of SOD1 and TDP43. Expression of these novel mutant proteins led to a reduction in aggregates in transfected HEK293 cells. In zebrafish, expressing the novel mutants via mRNA injection also reduced motor neuron toxicity compared to expression of wildtype or disease-related variants, as measured by motor neuron development and touch-evoked escape response (TEER). Application of candidate small drugs was found to reduce aggregation of SOD1 protein, and significantly reduced neuromuscular deficits in SOD1 and TDP43-injected embryos. These residues show promise as therapeutic targets and can elicit new questions about SOD1 misfolding, interactions with TDP43 and other proteins, and how these misfolded proteins lead to neuron death. Specifically, our novel SOD1 mutant seems to be beneficial or protective, in contrast to hundreds of disease-associated mutations, suggesting this residue may be relevant to protein misfolding in the context of other mutations.

Keywords: SOD1, TDP43, motor neuron, neurodegeneration
Cardiovascular development is a precisely regulated process involving multiple conserved signaling pathways. Perturbations of these pathways can lead to congenital malformations. In this study, we resorted to a Tol2-based protein trap system, a powerful forward genetics tool to mutate and functionally characterize zebrafish genes in cardiovascular system (Clark et al., 2011). Of these fish, we identified a mutant in which the Tol2 transgene was incorporated in the platelet-derived growth factor receptor alpha (pdgfra) gene, resulting in truncation and fusion of the native transcript to monomeric red fluorescent protein (mRFP). Platelet-derived growth factor receptor α (PDGFRα) is a highly conserved tyrosine kinase receptor essential for development and organogenesis. Disruption of Pdgfrα function in murine models is embryonic lethal due to severe cardiovascular defects, thus necessitating the use of alternative models to explore its precise function in cardiovascular development. Here, we show that zebrafish pdgfra mutants have cardiac defects as a result of abnormal endocardial and myocardial midline migration during the process of cardiac fusion, an essential step for cardiac assembly and development. We also show that heterozygous embryos initially expressed Pdgfra-mRFP in venous-derived endothelial cells, including lymphangioblasts, followed by progressive specification in lymphatic endothelial cells. Investigation of blood and lymphatic vessels in pdgfra mutants revealed significant defects in venous and lymphatic (secondary) sprouts, which appeared to result from misguided cell movements. Together, these findings suggest that pdgfra signaling is required for proper migration of myocardial and endothelial cells during cardiac fusion and lymphatic endothelial sprouting.

**Keywords:** Zebrafish, pdgfra, cardiac, venous- and lymphatic-angiogenesis.

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**Single cell functional and chemosensitive profiling of combinatorial colorectal therapy in zebrafish xenografts**

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Cancer is as unique as the person fighting it. With exception of few biomarker-driven therapies, patients go through rounds of trial-and-error approaches to find the best treatment. Using patient-derived cell lines, we show that zebrafish-larvae-xenotransplants constitute a fast and highly sensitive in vivo model for differential therapy response, with resolution to reveal intra-tumor functional cancer heterogeneity. We screened international colorectal cancer therapeutic guidelines and determined distinct functional tumor behaviors (proliferation, metastasis, angiogenesis) and differential sensitivities to standard therapy. We observed a general higher sensitivity to FOLFIRI than to FOLFOX, not only between isogenic tumors but also within the same tumor. We directly compared zebrafish-xenografts with mouse-xenografts and show that relative sensitivities obtained in zebrafish are maintained in the rodent model. Our data also illustrates how KRAS mutations can provide proliferation advantages in relation to KRASWT and how chemotherapy can unbalance this advantage, selecting for a minor clone resistant to chemotherapy. Zebrafish-xenografts provide remarkable resolution to measure Cetuximab sensitivity. Finally, we demonstrate the feasibility of using primary patient samples to generate zebrafish Patient Derived Xenografts (zPDX) and provide proof-of-concept experiments that compare response to chemo and biological therapies between patients and zPDX. Altogether, our results suggest that zebrafish-larvae-xenografts constitute a promising fast assay for precision medicine, bridging the gap between genotype and phenotype in an in vivo setting.

**Keywords:** zebrafish-xenograft, chemotherapy-functional-screening, colorectal-cancer, KRAS, metastasis

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Functional analysis of novel regulators of cardiac function in zebrafish

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Previously, our lab performed shotgun proteomics to enrich and identify membrane-associated proteins from primary mouse neonatal and human fetal ventricular cardiomyocytes. These studies identified a rank-ordered set of 555 differentially membrane-enriched protein clusters, prioritized by no previous links to cardiac function and enrichment, revealing several key cardiac proteins which required further study. To assess the function of the top candidate, Tmem65, invivo, morpholino-mediated depletion in zebrafish was conducted. Upon depletion of Tmem65, pericardial edema and altered cardiac morphology was observed at 96 hours-post-fertilization (hpf) and severity increased with development. Tmem65 morphants were not viable by 7 dpf, suggesting an essential function of Tmem65 in early stages of cardiac development. Detailed analysis of zebrafish hearts showed abnormal cardiac looping in the morphants. A significant decline in heart rate was also observed in Tmem65 morphants after 4.5 dpf and continued decreasing at 5 and 6 dpf. Knockdown of the second-highest ranked protein, REEP5, also caused morphological defects within the embryo. REEP5 morphant embryos demonstrated abnormalities in heart morphology with a lack of looping at both 48 and 72 hpf. These morphological defects were accompanied by irregularities in heart rhythm and a significant reduction in heart rate from 3-6 dpf. To characterize this, detailed analysis of the cardiac beating rhythm was performed using imaging analysis which demonstrated arrhythmogenic beating rhythms with asynchronous heart beats in REEP5 morphant embryos compared to wild-type controls. Altogether, our in-vivo analysis of REEP5 function in embryonic cardiomyocytes has demonstrated its critical role during the early stages of cardiac development.

Keywords: Cardiac, development, morpholino, proteomics

Zebrafish mbnl mutants model key molecular phenotypes of myotonic dystrophy

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The genetic disorder myotonic dystrophy (DM) is best known for causing muscle weakness and myotonia. Individuals with DM also often experience altered gastrointestinal tract motility and small intestinal bacterial overgrowth, but the underlying mechanisms are poorly understood. DM is caused by the expression of expanded CUG repeat RNAs, leading to sequestration of MBNL RNA-binding proteins and misregulation of alternative splicing. We generated stable zebrafish DM models through CRISPR-based mutation of the three mbnl genes, and are creating complementary models through transgenic overexpression of CUG repeats both globally and in specific tissues. Key DM-associated molecular phenotypes are recapitulated in zebrafish mbnl mutants, including changes in the auto-regulation of mbnl1 and mbnl2 alternative splicing. As in mouse DM models, mbnl1 and mbnl2 mutants display more pronounced alternative splicing defects than mbnl3 mutants, and compound mbnl mutants exhibit larger splicing changes than single mbnl mutants. Also resembling mouse models, splicing changes in zebrafish mbnl mutants are greatest in adult heart and skeletal muscle. The zebrafish DM models will afford the opportunity to characterize disease phenotypes in live animals, focusing on the digestive system. Specifically, we will use live microscopy to study whether and how gut motility is altered in DM model fish, we will investigate the cell types and underlying alternative splicing changes that contribute to gut phenotypes, and we will use germ-free fish to determine whether microbiota contribute to phenotypes. A long-term research goal is to use zebrafish models to screen for compounds that ameliorate DM-like phenotypes.

Keywords: myotonic dystrophy, alternative splicing, zebrafish disease model, gut motility, microbiota
A Novel Zebrafish Model of Neurodegeneration-Induced Astroglial Activation

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Astrocytes play an important role in the pathology of numerous neurodegenerative diseases, such as multiple sclerosis (MS), Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS) and schizophrenia. However, the molecular pathways controlling astrocyte function and activation are still relatively unknown. This has resulted in a lack of astrocyte-targeted therapies for the management of central nervous system (CNS)-related disorders. Current studies characterizing the molecular mechanisms of astrocyte involvement in CNS disorders will result in a large list of candidate genes and proteins, which need to be further validated in simpler experimental organisms. Both primary astrocytes and cell lines have been used for validation purposes. However, they lack influence over neighboring cells. Therefore, there is a clear need for new experimental models to study astrocytes in their physiological context. Zebrafish offers a superior platform for such validation studies, while it enables the use of both genetic and chemical screens to identify candidates of interest that can be further investigated in other model systems. We have developed a reporter zebrafish line to follow astroglial activation, and established a zebrafish model of inducible neurodegeneration. This reporter zebrafish line is useful to study astroglial signaling pathways controlling astroglial physiology. This zebrafish reporter line can be combined with existing zebrafish models of CNS-related disorders. Thus, this reporter zebrafish line is a unique tool that allows us to investigate in vivo targets and genes identified in genomic and transcriptional studies performed in murine and human models, and also to identify new pathways of interest.

Keywords: neurodegeneration, astroglia, inflammation

CRISPR/Cas9- induced mutation in cardiac Troponin T promotes cardiomyopathy in adult zebrafish

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Studying the molecular and cellular aspects of human pathologies predominantly relies on using animal models to better understand the disease mechanism. Over the years, the zebrafish (Danio rerio) has emerged as a favorable genetic model to study cardiac development and disease. By two days of fertilization, the heart fully develops, has a heart rate that is physiologically comparable to humans and is optically clear to allow the visualization of the progressing heart pathologies. In this study, we have taken advantage of both the zebrafish and CRISPR/Cas9 genome editing technology to target a sarcomeric cardiomyopathy gene, which is implicated in disease of the heart muscles in human patients. Patients with mutations in TNNT2, encoding for the sarcomere thin filament contractile protein cardiac troponin T, present various symptoms ranging from increased fibrosis, myocyte disarray, left ventricle wall and septum hypertrophy, enlarged left ventricle, disruption in contractility function to cases of sudden cardiac death. Typically, patients displaying these symptoms are screened at late stages of the disease, making treatment options limited. Here, we identified embryonic heterozygous zebrafish carriers of an in-frame deletion in tnt2a showing a decreased heart rate. Moreover, juvenile and adult zebrafish display an enlarged heart chamber size, accompanied with increased myocardial stress and fibrosis. Therefore, the ultimate goal of this study is to establish the zebrafish as a model for sarcomeric cardiomyopathies by further dissecting the mechanism behind the observed phenotypes as the disease progresses, and identify possible therapeutic compounds for the treatment of cardiomyopathies using our model.

Keywords: Zebrafish, CRISPR/Cas9, Cardiomyopathy, tnt2a, Heart rate
Zebrafish targeted mutagenesis to unveil normal physiological functions of, and interactions between, Prion Protein (PrP) and Amyloid Precursor Protein (APP): Relevance to Alzheimer’s Disease

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**Aims:** The normal roles of prion protein (PrPC) and amyloid precursor protein (APP) remain elusive despite intensive study of their misfolded forms in prion diseases and Alzheimer’s disease (AD), respectively. We previously expanded upon a role for PrPC in AD by demonstrating its interaction with APP, via co-IP and concerted knockdown and replacement of these gene products with cognate mRNA and mRNA of their mammalian homologs in zebrafish. Here we built upon the latter approach by engineering zebrafish mutants.  

**Methods:** prp1 and appa loss-of-function alleles were engineered using TAL effector nucleases. Compound prp1-/-;prp2-/- mutants and compound prp1-/-;appa-/- mutants were obtained through subsequent breeding. Morpholinos were used for acute gene knockdown. Results: Zebrafish prp1-/- and compound prp1-/-;prp2-/- mutants lacked overt phenotypes, resembling mammalian Prnp knockouts. This remarkably contrasts reports of severe developmental phenotypes when either prp1 or prp2 are knocked down using morpholinos. appa-/- and compound prp1-/-;appa-/- mutants also lacked overt phenotypes, but were smaller than wild type fish at some developmental stages. prp1-/- mutants, however, were more sensitive to appa knockdown than wild type fish, and both prp1 and mammalian Prnp mRNA rescued this effect.  

**Conclusion:** These results support a genetic interaction between prp1 and appa and raise questions about differences in acute versus long-term loss-of-function in these pathways. Awareness of how loss of normal protein function(s) contribute to AD and prion disease will likely inspire new therapies. Zebrafish provide a tractable platform to assess normal protein function via delivery of mRNAs accompanied by novel reporters of neuron function.

**Keywords:** Alzheimer’s disease, prion disease, targeted mutagenesis, mRNA rescue, neural development

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Developing Technologies for Whole-Brain Functional Mapping in Behaving Larval Zebrafish

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The rapid development of technology to monitor and manipulate the activity of individual neurons throughout the brain offers a unique opportunity to study the neural circuits underlying behaviour. However, designing and implementing these tools is challenging, as even the most fundamental behaviours require the coordinated activity of many neurons dispersed throughout multiple brain regions. The goal of our research is to develop whole-brain functional mapping technologies to study the neural circuits underlying visually-guided behaviours of larval zebrafish. We have designed a custom closed-loop behavioural feedback assay consisting of live video tracking integrated with dynamic closed-loop visual stimulation to determine the precise relationship between distinct visual features and discrete tail kinematics. We have also developed tools for recording tail kinematics during visual stimulation while simultaneously performing whole-brain two-photon calcium imaging. Both platforms are designed to be integrated with optogenetic stimulation and inhibition. These techniques should allow us to identify the functional connections between individual neurons, and may also reveal the behavioural roles of individual neurons or circuits. Overall, implementing these technologies will further assist in bridging the gaps between individual neurons, neural circuits, and behavioural outputs.

**Keywords:** Neuroscience, whole brain imaging, optogenetics
Rotenone as a model for dopamine neuron loss

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Rotenone is a common pesticide that has been linked to the development of Parkinson’s disease. Rotenone is believed to be mitochondrial complex I inhibitor, generating reactive oxygen species (ROS), and subsequently induces ROS-mediated apoptosis in dopaminergic neurons. To explore the mechanism of dopamine neuron death and development, we have generated two transgenic line in which the regulatory elements of the dopamine transporter (dat) gene respectively target Green Fluorescent protein Tg(dat:eGFP) and mCherry, after fusion with the mitochondrial localization signal (MLS) of Tom20 Tg(dat:Tom20 MLS-mCherry). We have found that Rotenone exposure induced dopaminergic neuron loss and locomotion deficits in zebrafish in a dose-dependent manner. The dopamine neuron deficits observed were most pronounced in the in the olfactory bulb and the ventral diencephalon after exposure of 100nM Rotenone, while there were no gross changes in other major neuron groups. There was also increased amount of mitochondrial oxidative stress and apoptosis markers observed in treated fish. Co-treatment with ascorbic acid partially rescued rotenone-induced dopamine neuron loss and locomotion phenotypes in zebrafish larvae. We also found that mitochondria in dopaminergic neurons are decreased in foci and are more fragmented. Adult zebrafish which were exposed to Rotenone during embryogenesis showed anxiety-like behaviors but did not show any deficits to their dopamine neuron distribution. These results suggest that Rotenone exposure can cause dopamine neuron death through ROS-mediated apoptosis, and can acts as a model of environmental factor of Parkinson’s disease.

Keywords: Rotenone; complex I inhibitor; Parkinson’s disease; dopamine neurons

Generating zebrafish models of human disease to facilitate drug discovery

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Advances in next generation sequencing have greatly accelerated the identification of novel disease-associated gene mutations. However, a prevailing challenge continues to be functional validation of these variants, particularly for rare diseases. We have established a Zebrafish Genetics and Disease Models Facility that combines shared expertise and infrastructure at the Hospital for Sick Children, creating a pipeline from gene identification to functional validation and drug discovery. Our facility aims to provide the services required to efficiently generate and analyze zebrafish models that accurately recapitulate human disease. We use a high throughput CRISPR-Cas9 mutagenesis system along with high resolution melt (HRM) analysis to generate mutations in zebrafish that are targeted to putative human disease loci. We also utilize transgenic techniques to create “humanized” models of disease, as well as CRISPR-Cas9 to develop knock-in models of human mutations at conserved zebrafish loci. Additionally, we offer phenotypic analysis and drug discovery services. We are currently developing models for a diverse set of diseases including inflammatory bowel disease, pediatric cancer, cardiac arrhythmia and childhood muscle disease. To date, we have worked with 13 individual labs to generate 40 targeted mutations in 25 genes with a success rate of >85%. In this study, we will present the results of our large-scale mutation generation effort, as well as the preliminary characterization of our first successful mutant strains.

Keywords: CRISPR/cas9, drug discovery, disease modeling
Expressing cardiac actin in Zebrafish

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Due to its many advantages such as optical transparency, rapid cardiovascular development and simple gene manipulation, zebrafish is a great in vivo model to study cardiovascular disease. My research is directed toward introducing mutations into zebrafish associated with cardiomyopathy to determine the molecular mechanisms of the disease. Different variants of sarcome re proteins have been linked to the development of both hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). One such protein is cardiac actin (ACTC), and to date there have been 16 ACTC mutations implicated in cardiomyopathy. Although the human genome contains a single ACTC gene, zebrafish have a partially duplicated genome. Currently, there are two confirmed cardiac-specific actin genes in the zebrafish genome (zfactc1a and zfacta1b), but genome and functional suggests an additional uncharacterized zfACTC gene which we call zfactc1c. Preliminary in situ hybridization experiments suggest that zfactc1c is expressed in zebrafish hearts. To express human ACTC mutants in zebrafish, ACTC genes are inserted into the zebrafish genome with Tol2 transposons and is expressed in the heart under the control of the cardiac myosin light chain promoter. We have generated a transgenic zebrafish line expressing wild type ACTC and a line expressing E99K mutant ACTC. Zebrafish expressing E99K ACTC display some cardiac phenotypes including a short bent tail and necrosis of the yolk leading to slow development. Using zebrafish to determine the molecular mechanism of cardiomyopathy development in humans will help advance therapies for patients.

Keywords: Cardiac actin, Cardiomyopathy, CRISPRs, Transposons

Aldh7a1 deficiency causes pyridoxine-dependent epilepsy and reduced GABA levels in zebrafish

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Pyridoxine-dependent epilepsy (PDE) is a rare disease characterized by intractable and recurrent neonatal seizures responsive to pharmacological dosages of pyridoxine (vitamin B6). Neurodevelopmental delay is observed in most PDE patients. Despite the identification of causal mutations in the lysine metabolism gene ALDH7A1 over a decade ago, no PDE in vivo models have been described so far. In this report, we describe a viable zebrafish model of aldh7a1 deficiency, displaying the key features of PDE. Mutant larvae displayed deficient lysine metabolism, characterized by accumulation of the intermediates aminoadipate semialdehyde (AASA) and piperideine 6-carboxylate (P6C); a toxic metabolite suggested to be the PDE pathogenic driver by inactivating pyridoxal 5'-phosphate (PLP), the active B6 vitamer. The aldh7a1-null larvae develop spontaneous seizures at 10 days post fertilization (dpf) leading to premature death within 4 days; earlier seizure onset and death was observed following lysine supplementation. Epileptiform electrographic activity was observed uniquely in the mutants as a series of population bursts in tectal recordings. Mass spectrometry analysis showed reduced B6 vitamers and low systemic GABA levels in the aldh7a1-/- fish, supporting a GABA-centric theory of PDE pathogenesis. Remarkably, as is the case in human PDE, the seizures show an almost immediate sensitivity to pyridoxine and PLP with a resulting extension in the lifespan of the mutant fish. GABA levels also normalize with pyridoxine treatment. This novel aldh7a1-null vertebrate model provides valuable insight into the pathophysiology of PDE; further research will lead to a deeper understanding of PDE and provide new opportunities for drug discovery.

Keywords: pyridoxine-dependent epilepsy, aldh7a1, zebrafish model, lysine metabolism, metabolic epilepsy.
Zebrafish primordial germ cells as a novel in vivo cancer model

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Accessible in vivo models for tumour progression can help developing therapies and identifying drugs that inhibit tumour growth and spread. To this end, we evaluated the potential of zebrafish primordial germ cells (PGCs) as a relevant model, by assessing the molecular similarity between PGCs and cancer cells. Interestingly, the mRNA expression profile of migrating PGCs is related to highly invasive cancers such as glioblastoma. Consistently, inhibition of pathways known to affect glioma progression using genetic mutations and small molecules impaired zebrafish PGC migration. Using this model, we determined, for the first time, the actual role proteins known to be involved in glioma etiology play in cell migration within the live tissue. Specifically, we find that Hsp90aa1.2 affects cell migration speed and displacement, as well as the progression through the cell cycle. Our results thus present zebrafish PGCs as a useful, cost-effective in vivo model for glioma. This model can provide a novel setup for large-scale small-molecule drug screens and facilitate better understanding of the progression of glioma and other types of invasive cancer.

Keywords: cancer, cell migration, drug screening, glioblastoma, zebrafish

Emerging roles of Pannexin1a in sensory motor integration in the zebrafish

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A coordinated process involving visual, acoustic, vestibular, and sensory-motor feedback guides efficient locomotion. Recent electrophysiological, behavioral, as well as genetic entry points highlight the importance of synaptic neuromodulation for coordinating the sensory and motor systems and modulating the final motor outputs. Synaptic modulation via membrane channels composed of Pannxin1 (Panx1) protein has currently received corroborations. Panx1 protein shows high expression and localization in the neurons of different regions in the nervous system including retina, hindbrain, and the spinal cord. Operating at the crossroad of major signaling pathways, foremost those involving intracellular calcium and extracellular ATP, underscores the potential relevance of Panx1 channel to synaptic transmission. Furthermore, distinct roles of the Panx1 protein in processing visual information in subsets of neurons in the rodent and zebrafish retina have been reported. Here, we describe role(s) of Panx1a protein in modulating visually driven motor behavior in a TALEN–mediated Panx1a knockout zebrafish model. To characterize the function of Panx1a with respect to the spontaneous motor activity of zebrafish, we examined the swimming behavior in Panx1a-deficient larvae. We found that larvae with a loss of function in Panx1a had a lower baseline activity compared with control wild-type larvae during the observation period. Light-induced locomotor (LLR) experiments showed that loss of Panx1a also results in reduced locomotor response to either light increments or decrements. Together, these data lend support to the hypothesis that Panx1a is required for the execution of visuomotor behaviours.

Keywords: Locomotion, Visual feedback, Pannexin1, TALEN, Zebrafish
Light-induced Generalized Epilepsy in Gabra1 Knockout Zebrafish: A Simple Model for Unravelling the Molecular Basis of Epilepsy and Screening for Anti-Epileptic Drugs

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Mutations in different subunits of the GABA receptor have been reported as predisposing for idiopathic generalized epilepsy. However, to date no functional in vivo studies reported tonic-clonic generalized seizures associated with mutation in alpha 1 subunit. More generally, animal models of spontaneous epilepsy are rare thus reducing the ease of studying the molecular origins of epilepsy. Here, we inserted a frame-shifting mutation in the zebrafish alpha 1 receptor (gabra1). This mutation leads to a complete loss-of-function as implied by the reduced mRNA level in homozygous fish. Gabra1/-/- embryos rarely reach adulthood suggesting that they die prematurely. More interestingly, gabra1/-/- fish undergo strong generalized seizures upon light exposure. Indeed, immediately after light, a first tonic-like phase occurs characterized by an arching of the body, a loss of swimming posture as well as jerks of the jaws. Then, in the following minute, the fish undergo a clonic-like phase with uncontrolled movement leading to an increased velocity and distance swam. Remarkably, generalized seizures can be triggered upon light exposure, which represents a major advantage that will greatly help our further investigation regarding the underlying molecular mechanisms. Moreover, we were able to detect an increased response to light in embryos as early as four days post-fertilization. As a result, our mutant line represents a model of choice for high-throughput drug-screen assays. Finally, we launched a transcriptomics assay comparing whole brain gene expression from gabra1/-/- larvae compared to wild-type siblings. This assay identified 460 differentially expressed genes that are candidate genes underlying gabra1 pathogenicity.

Keywords: Epilepsy, GABA, Transcriptomics

Using CRISPR-Cas9 to produce and characterize zebrafish cardiac actin knockout lines

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Cardiomyopathy is a common cause of heart failure, a growing epidemic in Canada. Two prevalent forms of cardiomyopathy are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), which are characterized by changes to the heart muscle (myocardium). The development of HCM and DCM has been associated with mutations found in genes encoding muscle proteins, including cardiac actin (ACTC1). An in vivo model is required to study the underlying molecular mechanisms leading to the development of cardiomyopathy. Three cardiac-specific actin genes in zebrafish (zfactc genes) have been identified through literature and phylogenetic analysis - zfactc1a, cardiofunk (zfacta1b), and zfactc1c. Research about the role of cardiac actin in cardiomyopathy development using zebrafish is translatable to humans because zebrafish and human ACTC proteins share an incredible 99% sequence identity. To validate zfactc genes as cardiac-specific, I have used CRISPR-Cas9 to produce and characterize zebrafish zfactc knockout lines. Phenotype trajectories of individual CRISPR injected embryos show four trajectories – (1) no clear dysfunction, (2) gradual decline towards death, (3) interval cardiac phenotypes, and (4) sudden death following a cardiac phenotype. Examples of cardiac phenotypes include blood accumulation, pericardial edema, and heart rate differences. This work will provide a zfactc knockout lines to determine the roles of zfactc genes in cardiac development and for future human ACTC rescue experiments. Generating a model for cardiovascular disease will allow scientists to target the associated dysfunctions with precision therapies leading to improved treatments and reducing the global burden of heart failure.

Keywords: CRISPR-Cas9, Cardiac actin, Cardiomyopathy
Acidosis prolongs APD in optically mapped adult zebrafish whole hearts as a result of hERG channel block

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During the early phase of myocardial ischemia, local extracellular acidosis (as low as pH 6.5) can play a part in the initiation of the ischemic cascade leading to cardiac arrhythmia. Previously, we characterized the effect of acidosis on hERG potassium channels in a heterologous expression system and showed that external protons modify numerous gating properties that result in hERG channel loss-of-function. In silico action potential simulations suggest that this could contribute to the development of arrhythmia. To explore this further, we investigated the effects of acidosis on action potentials recorded using optical mapping of isolated adult zebrafish whole hearts at 28oC. Zebrafish hearts represent a good model for human cardiac electrophysiological studies, since action potential morphology, intrinsic heart rate, and QT-interval are similar to those in human hearts, and repolarization is dependent upon hERG function, unlike in murine models. Measuring voltage transients using an optical dye (RH-237), we demonstrate that external acidosis (pHo 6.5) prolonged the rate-matched APD90 by 79 ± 10 ms (n=4). This effect occurred rapidly, within 1 min, and was readily reversed upon restoration of control pHo 7.4, indicative of a direct extracellular effect. Blockade of hERG channels by dofetilide (250 nM) blunted the action potential prolonging effects of acidosis by 55%, demonstrating a role for hERG channels in the acidosis-induced APD90 prolongation. These data indicate that hERG dysfunction during acute acidosis is partly responsible for prolongation of the action potential duration, which may contribute to arrhythmia generation.

Keywords: hERG, Acidosis, Zebrafish, Action potential

Zebrafish vars knockout model for investigating the molecular basis of aminoacyl-tRNA synthetase-related epilepsy

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Epilepsy is a heterogeneous group of seizure disorders affecting about 65 million people worldwide, making it one of the most common neurological diseases. The majority of patients suffering from epilepsy have an underlying genetic cause. Recessive variants in aminoacyl-tRNA synthetases (ARSs) such as glutaminyl-tRNA synthetase (QARS), alanyl- tRNA synthetase (AARS) and lysyl- tRNA synthetase (KARS) have been previously linked to epilepsy. More recently, also recessive variants in valyl-tRNA synthetase (VARS) were reported in patients with a similar phenotype with refractory seizures, developmental delay and microcephaly. Since there is a growing need to study the pathogenesis of epilepsy, we aim to model the disease in genetically modified ars mutant zebrafish lines. To clarify the role of VARS in epilepsy we generated a zebrafish vars knockout model by CRISPR/Cas9 technology. We have investigated the functional consequences of vars defect by performing morphological analysis, locomotor tracking, cognition assay and local field potential recordings. Our results showed that vars HO larvae died prematurely, had microcephaly and microphthalmia, their locomotor activity was decreased and had difficulties to adapt to new environmental conditions in comparison to heterozygote and wild type larvae. Moreover, investigation of abnormal brain activity demonstrated that recurrent spontaneous epileptiform events occurred in ~55% of all HO larvae. This provides a functional proof that vars knockout in a vertebrate in vivo system causes abnormal seizure-like behavior. Rescue experiments are ongoing in order to confirm the specificity of the knockout phenotype.

Keywords: epilepsy, VARS, seizures, CRISPR/Cas9, LFPs
Modeling C9orf72-associated amyotrophic lateral sclerosis in zebrafish

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Amyotrophic lateral sclerosis, a fatal disease associated with motor neuron degeneration, currently has limited therapeutic approaches to combat the disease. Intronic G4C2 expansions in C9orf72, an evolutionarily conserved gene of unknown function, have been identified in a large proportion of familial and sporadic ALS patients and is the most strongly associated gene in ALS/FTD across cohorts. Repeat-associated non-ATG-initiated (RAN) translation of these G4C2 repeats in the sense and antisense direction results in the generation of 5 different dipeptide repeats (DPRs): Poly (GA), (PA), (GR), (PR) and (GP), and these DPRs are a major cause of neurodegeneration observed in ALS. In order to study the toxicity of the DPRs in vivo, DPRs of different lengths (40, 200, 1000) were expressed in zebrafish embryos and the toxicity was scored according to their length. Since in vivo studies indicated GR and PR to be the most toxic DPRs, the first transgenic zebrafish line was generated expressing 100 GR repeats. Expression of the protein was confirmed by western blot analysis. Induction of (GR)100 expression resulted in developmental defects and an impairment of swimming. Studies are underway to analyze the consequences of DPR length, possible motor neuron and interneuron defects and rescue of these phenotypes using drugs tested in other ALS models. Hence, these transgenic lines could serve as useful models to study the pathobiology of C9orf72-associated ALS and for drug screening.

Keywords: Amyotrophic lateral sclerosis, C9orf72, zebrafish, dipeptide repeat toxicity, drug screening

The gonadal soma controls ovarian follicle proliferation through Gsdf in zebrafish

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Aberrant signaling between germ cells and somatic cells can lead to reproductive disease and depends on diffusible signals, including TGFB-family proteins. The TGFB-family protein Gsdf (gonadal soma derived factor) controls sex determination in some fish and is a candidate for mediating germ cell/soma signaling. Results: Zebrafish expressed gsd in somatic cells of bipotential gonads and expression continued in ovarian granulosa cells and testicular Sertoli cells. Homozygous gsd knockout mutants delayed leaving the bipotential gonad state, but then became a male or a female. Mutant females ovulated a few oocytes, then became sterile, accumulating immature follicles. Female mutants stored excess lipid and down-regulated aromatase, gata4, insulin receptor, estrogen receptor, and genes for lipid metabolism, vitellogenin, and steroid biosynthesis. Mutant females produced less estrogen and more androgen than wild types. Mutant males were fertile. Genomic analysis suggests that Gsdf, Bmp15, and Gdf9, originated as paralogs in vertebrate genome duplications. Conclusions: In zebrafish, gsd regulates ovarian follicle maturation and expression of genes for steroid biosynthesis, obesity, diabetes, and female fertility, leading to ovarian and extra-ovarian phenotypes that mimic human polycystic ovarian syndrome (PCOS), suggesting a role for a related TGFB signaling molecule in the etiology of PCOS.

Keywords: Gonad development, GDF9, BMP15, oogenesis, Polycystic ovarian syndrome (PCOS)
SESSION III: Mechanisms of Development & Organ Dysfunction

P3-30

Neurogenesis of Dopaminergic Neurons in a Genetic Model of Parkinson’s Disease

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Parkinson’s Disease (PD) is characterized by dopaminergic (DA) neuron loss in the substantia nigra. PD-genes, including pink1, may regulate neurogenesis, however it is unknown whether DA neurogenesis is altered in PD. The aim of our study was to determine whether zebrafish DA neurons are generated throughout life and to elucidate whether PINK1-deficiency results in impaired neurogenesis. Methods: DA neuron populations within the zebrafish posterior tuberculum (PT), homologous to the substantia nigra, were classified as previously described. EdU pulse-chase analyses, in combination with tyrosine hydroxylase-1 antibody labeling, was used to identify newborn DA neurons in the adult wild type PT (3-, 6- and 12-months of age) and in the pink1/- PT (3-months of age). EdU+/Th1+ neurons were quantitated in DA neuron subpopulations in pink1+/+ and pink1/- brain sections in 3-month-old zebrafish. Results: DA neurons are newly generated in the periventricular nucleus (TPp) and the paraventricular organ (PVO) at young adult stages (3- and 6-months). Other DA subpopulations within the PT (DC2/4) are not newly generated at these time points. TPp and PVO DA neurogenesis at later stages (12-months) is reduced, where no newborn DA neurons were observed in any subpopulation. PINK1-deficiency abrogates dopaminergic neurogenesis in both the TPp (p=0.0078) and the PVO (p=0.0017) at 3-months, and 2-year-old pink1/- zebrafish have fewer DA neurons in all PT subpopulations. Discussion: Our observations (of impaired adult neurogenesis in PINK1-deficient zebrafish) suggests the possible relevance of impaired neurogenesis, including early adult neurogenesis, in PINK1-related PD, which may contribute to disease onset or progression.

Keywords: Zebrafish, Parkinson's, Neurogenesis, Dopamine, Pink1

P3-31

Analysis of Angiopoietin-1 function during zebrafish embryonic neurogenesis

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Growing evidence suggests that angiogenesis and neurogenesis share common regulatory mechanisms during embryonic and postnatal development. Angiopoietin-1 (angpt1) and its receptor tie2 play important roles in the regulation of angiogenesis in mammals. In this study, we first revealed the detailed spatiotemporal expressions of angpt1/2a/2b and their receptors tie1/2. All angiogenic factors displayed a ubiquitous distribution from blastula to gastrula period. At later development stages, more restricted expression was found for both in the head and in the cardinal veins of the trunk. By studying the zebrafish angpt1 mutant line, carrying a premature stop codon causing loss of the fibrinogen-C-terminal domain in angpt1, we observed that angpt1 KO mutants showed a smaller sized brain, eyes and body length, impaired cardiovascular circulation including brain haemorrhage, cardiac edema, significantly slower heart rate and blood flood rate and died within 5 dpf. Aberrant cerebrovascular formations were found in the angpt1 KO mutant while no overt defects were morphologically detected in the trunk vessels. Notably, severe malformations of reticulospinal neurons and hindbrain rhombomeres were also revealed in angpt1 KO mutants. By WISH and qPCR, expression levels of genes related to neurogenesis such Notch1a, nestin, wnt1 and pcna were significantly downregulated whereas the expression of glial markers apoeb and gfap were upregulated. Taken together, in zebrafish targeted destruction of angpt1 causes severe distortions of cerebrovascular development and hindbrain patterning, suggesting that angpt1 acts as a crucial regulator not only in angiogenesis but also in neurogenesis.

Keywords: Angiopoietin-1, neurogenesis
Mechanistic studies of humoral factors from remote ischemic conditioning on inhibition of neutrophil migration using zebrafish models

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To investigate the mechanisms by which remote ischemic conditioning (RIC) exerts its anti-inflammatory effects in zebrafish models. Background: Ischemia/reperfusion from hemorrhagic shock/resuscitation in trauma patients contributes to organ dysfunction. Previous zebrafish studies have shown that RIC protects against organ injury through a humoral factor(s) which alters neutrophil function. We hypothesized that RIC inhibits neutrophil function through a decrease in reactive oxygen species (ROS) production via the up-regulation of the transcription factor Nrf2 and downstream anti-oxidative genes. Methods: Plasma from mice subjected to RIC (4 cycles of 5-min hindlimb ischemia/reperfusion) was microinjected into zebrafish, and neutrophil migration was assessed after tailfin transection. Survival experiments were conducted under lipopolysaccharide (LPS) or H2O2 treatment. Morpholino knockdown of Nrf2a and Nrf2b was used to investigate the relationship between Nrf2 and RIC-induced neutrophil inhibition. Analysis of oxidative stress genes was conducted using the anti-oxidative stress qRT-PCR array. Results: Zebrafish injected with RIC plasma had reduced neutrophil migration and higher survival rates following LPS or H2O2 treatment when compared with control zebrafish. In addition, knockdown of Nrf2a attenuated the anti-inflammatory effect of RIC plasma on neutrophil migration. Moreover, RIC treatment led to up-regulation of anti-oxidative genes including Duox, Myoglobin, and Hmox1. Conclusion: RIC protects against trauma induced inflammation, in part, through a humoral factor(s), which down-regulates neutrophil activity. The inhibition of neutrophil migration may be attributed to the up-regulation of anti-oxidative genes, potentially leading to a decrease in ROS production. Our data suggest that clinical RIC protocols may offer significant clinical benefits to trauma patients.

Keywords: ischemia/reperfusion, remote ischemic conditioning, nrf2, anti-oxidative stress

The Role and Mechanism of Zinc in Regulating Oocyte Maturation in Zebrafish

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Zinc is an important metal element serving both as a structural component, and also as a signaling molecule. Zinc availability plays an important role in oocyte development and function. Our initial data indicated an entirely different mode of action of zinc on meiotic resumption from meiosis I in zebrafish oocyte maturation as compared to that in mammals. Total zinc concentration increased during oocyte maturation as measured by inductively-coupled plasma mass spectrometry. In vitro treatment with ZnCl2 or ZnSO4 of either intact or denuded full grown oocytes significantly induced germinal vesicle breakdown (GVBD) in a time-, dose- and stage-dependent manner. Zinc depletion caused by TPEN significantly blocked spontaneous GVBD, and this could be rescued when zinc was added back into the system. The maturation defect phenotype observed in the luteinizing hormone beta-subunit homozygous mutant (lhb-/-) could also be rescued by zinc. Testosterone treatment significantly increased total zinc content in the maturing oocyte and follicular cells, and the maturing action of zinc could be blocked by TPEN. These results suggest the potential involvement of zinc in androgen-mediated oocyte maturation in zebrafish. Analysis of a membrane androgen receptor/zinc importer ZIP9 showed that it is highly localized in the ovarian follicular cells and its expression in the follicles is highly responsive to hCG administration in zebrafish in vivo. Further experiments are required to confirm the potential involvement of ZIP9 in this zinc-dependent androgen-mediated oocyte maturation in zebrafish. Our study provides evidence for a novel pathway in regulating oocyte maturation using zebrafish as a model.

Keywords: Zinc, ZIP9, androgen, oocyte maturation, zebrafish
Function of Transmembrane 88, a novel Wnt regulator, during heart development

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Transmembrane protein 88a (Tmem88a) was identified in a Gata5/6 double knockdown screen as a downstream target of GATA factors during cardiac progenitor specification in zebrafish. We showed that tmem88a morphant fish display cardiomyopathy that can be rescued by forced expression of the WNT inhibitor Dkk1. Previous cell culture studies suggested that the C-terminal PDZ binding motif of Tmem88 can bind to Dvl, a positive WNT signaling transducer, leading to a hypothesis that Tmem88 sequesters Dvl and inhibits it from transducing WNT signaling. However, we discovered that Dvl is dispensable for the ability of Tmem88 to suppress WNT signaling, and provide evidence that Tmem88 functions downstream of Dvl to activate GSK3β, which raises interesting questions regarding how a transmembrane protein localized at the plasma membrane activates a cytosolic complex. We generated a targeted null mutation of tmem88a in zebrafish, which unlike morphant knockdowns is surprisingly tolerated and mutant fish appear phenotypically normal. However, we discovered a novel transmembrane WNT inhibitor, Igsf9, that is strikingly up-regulated in the tmem88a mutants and may compensate for loss of Tmem88a. Igsf9 also has a C-terminal PDZ-binding motif, but this appears to be dispensable for modulating WNT signaling. Structure/function comparisons of Tmem88 and Igsf9 will provide insight into how transmembrane proteins regulate downstream signaling in the WNT pathway and their roles in the heart development.

Keywords: Tmem88, Wnt signaling, heart development

A metabolic switch controls intestinal differentiation downstream of Adenomatous Polyposis Coli (APC)

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Elucidating signaling pathways that regulate cellular metabolism is essential for a better understanding of normal development and tumorigenesis. Recent studies have shown that Mitochondrial Pyruvate Carrier 1 (MPC1), a crucial player in pyruvate metabolism, is downregulated in colon adenocarcinomas. Utilizing zebrafish to examine the genetic relationship between MPC1 and APC (Adenomatous Polyposis Coli), a key tumor suppressor in colorectal cancer, we found that apc controls the levels of mpc1 and that knock down of mpc1 recapitulates phenotypes of impaired apc function including failed intestinal differentiation. Exogenous human MPC1 RNA rescued failed intestinal differentiation in zebrafish models of apc deficiency. Our data demonstrate a novel role for apc in pyruvate metabolism and that pyruvate metabolism dictates intestinal cell fate and differentiation decisions downstream of apc.

Keywords: MPC1, APC, metabolism, differentiation, cancer
Dissecting Gata5/6 mediated regulatory mechanism of early cardiac lineage commitment

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Deciphering the earliest events of heart development is crucial for developing treatments to cardiovascular disease. The Gata5/6, zinc finger transcription factors have been shown to be key regulators in vertebrate heart development, with simultaneous loss of gata5/6 in zebrafish resulting in a heartless phenotype. While several studies have shown that gata5/6 regulate later stages of heart development, the regulatory mechanism of Gata5/6 activity in early cardiac lineage, prior expression of nkx2.5 remains unknown. Here we investigated gata5/6 mediated regulatory mechanisms by a combination of developmental biology and genomics approaches. By live imaging and quantitative analysis, we showed that there is a delayed arrival of mesendoderm cells to midline when gata5/6 are knocked down or mutated. To further understand how gata5/6 regulates cell migration during gastrulation, we conducted bulk population mRNA-seq and identified down-regulation of several genes encoding cell adhesion molecules. Currently we are characterizing their roles in cell migration during gastrulation. We next aimed to understand how Gata5/6 exert cardiac-specific activities while being broadly expressed in mesendodermal cells. We conducted single-cell mRNA-seq on a mesendoderm population from multiple gastrulation stages. We identified several novel candidate cardiac genes, the expression of which was confirmed by RNA in situ. Upon gata5/6, we observed that the expression pattern of these candidate genes were greatly diminished. We are characterizing the functional roles of several candidate genes by generating zebrafish mutants using CRISPR/Cas9. Overall, our study provided further insights into the role of Gata5/6 in early heart development.

Keywords: Gata5/6, cardiac lineage, migration, specification

The Apelin receptor modulates Nodal signaling to direct the development of cardiac progenitors

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Cardiogenesis requires a complex program of transcriptional and epigenetic events to specify cardiac progenitor cells (CPCs) from the bipotent mesendoderm. Previous work has shown that the Apelin receptor (Aplnr) plays a key role in this process as loss of Aplnr function prevents heart development. Aplnr mutants show a loss of CPCs as well as a reduction in endodermal progenitor cell number. These consequences have been attributed to migratory failures of presumptive CPCs as well as decreased levels of Nodal signaling. Interestingly, Nodal signaling is critical in patterning the mesoderm and endoderm from the bipotent mesendoderm. Precisely how Aplnr functions to direct CPC development at a molecular level is currently not understood. We have studied the role of Aplnr and its ability to modulate Nodal signaling in directing the development of CPCs from the bipotent mesendoderm in zebrafish. Using a gata5:EGFP transgenic line, which marks the mesendoderm during gastrulation, in combination with Aplnr knockdown we are able to investigate changes in the transcriptome and epigenetic landscape of mesendoderm cells that are responsible for disrupted cardiac development. Loss of Aplnr or its pertinent ligand, Elabela, decreases the intensity of the Nodal signaling gradient during the initiation of gastrulation. Results from single cell RNA-sequencing and epigenetic profiling of Aplnr loss of function embryos will be presented.

Keywords: cardiovascular development, cardiac progenitors, Apelin receptor, Nodal signaling
Vision and systemic defects associated with loss of Vps11 function

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Leukodystrophies (LD) and genetic Leukoencephalopathies (gLE) are genetic disorders affecting the white matter (myelin) in the central nervous system. LDs and gLEs progressively affect the motor and sensory systems, including the visual systems. Parents of affected children first note visual problems as a gradual loss in the ability of their child/children to track visual cues. Vision slowly worsens over subsequent years, likely due to the loss of myelin in the optic nerve and brain, termed cortical blindness. An MRI is typically diagnostic for myelination defects, but a clear diagnosis of disease-specific LDs and gLEs remains a challenge, and the majority of LDs and gLEs have an unknown genetic origin. We recently identified a mutation in VPS11 as a causative allele in the gLE phenotypes observed in five individuals from three unrelated Ashkenazi Jewish (AJ) families. Our analysis indicates a carrier rate of 1:250 AJ individuals. VPS11 functions in a complex of four C-VPS proteins, which are conserved from yeast to humans, and control critical cellular processes in the endolysosomal and autophagy pathways. We previously characterized a zebrafish vps11 mutant and have recently discovered that it shares many of the phenotypes of affected human patients. Current work focuses on utilizing the zebrafish model to characterize the pathology underlying the vision loss associated with loss of C-Vps function. This includes characterizing the defects in endolysosomal and autophagy pathways, myelination defects in the CNS, loss of retinal and CNS neurons, and motor defects.

Keywords: myelin, leukoencephalopathy, lysosome, vision, vps11

The Calcium Binding Proteins S100A1 and S100B Modulate Cardiac Development in the Zebrafish

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Zebrafish are a superior model for studying vertebrate embryonic development and gene function. The S100 family of calcium binding proteins consisting of 30 members of which S100A1 and S100B are the most extensively studied, regulate a variety of intracellular functions including cell proliferation, differentiation, calcium homeostasis, inflammation and cell death. The functional roles of S100A1 and S100B during in vivo cardiac development are yet to be determined. We examined the consequences of morpholino (MO)-induced single and double gene knockdown of S100A1 and S100B during zebrafish development. Single microinjection of MO for S100B and S100A1 resulted in swelling of the body cavity with a fluid-filled like sac reminiscent of edematous changes in mammalian models of heart failure. Additionally, 5 day old MO-zebrafish displayed atypical positioning of the heart chambers and abnormal cardiac looping is present such that the normal left right orientation of the two chambered heart is lost with atria and ventricle appearing in a single plane. MO-zebrafish displayed cardiac apoptosis as quantitated by an increase in the BAX/BCL2 apoptotic ratio. Functionally, a reduction in ventricular calcium transients is demonstrated in MO-zebrafish that may explain the observed reduced heart rate compared to wild type (WT). In the heart of WT zebrafish, S100A1 and S100B heterodimerize and interestingly, double knockdown of S100A1 and S100B resulted in a WT phenotype with preserved ventricular calcium transients. These results are in line with better survival and improved cardiac function observed in S100A1-S100B (double)-knock out (KO) mice compared to single gene KO following myocardial infarction.

Keywords: Cardiac development, S100 calcium binding proteins, Calcium transients, Apoptosis
Endoplasmic Reticulum Resident Protein 44 (ERp44) Deficiency in Mice and Zebrafish Leads to Cardiac Developmental and Functional Defects

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Background Endoplasmic reticulum (ER) resident protein 44 (ERp44) is a member of the protein disulfide isomerase family, is induced during ER stress, and may be involved in regulating Ca²⁺ homeostasis. However, the role of ERp44 in cardiac development and function is unknown. The aim of this study was to investigate the role of ERp44 in cardiac development and function in mice, zebrafish, and embryonic stem cell (ESC)-derived cardiomyocytes to determine the underlying role of ERp44. Methods and Results We generated and characterized ERp44−/− mice, ERp44 morphant zebrafish embryos, and ERp44−/− ESC-derived cardiomyocytes. Deletion of ERp44 in mouse and zebrafish caused significant embryonic lethality, abnormal heart development, altered Ca²⁺ dynamics, reactive oxygen species generation, activated ER stress gene profiles, and apoptotic cell death. We also determined the cardiac phenotype in pressure overloaded, aortic-banded ERp44+/− mice: enhanced ER stress activation and increased mortality, as well as diastolic cardiac dysfunction with a significantly lower fractional shortening. Confocal and LacZ histochemical staining showed a significant transmural gradient for ERp44 in the adult heart, in which high expression of ERp44 was observed in the outer subepicardial region of the myocardium. Conclusions ERp44 plays a critical role in embryonic heart development and is crucial in regulating cardiac cell Ca²⁺ signaling, ER stress, ROS-induced oxidative stress, and activation of the intrinsic mitochondrial apoptosis pathway.

Keywords: apoptosis • Ca²⁺ • ERp44/ • ESC-derived cardiomyocytes • heart development and cardiomyopathy

The overlapping functions of gonadotropin signaling in zebrafish spermatogenesis

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Gonadotropin signaling plays an important role in spermatogenesis of vertebrates. However, most of the information comes from studies in mammals. Using a TALEN-mediated gene knockout approach, we have successfully generated four gonadotropin- or receptor-knockout zebrafish lines (lhb, fshb, lhr and fshr). Interestingly, all male fish of the four mutant lines are fertile, a phenomenon which is very different from that in mammals. To study the role of Lh and Fsh signaling in zebrafish spermatogenesis, we have generated two double knockout mutant lines (lhb;lhr and fshb;fshr). The male fish of both double mutant lines are morphologically and histologically normal as well as functionally fertile, indicating that the two signaling pathways could compensate each other, and either Lh or Fsh signaling alone is sufficient to support spermatogenesis in zebrafish. Moreover, the specificity of the two gonadotropins on the two receptors was also explored. Investigations on two other double mutant lines (fshb;lhr and lhb;fshr) revealed that the males of both lines are fully fertile with normal spermatogenesis, suggesting cross-activation between the ligands and the receptors. Such cross-reactivity was further substantiated by three different assay systems including transient transfection in cells, stable transfection in cells and microinjection into zebrafish embryos. Our results suggest the highly overlapping functions of gonadotropin signaling in zebrafish spermatogenesis. This project is supported by the Hong Kong Research Grants Council (CUHK 14103014).

Keywords: Lh, Fsh, testis, spermatogenesis, zebrafish
The investigation into the role of dre-miR-146b in zebrafish ovulation

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MicroRNAs are small non-coding RNAs which are usually about 22nt in length and mediate gene regulation post-transcriptionally through binding to the 3’UTR of target mRNAs. In mammal, the roles of microRNAs in reproduction have been widely studied. However, evidence regarding the involvement of microRNAs in fish reproduction is scarce. Here we investigated the role of microRNAs in zebrafish ovulation. Previously we have established a luteinizing hormone beta-subunit homozygous mutant (lhb-/-) zebrafish line and found that the follicles of the mutant females were arrested at the full grown (FG) stage and could not undergo the subsequent maturation and ovulation stages. From our microarray results, we have identified a dre-miR-146b with an increased expression level in the lhb-/- female zebrafish when compared to the wild type control. This was further confirmed by real time PCR. Thus we postulated that a low level of dre-miR-146b might be necessary during normal development of the zebrafish ovary. After in vivo administration of hCG, which can induce ovulation, into wild type zebrafish, the expression of dre-miR-146b was significantly decreased and maintained at a very low level until ovulation. Meanwhile, we have also injected dre-miR-146b mimics into wild type zebrafish, and observed significant ovulation failure. Further studies will focus on the targets of dre-miR-146b and the construction of the dre-miR-146b overexpression zebrafish model. This study will provide insights into the role of dre-miR-146b in fish reproduction.

**Keywords:** dre-miR-146b, ovulation, zebrafish

Ancient enhancers drive cardiac expression patterns during early vertebrate heart development

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Highly conserved transcription factor networks control heart development, however the cis-regulatory elements (CREs) that control the spatial and temporal cardiac gene expression are only beginning to be uncovered. Here we set out to discover CREs active at the earliest stages in heart development in vivo using comparative epigenomics and the zebrafish model system. To isolate early cardiac progenitor cell populations in zebrafish we used a recently characterized mouse enhancer to drive GFP expression in zebrafish immediately after gastrulation. Results obtained using open chromatin profiling, single cell mRNA-seq, lineage tracing, in situ hybridization, and cardiac transcription factor Gata5/6 knockdowns, support that we purified a population of cells enriched for cardiac progenitor cells. We uncovered several thousand open chromatin regions specific to these GFP positive cells, ~125 of which could be aligned to human and/or mouse non-coding open chromatin regions using both direct and indirect alignments. These anciently conserved open chromatin regions were enriched for Polycomb repressive complex 2 binding and developmentally active transcription factors. In vivo assays confirmed 17/21 of these anciently conserved open chromatin regions drive cardiac related gene expression patterns during early zebrafish development. While more than a third of these ancient enhancers could only be aligned indirectly to human, many of these human orthologous CREs recapitulated zebrafish enhancer expression patterns indicating a high degree of functional conservation in the absence of overt sequence conservation. Our study adds a set of ancient enhancers to the lexicon of CREs that are active during the early stages of vertebrate heart development.

**Keywords:** heart development, ATAC-seq, cis-regulatory elements, comparative epigenomics
Cardiac transcriptome profiling during regeneration in zebrafish

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Myocardial infarctions (MIs) are a prevalent form of cardiovascular disease (CVD) and are a leading cause of global mortality. MI induces cardiomyocyte (CM) necrosis and fibrosis after ischemic injury. Therapeutics can attenuate MI progression, but they fail to induce CM proliferation and leave patients at risk for recurrent MI and additional CVDs. Mammalian CMs proliferate during development, but become post-mitotic shortly after birth and fail to proliferate after injury. Understanding which genes are active during development that regulate CM proliferation may offer insights into stimulating mitosis in adult CMs. Unlike mammals, adult zebrafish possess robust cardiac regeneration, but also share considerable homology with mammalian genes and are excellent genetic models. Next generation sequencing studies demonstrate that many conserved genes become upregulated after injury in zebrafish, but their exact functions are not completely characterized. In this study, we performed RNA-seq on uninjured and amputated (3dpa) AB* ventricles and validated gene upregulation using semi-quantitative PCR. foxm1 and cenpf were among the genes upregulated after amputation. Foxm1 is a transcription factor that regulates the expression of pro-mitotic genes and Cenpf is a kinetochore-binding protein that assists in mitosis, vesicle trafficking, and cell migration. These genes are expressed in mammalian CMs during development, but are significantly downregulated 7 days after birth. We hypothesize that foxm1 and cenpf are critical for CM proliferation and their loss will affect cardiac development and regeneration in zebrafish. Currently, we are characterizing the regenerative profile of cenpf-/- adults after ventricular amputation by quantifying CM proliferation and scar resolution.

**Keywords:** cardiac regeneration foxm1 cenpf RNA-seq

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**SESSION V: Chemical Biology, Pharmacogenetics & Drug Discovery**

Tools for brain-wide mapping, improved surface ECG and clarifying organs in the zebrafish

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Zebrafish has been used extensively as an animal model of human diseases. We have worked as a multidisciplinary team to develop tools to enable us to explore the use of zebrafish as a model for personalised and precision medicine. We used these tools to investigate the dynamics of the zebrafish embryonic development and adult organ regeneration. We fabricated a microfluidics chip by incorporating fluid dynamics and enabled the processing of tens of larvae simultaneously for microscopic imaging at single-cell resolution involving no gel-fixation nor anesthesia. We named this device the “Fish-trap technology” and used it to record global activities at single-cell resolution in the brains of the transgenic zebrafish with the calcium sensor (GCaMP5G) coupled with the reporter GFP. We improved on the design and developed a versatile Fish-on-Chip platform to record other parameters, such as larval behavior and heart beats in defined orientations for arrays of larvae. We also improved the surface ECG recording for adult zebrafish and showed that both ventricular amputation and cryoinjury induced ST segment depression and affected QRS duration. Interestingly, only cryoinjury, and not amputation, decelerated the heart rate. Recently, we have improved on clarification protocols to enable whole-heart 3D imaging at single-cell resolution with the lightsheet microscope and showed that revascularization of the cryoinjured heart is initiated within 24 hours after injury. Furthermore, we have developed a clearing protocol which allows 3D heart imaging in an intact adult fish.

**Keywords:** brain-wide activity, microfluidics chip, surface ECG, clarified organs, lightsheet microscopy
Deciphering histone deacetylase 6 (HDAC6) interaction with human Tau using zebrafish

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In Alzheimer’s disease (AD) patients, it has been shown that HDAC6 is functionally impaired and that Tau binds and inhibits its catalytic activity, as revealed by hyperacetylation of its main target, α-tubulin (Ding et al., 2008; Perez et al., 2009). Therefore, disrupting the interaction between Tau and HDAC6 in order to restore its activity could be of therapeutic interest. Here we characterized the functional link between Tau and HDAC6 in zebrafish (zf) larvae. First, the conserved role of zfHDAC6 in tubulin deacetylation was verified by western blot using either pharmacological inhibition or morpholino-mediated knockdown of zfHDAC6. Furthermore, overexpression of human HDAC6 decreased the acetylation of zebrafish α-tubulin. Second, co-immunoprecipitation experiments suggest that zfHDAC6 interacts in vivo with human Tau in transgenic zfTAUP301L larvae, a zebrafish tauopathy model (Paquet et al., 2009). Interestingly, data from human cell lines and cell-free assays also supported a physical interaction between these two proteins. Third, zfHDAC6 morpholino-induced knockdown did not modify phenotypes of zfTAUP301L, namely neuronal cell death and shortened motoneuron axonal extensions. Finally, pharmacological inhibition of HDAC6 in tauopathy fish did not modify axonal length defects and - not in line with the working hypothesis - rescued neuronal death. As such, our zebrafish results do not support a role for HDAC6 in modulation of Tau pathology, in line with recent data from AD brain samples also pointing to an absence of any functional link between AD and HDAC6 dysfunction. Together, our data illustrates how zebrafish model organism can contribute to proteinopathy drug discovery programs.

Keywords: Alzheimer's disease, HDAC6, Tau, proteinopathy

Validating Nuclear Receptor Activity in Unique Zebrafish Model of InDanio Bioscience Inc. and Highlighting Commercial and Research Applications

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The nuclear receptor (NR) superfamily acts as a ligand-mediated transcription factor responsible for altering gene expression. Targeting NRs have shown great therapeutic success and the investigation for novel ligands is in demand. InDanio Bioscience Inc. provides researchers a unique zebrafish model based on a heat pulse-induced gene expression of the human NR ligand-binding domain with a GAL4 DNA-binding domain. When activated, the protein binds to the GAL4 activating sequence and expresses green fluorescent protein (GFP). A validation study was conducted to assess the fidelity of the transgenic model. Zebrafish DNA was isolated from the dorsal fin. PCR was conducted to identify GFP+ zebrafish. GFP+ zebrafish were crossed with wild type fish on day 1 and embryos were collected on day 2. On day 3, zebrafish embryos were treated with known agonist and incubated in 37°C for 1 hour and were observed under fluorescent microscopy on day 4 for GFP expression. Glucocorticoid receptor (GR), thyroid receptor beta (TR-beta), and retinoic acid receptor gamma (RAR-gamma) lines were used for this study. Zebrafish embryos showed GFP expression upon activation of NR when crossed with GFP+ zebrafish and wild type. Embryos exhibited GFP expression upon treatment of 1 uM dexamethasone for GR line, 1 uM TRIAC for TR-beta line, and 1 uM TTNPB for RAR-gamma line. A protein purification and antibody-based assay may further support validating the model. Downstream applications include, but are not limited to, drug screening, investigation of orphan NRs, and isolation of ligand-trapped NR for drug validation and structural studies.

Keywords: nuclear receptor; drug discovery; transgenic model; drug screening; metabolic disease
Towards the generation of a digital adult zebrafish brain atlas

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Adult zebrafish are an increasingly popular and important animal model in neuroscience research. As a genetically tractable vertebrate with a sophisticated behavioral repertoire and high genetic similarity to humans (70%), zebrafish are an ideal system for the study of neurobiology and behavior in both health and disease. However, although a comprehensive brain atlas for adult zebrafish was published over 20 years ago in book form, the field is lacking a digital brain atlas necessary for automated whole brain studies. Here, we report on our progress in creating such an atlas by combining tissue clearing techniques, fluorescent labelling, light sheet microscopy, automated image registration, and manual parecellation. We anticipate that the generation of such an atlas will significantly increase the utility and sophistication of adult zebrafish as an animal model in neuroscience research.

**Key words:** adult, brain, tissue clearing, light sheet microscopy

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Developing a zebrafish xenotransplant model of neuroblastoma

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Neuroblastoma is the most common extracranial solid tumour in children. While low-risk disease has a good prognosis, patients with high-risk disease have a long-term survival rate of <50%. Treatment includes multimodal chemotherapy, including cisplatin, with risk of hearing and kidney toxicity in survivors. Zebrafish xenotransplantation has emerged as a robust preclinical platform for human cancer. Xenotransplantation has been used by our group to study cancer cell proliferation and in vivo drug response in lung cancer, prostate cancer, Ewing sarcoma, and neuroblastoma (El-Naggar et al., 2015; Melong et al. submitted; Leung et al., submitted). Post-injection, cisplatin treatment in the embryo water was superior to other routes of administration. Zebrafish larvae (48 hours post-fertilization) were xenotransplanted with fluorescently labelled human neuroblastoma SK-N-AS, IMR-32 or SK-N-SH cell lines in the yolk sac. SK-N-AS and IMR-32 cells proliferate over 48h. The addition of cisplatin (0.2mM) to the embryo water attenuated SK-N-AS cell growth more significantly than that of the IMR-32 cells. Preliminary results suggest that SK-N-SH cells follow a similar trend. Current efforts are aimed at correlating cisplatin sensitivity in the xenotransplantation model with in vitro EC50 for these cell lines. As we have done previously with patient-derived leukemia samples (Bentley et al., 2015), neuroblastoma bone marrow metastases will be transplanted into the zebrafish model, to determine if these results are conserved in a more heterogeneous tumour. These studies will establish zebrafish xenotransplantation as reliable assay for identifying adjuvant compounds that can protect against cisplatin-induced toxicities without compromising efficacy in neuroblastoma.

**Keywords:** Neuroblastoma/xenotransplantation/cisplatin/chemotherapy/pediatric cancers
A simple automated system for appetitive conditioning of zebrafish in their home tanks and studying underlying neural activation

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Zebrafish are emerging as a novel model for studying learning and memory due to the accessibility of molecular tools, rich repertoire of behaviours, relatively simple neuronal circuits, reasonable cost, and usefulness for high-throughput screens. However, the number of behavioural paradigms that minimize handling stress and are well suited to the social nature of these fish is limited. We developed an automated learning paradigm to condition groups of adult and juvenile zebrafish rapidly in their home tanks in a standard zebrafish facility. Fish exhibited significant conditioned responses as early as the 5th trial, learning that the auditory stimulus (20 seconds of alternating half-second ascending and descending 100-1000 Hz sweeps) was a predictor for the presentation of food at the water surface at one end of the tank. Control zebrafish, for which the auditory stimulus was explicitly unpaired with food, displayed no comparable responses. Memory of the association persisted for at least 2 days after training when fish were tested either as groups or as individuals. The 2 day retention in juveniles (30 days post-fertilization) was associated with increased immunoreactivity to phosphorylated extracellular signal-regulated kinase (pERK), a known marker of neural activity, in the dorsolateral telencephalon. This simple paradigm permits scalable conditioning of zebrafish with minimal human intervention and reduced variability. In addition, these results support the use of pERK to examine the neural correlates of learning and memory.

Keywords: High-throughput, Screening, Learning, Memory, Behaviour

Zebrafish Reverse Translation Models for Major Depressive Disorders (MDD)

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Zebrafish has emerged as a robust vertebrate model for depression studies given the physiological similarity to humans in neurotransmitter systems, neuroanatomical structures, and genetic overlap. This study applies a Reverse Translation strategy to validate clinically identified MDD biomarkers in zebrafish and generate models and reporter line for mechanistic studies and drug screens. From a Canadian Biomarker Integration Network in Depression Duloxetine patient study, a number of microRNA biomarkers have been identified as antidepressant responders, including miR-146a, miR-146b and miR-24 that are conserved in zebrafish. In zebrafish, we validated that these miRNA are duloxetine responsive by real-time PCR. The goal of this study is to generate zebrafish models and reporter lines that mimic genetic changes in MDD patients with easy biological readout for drug screens. Using Tol2 transgenesis, we are generating zebrafish transgenic lines containing miR-24 target recognition sequence downstream of GFP. The endogenous zebrafish miR-24 will regulate the expression of GFP reporter, which can serve as an efficient readout for drug screens. Since the zebrafish miR-146a and miR-146b have the same target site, we focus our study on miR-146a. We are currently working on generating a transgenic line by knocking in a fluorescent gene downstream of miRNA-146a using the CRISPR/Cas9 system. This line will report endogenous miR-146a expression by measuring fluorescent intensity. The use of zebrafish as a preclinical model to test MDD targets would represent a significant advancement in the field. Generation of transgenic zebrafish with fluorescent readout will provide an opportunity for rapid drug screening against MDD.

Keywords: Zebrafish, CRISPR, Transgenics, Micro RNA, Drug Screening
Girinimbine from Murraya koenigii - currying therapy for cancer

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Therapy that can potentially target apoptosis, inflammation and/or angiogenesis is now being strongly considered in treatment of cancer. Murraya koenigii, the curry leaf tree, is a tropical to sub-tropical shrub from the Rutacea family. Its leaves are an important ingredient in curries and other South and South-East Asian dishes and has also been used in Ayurvedic medicine for centuries. Here we report that girinimbine, a carbazole alkaloid isolated from M. koenigii, induced apoptosis and inhibited inflammation and angiogenesis in vitro as well as in vivo. In human colon cancer cells (HT-29), girinimbine induced apoptosis without any significant cytotoxicity in normal colon cells. In vitro anti-inflammatory action was evidenced by significant dose-dependent girinimbine inhibition of nitric oxide production in LPS/IFN-gamma-induced cells while anti-angiogenic activity was confirmed by girinimbine inhibition of HUVECs cell proliferation, and endothelial cell invasion, migration, tube formation, and wound healing. In vivo studies using girinimbine showed a significant number of apoptotic cells in zebrafish embryos after a 24-hour treatment period, while in carrageenan-induced peritonitis in mice, oral pre-treatment with girinimbine inhibited neutrophil migration and reduced pro-inflammatory IL-1β and TNF-α cytokine levels in peritoneal fluid. The anti-angiogenic potential of girinimbine was evidenced by inhibition of blood vessel formation in zebrafish embryos. Taken together, these results showed that girinimbine could effectively induce apoptosis, as well as suppress inflammation and angiogenesis, which strongly suggest that girinimbine could be a potential chemopreventive and/or chemotherapeutic agent.

Keywords: targeted therapy, cancer, apoptosis, inflammation, angiogenesis, girinimbine, natural products

Towards a zebrafish serotonin receptor pharmacology

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Potential new pharmacotherapeutics for smoking cessation therapy can be identified in simple neurobehavioral assays using larval zebrafish. In mammals, serotonin receptors (htr)s of type 2a (htr2a) and 2c (htr2c) have been implicated in regulating different states of tobacco dependence, thus representing potential targets for treating tobacco dependence. In larval zebrafish htr2a and htr2c receptors could be involved in attenuating the acute response to nicotine as behavioral experiments indicate. However, the pharmacological specificity of zebrafish htrss is unknown. In addition, because of a teleost genome duplication event several but not all human htr genes have duplicate orthologs in the zebrafish genome including the type 2a and type 2c (htr2aa, htr2ab, htr2cl1 and htr2cl2). Cloning and sequencing of entire coding regions of zebrafish serotonin receptor genes (htr2cl1, htr2aa, htr2ab) confirmed a 55 to 70% identity between the deduced amino acid sequences of zebrafish with mouse and human amino acid sequences. A syntenic analysis provides limited information regarding the similarity between human and zebrafish serotonin receptors. Expression of zebrafish htrss in cultured cells will enable the synthesis and isolation of htr proteins for pharmacological binding assays which are being carried out to determine the specificity of htr2a and htr2c serotonin receptor agonists and antagonists. Thus, larval zebrafish show promise for the development of new serotonin receptor pharmacotherapeutics for smoking cessation therapy. A pharmacological characterization will provide insight into functional similarities between human and zebrafish serotonin receptors.

Keywords: serotonin, receptor, nicotine, behavior, screening
Studying Depression with Zebrafish

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Antidepressants (ADs) are the most common treatment for major depressive disorder (MDD). However, only about 30% of patients experience adequate response after a single AD trial, and this variability remains poorly understood. Here, we investigated microRNAs (miRNAs) as biomarkers of AD response, and their applicability for reverse translation and high-throughput drug discovery in zebrafish. Targets were discovered by small RNA-sequencing in paired samples from MDD patients enrolled in a large, randomized placebo-controlled trial of duloxetine collected before and eight weeks after treatment. Using a combination of bioinformatics, mRNA studies and functional in vitro experiments, we also identified downstream genes of the MAPK/Wnt systems with significant dysregulation. These results were replicated for both zebrafish subjected to a novel behavioural model which can differentiate depression-like symptoms from anxiety, and validated with 7 clinically used antidepressants. Phenotypic target reporter fish in development utilize a fluorescent reporter with 3’ untranslated region anti-target sequences that are degraded without treatment.

Keywords: Depression, behavior, miRNA

Validation and Mechanistic Studies of Gluconeogenesis Regulators Identified from Zebrafish Chemical Genetic Screens

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High levels of phosphoenolpyruvate carboxykinase 1 (pck1) are associated with the development of type 2 diabetes while improved insulin sensitivity is observed in mice with decreased pck1 level. Using pck1 reporter zebrafish lines, we have previously screened 727 FDA-approved compounds and identified ten lead compounds with potential of decreasing pck1 gene expression. We used luminescent and fluorescent zebrafish, Tg(pck1:Luc2) and Tg(pck1:Venus), to rank the compounds, and used diabetic mouse models for further validation. Ten lead compounds were ranked based on their ability to down-regulate pck1 gene expression and lower glucose levels in larval zebrafish. Two top lead compounds (E-1 and E-2) were selected for further validation studies in mouse models. 6-week old mice were on high fat diet (HFD) feeding for 3 weeks to mimic the pre-diabetic conditions followed by five consecutive intraperitoneal (ip) STZ injections (50mg/kg). Both 12-week old healthy and diabetic mice were treated with E-1 (3 or 15mg/kg) through ip injection (once/day for 5 days). On the last day of the treatment, mice were subjected to intraperitoneal pyruvate tolerance test (iPTT). Negative control group received saline (10ul/g) and metformin (50mg/kg) was used as a positive control. E-1 treatment led to significantly improved glycemic control in diabetic mice as demonstrated by pyruvate tolerance tests. Here we show one possible pathway of E-1; regulation of hepatic gluconeogenesis through insulin-dependent down-regulation of FoxO-1 and thus pck1. Experiments are current underway to examine the effects of E-2 on glucose metabolism in mice. Our study has potential to develop new anti-diabetic drugs.

Keywords: gluconeogenesis, pck1, zebrafish, pyruvate tolerance test, diabetes
Floor Plan

2nd Floor Exhibition Hall

Li Ka Shing Knowledge Institute, St. Michael’s Hospital

209 Victoria Street, Toronto, ON, M5B 1T8

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* Presenters may use Room 213 to test out their presentation slides