Transgenerational analysis of embryonic heat exposure in Japanese quail

Anais Vitorino Carvalho, Coralie Gimonnet, Christelle Hennequet-Antier, Benoit Piegu, Aurélien Brionne, Sabine Crochet, Nathalie Couroussé, Thierry Bordeau, Julie Lemarchand, Paul Constantin, et al.

www.epigenetic-inheritance-zurich.ethz.ch

EPIGENETIC INHERITANCE SYMPOSIUM 2019

Impact for Biology and Society

26-28 August 2019
ETH Zurich, Switzerland

Universität Zürich[UZH] | ETH zürich
JOHN TEMPLETON FOUNDATION
FNSNF
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Important information

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Venue

Auditorium Maximum (HG F 30)
ETH Zurich Main Building
Rämistrasse 101
8092 Zurich
www.ethz.ch

Summary of the previous symposium

Transgenerational epigenetic inheritance: from biology to society — Summary Latsis Symposium Aug 28–30, 2017, Zürich, Switzerland

Johannes Bohacek, Olivia Engmann, Pierre-Luc Germain, Silvia Schelbert, Isabelle M Mansuy

Environmental Epigenetics
Volume 4, Issue 2, April 2018
DOI: 10.1093/eep/dvy012

WELCOME

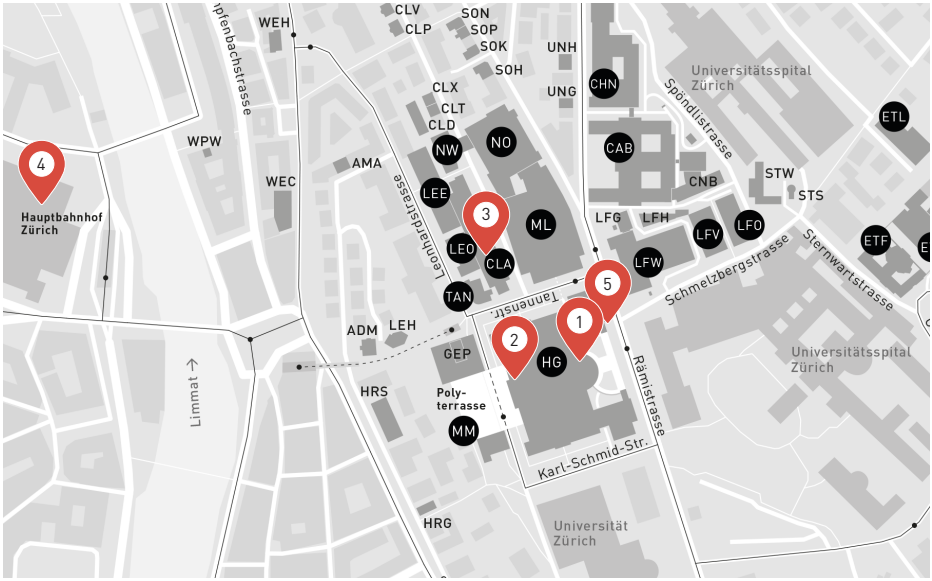
Dear colleague, student and friend,

It is a great pleasure to welcome you to the 2019 Epigenetic Inheritance Zurich symposium, as a follow-up of the Latsis symposium that we organized in August 2017. This year again, the symposium will feature major aspects of epigenetic inheritance across different disciplines, from genetics/epigenetics to metabolism, behavioral science, bioinformatics and social science, in humans and various animal models. It will discuss new findings and discoveries, highlight challenges of the discipline and reflect on perspectives for biology, medical research and the society. It will offer keynote lectures from leaders in the field, short and flash talks, poster sessions with an award to the best poster, a workshop "Meet the Experts", and a guided tour to the Functional Genomics Center Zurich.

I hope that you'll enjoy the symposium, and find it inspiring for your research and your thinking about the biology of heredity. I wish you a great and productive time in Zurich and warmly thank you for participating.

Isabelle Mansuy

VENUE



1. Epigenetic Inheritance Symposium, ETH Zurich, HG F 30, Rämistrasse 101, 8092 Zurich
2. Terrace Dinner, ETH Zurich, HG K 30.5, Rämistrasse 101, 8092 Zurich (upon registration only)
3. Workshop, ETH Zurich, CLA J 1, Tannenstrasse 3, 8092 Zurich (upon registration only)
4. Zürich HB / Zurich main station
5. PubliBike station

Direction for Public Transportation
Tram Nr. 6, 10, 3 || From Zurich main station to ETH/Universitätsspital || Ticket: Zone 110

PROGRAM

Monday 26.08.2019

07:45 – 08:45	Registration

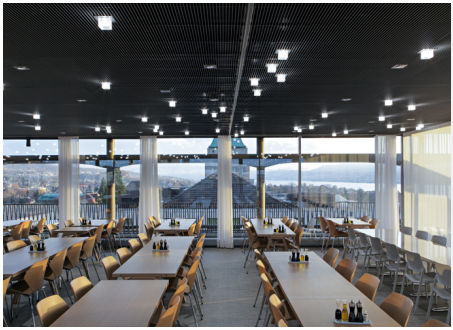
Introduction

08:45 – 09:00	Isabelle Mansuy, Professor in Neuroepigenetics, University and ETH Zurich

Session 1 — Epidemiological evidence and animal models
Chair: Isabelle Mansuy, University and ETH Zurich, CH

09:00 – 09:40	Epigenetic variation in human sperm under lifestyle influences Romain Barrès, University of Copenhagen, DK
09:40 – 10:20	Environmentally induced epigenetic transgenerational inheritance of disease and phenotypic variation: Ancestral ghosts in your genome Michael K. Skinner, Washington State University, US
10:20 – 10:50	*Coffee Break - Floor F, Uhrenhalle*
10:50 – 11:30	Human germline: specification, programming and epigenetic inheritance Azim Surani, University of Cambridge, UK
11:30 – 11:45	Dissecting the epigenetic machinery underlying the memory of chemical exposure Patrick Allard, University of California, US
11:45 – 12:00	Heritable effects of general anesthesia Jill Escher, Escher Fund for Autism, San Francisco, US
12:00 – 13:30	*Lunch Break - Floor F, Uhrenhalle*

Session 2	Epidemiological evidence and animal models Chair: Michael K. Skinner, Washington State University, US
13:30 – 14:10	Role of sperm miRNAs in the transmission of the effects of stress across generations in mice and men Larry A. Feig, Tufts University, US (Talk sponsored by the Institute for Neuroscience, D-HEST, ETHZ)
14:10 – 14:50	Variable silencing of the repeat genome - implications for non-genetic inheritance Anne Ferguson-Smith, University of Cambridge, UK
14:50 – 15:05	Comparable molecular signatures of early life trauma in mice and humans: Potential implications for epigenetic inheritance Ali Jawaid, University and ETH Zurich, CH
15:05 – 16:30	*Coffee Break and Poster Session I - Floor F, Uhrenhalle and Gallery*
16:30 – 17:10	Epigenetic underpinnings of metabolic disease heterogeneity (video lecture) J. Andrew Pospisilik, Van Andel Research Institute, US
17:10 – 17:25	Ancestral stress accelerates biological aging via epigenetic regulation Gerlinde A.S. Metz, University of Lethbridge, CA
17:25 – 17:40	Cannabis use and altered sperm DNA methylation at autism candidate *DLGAP2* Rose Schrott, Duke University, US
18:30 – 22:00	Terrace Dinner at ETH Dozentenfoyer (upon registration only)



ETH Dozentenfoyer

ETH Zurich
HG K 30.5
Rämistrasse 101
8092 Zurich

PROGRAM

Tuesday 27.08.2019

08:30 – 09:00	Registration

Session 3	Transmission mechanisms Chair: Martin Roszkowski, University and ETH Zurich, CH
09:00 – 09:40	Sperm RNA code: how many secrets in programming offspring phenotypes? Qi Chen, University of California, US
09:40 – 10:20	Mechanisms of transgenerational inheritance of obesity epiphenotypes Victor G. Corces, Emory University, US
10:20 – 10:50	*Coffee Break - Floor F, Uhrenhalle*

Session 4	Transmission mechanisms Chair: Kristina Thumfart, University and ETH Zurich, CH
10:50 – 11:30	Intergenerational epigenetic inheritance by sperm small RNAs Upasna Sharma, University of California Santa Cruz, US
11:30 – 11:45	Zebrafish as a model for induction of epigenetic inheritance of disease in response to environmental contaminants Tracie R. Baker, Wayne State University, US
11:45 – 12:00	Epigenetic reprogramming of primordial germ cells and the reprogramming-resistant DNA methylation marks in medaka fish Ramji K. Bhandari, University of North Carolina at Greensboro, US
12:00 – 13:30	*Lunch Break - Floor F, Uhrenhalle*

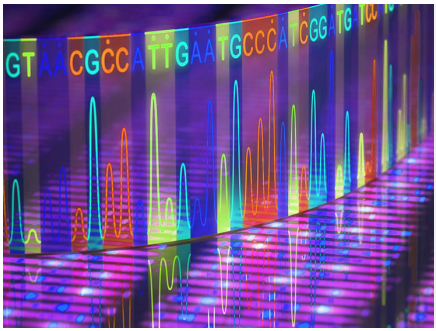
Session 5	Transmission mechanisms Chair: Anastasiia Efimova / Irina Lazar-Contes, University and ETH Zurich, CH
13:30 – 14:10	The role of epigenetic regulators in early development Alexander Meissner, Max Planck Institute for Molecular Genetics, DE
14:10 – 14:50	Aberrant paternal transmission of nucleosomes impairs transcription in and development of early mouse embryos Antoine Peters, Friedrich Miescher Institute, CH
14:50 – 16:30	*Coffee Break and Poster Session II - Floor F, Uhrenhalle and Gallery*
16:30 – 17:10	Paternal effect genes in the mouse Lucia Daxinger, Leiden University Medical Center, NL
17:10 – 17:25	Sperm RNAs are required for establishment but not long-term maintenance of paternal epigenetic inheritance of metabolic changes in mice Valérie Grandjean, University of Nice Sophia Antipolis, FR
17:25 – 18:00	6 flash talks by poster presenters - Unravelling the role of low-grade chronic inflammation in epigenetic inheritance of paternal obesity Stine Thorhauge Bak, Aarhus University, DK - Long-term and transgenerational neurobehavioral effects of the insecticide permethrin in zebrafish Mélanie Blanc, Örebro University, SE - The vulnerability of the germline: ethics in the time of epigenetics Anne Le Goff, University of California Los Angeles, US - Molecular characterization of transgenerational epigenetic phenomena after genistein exposure in quail Frédérique Pitel, Université de Toulouse, FR - Adolescent THC exposure reduces microRNA levels in sperm Gregory Rompala, Icahn School of Medicine at Mount Sinai, US - Epigenetic inheritance of the cardiac regenerative capacity in the zebrafish Andres Sanz-Morejon, University of Bern, CH

PROGRAM

Wednesday 28.08.2019

Session 6	Conceptual challenges and computational aspects of epigenetic mapping Chair: Deepak Tanwar, University and ETH Zurich, CH
09:00 – 09:40	Sperm-mediated transgenerational inheritance: role of LINE-1 expression in the genesis and assimilation of epigenetic variations Corrado Spadafora, National Research Council, IT
09:40 – 10:20	Analysis of allele-specific methylation from bisulphite sequencing data Mark D. Robinson, University of Zurich, CH
10:20 – 10:50	*Coffee Break - Floor F, Foyer Audimax*

Session 7	Impact on society and evolution Chair: Pierre-Luc Germain, University and ETH Zurich, CH
10:50 – 11:30	Transgenerational epigenetics, social complexity, and responsible research Michael Penkler, Technical University of Munich, DE
11:30 – 12:10	Transgenerational effects of age in the wild – patterns and consequences Julia Schröder, Imperial College London, UK
12:10 – 12:30	Poster prize (offered by the Institute for Neuroscience, D-HEST, ETHZ) / Closing



Tour of the Functional Genomics Center Zurich

Wednesday 28.08.2019
13:30 - 15:30

Meeting Point: Registration desk in the Foyer Audimax

Limited to 8-10 participants. Free of charge, first come first serve.

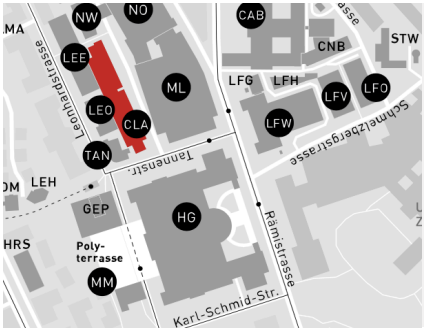
WORKSHOP

Wednesday 28.08.2019

Workshop

13:00 – 14:00	*Lunch - CLA glass hall (transition CLA / LEE)*
14:00 – 17:00	«Meet the experts: Questions and Answers»
	Victor G. Corces, Emory University, US
	Anne Ferguson-Smith, University of Cambridge, GB
	Upasma Sharma, University of California, US
	Michael K. Skinner, Washington State University, US

Moderated by Gretchen van Steenwyk, University and ETH Zurich, CH
Katharina Gapp, ETH Zurich, CH



Workshop

ETH Zurich
Building CLA J 1
Tannenstrasse 3
8092 Zurich

SPEAKERS

Epigenetic variation in human sperm under lifestyle influences

Romain Barrès - Professor of Metabolic Epigenetics, University of Copenhagen, DK

The developmental programming of the embryo is controlled by genetic information but also dictated by epigenetic information contained in spermatozoa. Lifestyle and environmental factors not only influence health in one individual but can also affect the phenotype of the following generations. This is mediated via epigenetic inheritance i.e., gametic transmission of environmentally-driven epigenetic information to the offspring. Evidence is accumulating that preconceptional exposure to certain lifestyle and environmental factors, such as diet, physical activity, and smoking, affects the phenotype of the next generation through remodelling of the epigenetic blueprint of spermatozoa. This presentation will summarize our recent understanding of the different epigenetic signals in sperm that are responsive to environmental and lifestyle factors and are capable of affecting embryonic development and the phenotype of the offspring later in life.

Environmentally induced epigenetic transgenerational inheritance of disease and phenotypic variation: ancestral ghosts in your genome

Michael K. Skinner - Center for Reproductive Biology, Washington State University, US

Transgenerational effects of environmental toxicants, nutrition or stress significantly amplify the biological impacts and health hazards of these exposures. One of the most sensitive periods to exposure is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation occurs. Previous studies have shown that toxicants can cause a transgenerational increase in adult onset disease such as infertility, prostate, ovary and kidney disease, cancers and obesity. The transgenerational epigenetic mechanism appears to involve the actions of an environmental exposures at the time of sex determination to permanently alter the epigenetic (e.g. DNA methylation, ncRNA, and histone modifications) programming of the germ line that then alters the transcriptomes of subsequent generations developing organs to induce disease susceptibility. The developmental origins of these epimutations appear in the primordial germ cells, spermatogenic cells, and developing sperm. A variety of different environmental compounds have been shown to induce this epigenetic transgenerational inheritance of disease. Epigenetic biomarkers for specific diseases have been identified. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational inheritance of disease and phenotypic variation is a novel paradigm in disease etiology that is also relevant to other areas such as evolution.

Human germline: specification, programming and epigenetic inheritance

Azim Surani - Wellcome Trust Cancer Research UK Gurdon Institute, University of Cambridge, UK

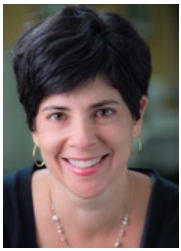
Specification of human primordial germ cells (PGCs) occurs during pre-gastrulation on ~day 17 of postimplantation development, followed by a dynamic and comprehensive epigenetic resetting. We recently developed robust in vitro models to simulate the origin of hPGCs and early human development with pluripotent stem cells (hESCs and hiPSC). The in vitro hPGCs share key characteristics with the authentic hPGCs from Wk5-Wk7 human fetuses. The analysis unexpectedly revealed divergence from the mouse, with SOX17 as the critical regulator of hPGC fate. SOX17-BLIMP1 also probably contributes to the initiation of epigenome resetting in the early human germline. Crucially, PGC specification is linked intimately with the initiation of the epigenetic program, comprehensive erasure of DNA methylation, including resetting of genomic imprints, a well-established source of epigenetic inheritance in mammals. Notably, we also detect young hominoid-specific loci that resist complete germline reprogramming. The functions, if any, of the probable transmission of this epigenetic information to subsequent generations through the 'immortal' germline in great apes, may have been important for human development, evolution and disease.

Dissecting the epigenetic machinery underlying the memory of chemical exposure

Patrick Allard - University of California, US

Germ cells are the bridge between generations and their integrity is paramount to the health and viability of all organisms. However, how germ cells integrate environmental cues and mediate transgenerational inheritance are still poorly understood. Here, we describe the use of several model systems, including *C. elegans* and ES cells-derived mammalian germ cells, to better understand how the complex developmental program unique to germ cells is affected to environmental exposures. The combination of these models allows us to dissect the genetic pathways at the root of germline dysfunction, epigenetic impact, and transgenerational effects. We will present our work showing that BPA causes a transgenerational redistribution of repressive histone modifications such as H3K9me3 and H3K27me3 that correlates with reproductive toxicity effects (including germ cell apoptosis and embryonic lethality). Furthermore, we show that a network of H3K9 and H3k27 histone demethylases acts to sustain the germline toxicity response elicited by Bisphenol A across 5 generations. We will also present our work showing that the same marks are altered in mouse primordial germ cells. Together, our work highlights the value of leveraging multiple platforms in the fields of genetics and stem cell biology to uncover new mechanisms of transgenerational inheritance.

Heritable impacts of general anesthesia



Jill Escher - Escher Fund for Autism, San Francisco, US

Volatile general anesthetic gases (GA) are small lipophilic molecules used in clinical practice since the late 1950s. They now are perhaps the most common highly toxic (at standard doses) exposure reaching human germ cells, yet there have been no studies on the heritable effects of GA in humans.
Only four mammal studies have explored this question. In the early 80s two mouse studies found that progeny of the GA exposed germ cells exhibited learning impairments. Two recent studies in rats found similar effects, and connected the behaviors to epigenetic modifications in exposed germline and progeny brain. In somatic cells, GA is a powerful modulator of chromatin remodeling that leads to a cascade of events resulting in the dysregulation of gene expression necessary for proper neurodevelopment, including cytoskeleton, neural migration, and synapse formation. Changes in transcription factors, histone modifications, and DNA methylation have been implicated in these effects. The agents also act as hormone disruptors and cause DNA damage. Based on family histories of GA exposure, in combination with scientific literature, the Escher Fund for Autism funds and advocates for research on heritable impacts of germ cell toxicant exposures, including general anesthesia, maternal smoking, HDAC inhibitors, and synthetic steroid drugs.

Role of sperm miRNAs in the transmission of the effects of stress across generations in mice and men

Larry A. Feig - Tufts University, US

Susceptibility to psychiatric disorders derives from both personal experiences and gene alleles inherited from one's parents. However, recent experiments have suggested that this susceptibility also derives from the effects of one's parents' experiences transmitted by epigenetic inheritance. In male mice, this concept has been documented by studying the transgenerational effects of stress that are mediated by changes in the content of specific sperm micro miRNAs. In mice, we have studied the paternal transmission across multiple generations of elevated anxiety and sociability defects that occur specifically in female offspring of male mice exposed to chronic social instability stress. In this system, we have implicated a set of sperm miRNAs in this process by the fact that they are also altered in male offspring across multiple generations. In addition, some of these miRNAs also display altered levels in early embryos derived from stressed males. In men, we have focused on the effects of exposure to abusive and/or dysfunctional family life as children. Remarkably, the same sperm miRNA changes occur in these men as we found in stressed male mice. I will present data in mice reinforcing the idea that these miRNA changes early embryos can impact the stress-associated phenotypes found in offspring of stressed males.

Variable silencing of the repeat genome - implications for non-genetic inheritance

Anne Ferguson-Smith - Department of Genetics, University of Cambridge, UK

Genetic models of epigenetic inheritance can provide useful insights into the mechanisms, stability and heritability of modified states. Endogenous retroviruses (ERVs) are a class of LTR retrotransposons representing around one quarter of the repetitive elements in the murine genome. Most are epigenetically silenced. However, in two classic mouse models, Agouti viable yellow (Avy) and Axin fused (Axin(Fu)), a member of the IAP class of ERV has inserted in the vicinity of the agouti and axin genes respectively, and been variably DNA methylated between individuals; this is associated with transcriptional variability of the associated genes, and non-genetically conferred phenotypic variation which can be transmitted across generations. Such alleles are known as metastable epialleles. We have conducted a genome-wide systematic screen for metastable epialleles at ERVs using mouse strain-specific datasets that we generated as part of the BLUEPRINT reference epigenome project (EUFP7 BLUEPRINT grant HEALTH-F5-2011-282510). The properties, impact and heritability of these novel variably methylated IAPs provides useful insights into the impact of the epigenetic control of repetitive elements on the mammalian genome and transgenerational epigenetic inheritance. In particular, the variable methylation state is locus-specific within an individual and very few act as heterologous promoters for adjacent genes. Of interest, variably methylated IAPs are enriched for the methylation-sensitive DNA binding factor CTCF which may influence the establishment/maintenance of the metastable state as well as contribute longer range functional effects. Variably methylated IAPs are reprogrammed after fertilization and re-established as variable loci in the next generation indicating reconstruction of metastable epigenetic states. Finally, we explore the sensitivity of metastable epialleles to environmental effects to determine the extent to which they can act as biosensors of environmental compromise and mediators of environmental effects on genome function.

Comparable molecular signatures of early life trauma in mice and humans: Potential implications for epigenetic inheritance

Ali Jawaid - Laboratory of Neuroepigenetics, University and ETH Zurich, CH

Traumatic experiences in early life have a persistent impact on physiological and psychological functioning. The mechanisms underlying such effects are not known and are difficult to study in humans. Animal models provide useful means to study these mechanisms and gain translational insight to better understand pathological traits in human. Here, using a mouse model of postnatal trauma based on unpredictable maternal separation combined with unpredictable maternal stress (MSUS), we demonstrate that several physiological alterations in MSUS mice can be validated in a human cohort of 6-12 years old children (n=26) who were exposed to early life trauma in the form of paternal loss and maternal separation (PLMS). Compared to matched controls (n=16), these children have depressive symptoms and reduced level of serum high-density lipoproteins (HDL), similar to MSUS mice. Altered expression of HDL-associated miRNAs in MSUS serum (miR-16, miR-29a, miR-375) is also apparent in both serum and saliva of PLMS children. In particular, miR-375 is upregulated in serum, corresponding to previous reports of its upregulation in MSUS sperm. Treatment of developing germ cell-like cells in vitro with serum from MSUS mice increases miR-375, which is reversed after siRNA-mediated knockdown of the HDL receptor, SCARB1. These results suggest that similar behavioral and metabolic effects and vectors of non-genomic inheritance of early trauma may exist in humans and mice.

Epigenetic underpinnings of metabolic disease heterogeneity

J. Andrew Pospisilik - Van Andel Research Institute, US

Complex trait diseases afflict >2 billion people worldwide. The rapid rise in particular of early life disease carries long-term health burdened including heart disease, diabetes and stroke, making the issue one of the world's chief economic and health care challenges of the day. Whereas our understanding of the genetic framework for complex disease has expanded dramatically the last decades, our understanding of causal epigenetic mechanisms of disease remain poorly understood. Our focus has been to mine and understand the mechanisms underpinning non-genetic disease heterogeneity and thus understand the spectrum of disease potential that lies within each individual. I will discuss how these efforts have uncovered a chromatin (PRC2) based mechanism buffering beta-cell dedifferentiation, as well as signalling modules that drive and potentiate browning of adipose tissues. Also how they have revealed mechanistic underpinnings for intergenerational control of non-genetic variation and what we believe to be the first stochastic disease 'switch' yielding distinct phenotypic 'on' and 'off' states in mouse and potentially man. The data suggest a highly regulated landscape of non-genetic phenotypic variation defines mammalian disease.

Ancestral stress accelerates biological aging via epigenetic regulation

Gerlinde A.S. Metz - Canadian Centre for Behavioural Neuroscience, University of Lethbridge, CA

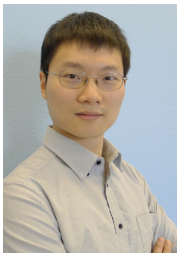
Prenatal and ancestral stresses are among the most significant risk factors for adverse maternal and child health outcomes. Ancestral stress may also lead to greater risk of disease in later life and compromise chances of successful aging. Here we investigated if the cumulative impact of multigenerational prenatal stress (MPS) affects age-dependent profiles of physical and mental health, stress response, and epigenetic regulation by microRNA (miRNA) expression. Fourth (F4) generation male and female MPS offspring were derived from a lineage whose female ancestors (F0-F3) were exposed to gestational stress. Endocrine and brain functions were assessed across the lifespan. The results revealed that MPS accelerates the mental and physiological health decline during aging. MPS also increased the incidence of diseases such as renal failure, inflammatory disease and tumors. Unbiased deep sequencing revealed mediating factors such as miRNAs that target synaptic plasticity and endocrine and immune regulators. These findings suggest that cumulative ancestral stress is a significant determinant of lifetime physical and mental health trajectories and a risk factor for common age-related diseases via epigenetic regulation with significant sexual dimorphisms. (Funded by AI-HS, CIHR and NSERC)

Cannabis use and altered sperm DNA methylation at autism candidate *DLGAP2*

Rose Schrott - Nicholas School of the Environment, Duke University, US

Cannabis sativa is one of the most commonly used illicit psychoactive drugs, with men comprising 67% of the recreational cannabis market. While the reproductive consequences of cannabis use remain poorly understood, increased use in men is particularly relevant to potential fathers. Parental cannabis use is associated with adverse neurodevelopmental outcomes in offspring, but how phenotypes are transmitted remains unknown. We recently reported associations between male cannabis use, significantly reduced sperm counts, dose-dependent DNA methylation changes, and hypomethylation in sperm. Discs-Large Associated Protein 2 (*DLGAP2*) exhibits significant hypomethylation in sperm of cannabis-exposed men relative to controls ($p<0.05$) at 17 CpG sites. *DLGAP2* is involved in synapse organization in the brain, and dysregulation is associated with autism. Quantitative bisulfite pyrosequencing validated differential methylation in sperm ($p<0.05$) for 9 CpG sites in intron 7. We confirmed an inverse relationship between intron 7 DNA methylation and *DLGAP2* transcript levels in human conceptal brain tissues ($p<0.01$). Male rats exposed to delta-9-tetrahydrocannabinol (THC) showed differential methylation at *Dlgap2* in sperm ($p<0.03$), as did the nucleus accumbens of rats whose fathers were preconceptionally exposed to THC ($p<0.05$). These results warrant further investigation into effects of preconception cannabis use in males and potential effects on subsequent generations.

Sperm RNA code: how many secrets in programming offspring phenotypes?



Qi Chen - University of California Riverside, US

Mammalian sperm RNA is increasingly recognized as an additional paternal hereditary information beyond DNA. Environmental inputs, including an unhealthy diet, mental stresses and toxin exposure, can reshape the sperm RNA signature and induce offspring phenotypes relating to paternal environmental stressors. The expanding categories of sperm RNAs (such as tsRNAs, miRNAs, rsRNAs and lncRNAs) and associated RNA modifications have begun to reveal the functional diversity and information capacity of these molecules. However, the coding mechanism endowed by sperm RNA and RNA modification-induced structure effects remains unknown, and how sperm RNA-encoded information is decoded in early embryos to control offspring phenotypes remains unclear. I discuss these issues in light of emerging data.

Mechanisms of transgenerational inheritance of obesity epiphenotypes

Victor G. Corces - Emory University, US

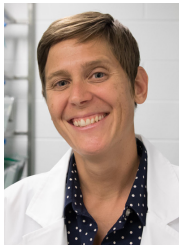
Mechanisms by which epiphenotypes are transmitted between generations through the paternal germline remain poorly understood. Most promoters in mouse sperm contain RNAPII and are flanked by positioned nucleosomes marked by a variety of active histone modifications. The sperm genome is bound by transcription factors, including Mediator, FoxA1, ERa and AR. These proteins are found at promoters, enhancers, and super-enhancers. CTCF and cohesin are also present in sperm DNA, where they mediate interactions that organize the sperm genome into domains overlapping extensively with those found in mESCs. This information suggests that epigenetic information present in mammalian sperm could be altered by environmental factors to cause novel phenotypic outcomes in the next generation. When pregnant females are exposed to endocrine disruptor chemicals, their progeny show a variety of phenotypes, including obesity. This phenotype is transmitted between generations in the absence of further exposure. Approximately 68 new protein binding sites are present in the sperm and fat tissue of obese mice from the F1 through the F4 generations. These new binding sites correspond to CTCF, RA, and ERa, suggesting that effects of these proteins on 3D chromatin organization and transcription of specific genes are responsible for the establishment and transmission of epiphenotypes.

Intergenerational epigenetic inheritance by sperm small RNAs

Upasna Sharma - Department of Molecular, Cell, and Developmental Biology, University of California Santa Cruz, US

Although there is mounting evidence from worms to humans suggesting that parental environment can influence phenotypes in offspring, the mechanism of such transgenerational inheritance remains deeply mysterious. Male mice fed a low protein diet sire offspring with altered lipid and cholesterol biosynthesis. Here, we examine the mechanistic basis of epigenetic inheritance of paternal dietary effects. We show that protein restriction affects small RNA levels in mature sperm, with increased amounts of 5′ fragments of glycine transfer RNAs (tRF-GlyGCC). Moreover, we found that tRF-GlyGCC represses genes associated with the endogenous retroelement MERVL, both in embryonic stem cells and in preimplantation embryos. Together, these studies suggest that sperm small RNAs potentially mediate epigenetic inheritance of paternal dietary effects. Intriguingly, our studies revealed surprising dynamic aspects of small RNA biogenesis during sperm maturation in the epididymis. We find that a subset of sperm small RNAs are synthesized in the somatic epididymis epithelial cells and shipped to sperm via extracellular vesicles. These studies revealed a role for soma-to-germline trafficking in shaping the RNA payload of mammalian sperm. Current studies are focused on elucidating the mechanism of this RNA-mediated soma-germline communication and its consequences to offspring development.

Zebrafish as a model for induction of epigenetic inheritance of disease in response to environmental contaminants



Tracie R. Baker - Institute of Environmental Health Sciences and Department of Pharmacology, Wayne State University, US

We have shown that zebrafish (*Danio rerio*) are an ideal model for evaluating transgenerational effects of certain toxicants and their role in the fetal basis of adult disease. We utilized this model to identify transgenerational effects of a known endocrine disrupting chemical (EDC), dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD). Exposure to TCDD early in development produces reproductive abnormalities in adulthood and decreased reproductive capacity that persists for multiple, unexposed generations, and appears to be mediated through the male germline. Thus, testicular tissue from each generation was analyzed for histologic, transcriptomic, and epigenetic changes. We identified significant changes indicating delayed spermiation in TCDD-exposed males and their offspring. Gene expression analysis revealed TCDD-altered spermatogenesis, steroidogenesis, lipid metabolism, and xenobiotic metabolism pathway responses in all generations. Whole-genome bisulfite sequencing identified gene-specific methylation changes associated with significantly altered gene expression in all generations. In response to TCDD exposure, multiple genes were differentially methylated; many of which are involved in reproductive processes or epigenetic modifications, suggesting a role of DNA methylation in later-life health outcomes. Using molecular tools in the zebrafish model, our research aims to uncover critical genes and changes in epigenetic regulation required for adverse transgenerational, reproductive endpoints of EDC exposure.

Epigenetic reprogramming of primordial germ cells and the reprogramming-resistant DNA methylation marks in medaka fish

Ramji K. Bhandari - Department of Biology, University of North Carolina Greensboro, US

Primordial germ cells (PGCs) undergo epigenetic reprogramming during their cell fate determination. How the fish PGCs epigenetically reprogram during early embryonic development is currently unknown. Here we show that medaka PGCs undergo a global demethylation in a two-step strategy. The first step occurs between the blastula and 8-dpf stages, and the second step occurs between the 10-dpf and 12-dpf stages. Both demethylation processes are global, except for CGI promoters, which remain hypomethylated throughout the stage of PGC specification. We also show that the endocrine disruptor bisphenol A (BPA)-induced epimutations can survive PGC reprogramming in embryo, and additional unique epimutations are established de novo during gametogenesis. Only a fraction of the BPA-induced reprogramming-resistant epimutations are transmitted to sperm in adulthood. Interestingly, approximately 80% of BPA-specific epimutations found in sperm were unique and mechanisms underlying their establishment in sperm is currently unknown. We are currently examining the status of the BPA-specific epimutations in somatic cells in the offspring past somatic reprogramming and in sperm past second round of PGC reprogramming. Our results provide a line of evidence for inheritance of the exposure-specific, reprogramming-resistant epigenetic memory by sperm and their distribution to soma and germ cells in offspring. - Supported by US NIEHS (R21ES027123)

The role of epigenetic regulators in early development

Alexander Meissner - Dept. Genome Regulation, Max Planck Institute for Molecular Genetics, DE

Ontogeny describes the orchestrated assembly of a complex multicellular organism from a single totipotent zygote. As the embryo grows, proliferating cells become restricted in their fate to establish one of many encoded phenotypes, which is realized through the combined action of cell-type specific transcription factors and ubiquitous epigenetic machinery. Substantial work has been done to connect molecular observations made from in vitro differentiation models to the morphological features of mutant animals. However, the complete and unbiased characterization of any given epigenetic regulator's role in body plan assembly has been hampered by their early activity, which often leads to diverse or lineage-specific effects from gastrulation onwards. Here we report a zygotic sgRNA/Cas9-based perturbation platform where the status of many embryos can be assayed simultaneously and at single cell resolution. We believe our experimental framework provides a general opportunity to study how cellular diversity emerges from a single genome *in vivo*.

Aberrant paternal transmission of nucleosomes impairs transcription in and development of early mouse embryos

Antoine Peters - Friedrich Miescher Institute, CH

We are interested in understanding the relevance of paternal transmission of chromatin states to fitness and survival of offspring. Towards this, we established a micromanipulation assay in which nuclei from immature haploid round spermatids containing nucleosomes genome-wide are injected into eggs ("ROSI"). The developmental potential of such ROSI embryos is impaired compared to those having been injected a nucleus of mature sperm (via intra cytoplasmic sperm injection; "ICSI"). Single embryo sequencing indicates that ROSI embryos express aberrantly a significant number of genes and display altered levels of histone and DNA methylation. Our data support the notion that the histone-to-protamine exchange process contributes to proper gene expression and embryonic fitness post fertilization.

Paternal effect genes in the mouse

Lucia Daxinger - Leiden University Medical Centre, NL

Repression of transposons is a major function of the epigenome and to achieve this multiple silencing mechanisms are being used. In some rare cases, termed metastable epialleles, a transposon insertion can control the activity of a locus and influence an individual's phenotype and inheritance patterns. We previously reported a group of factors involved in transposon repression via DNA methylation and/or histone modification pathways, termed Modifiers of murine metastable epialleles Dominant (MommeD). Within this group, we identified *Setdb1*, *Dnmt1* and *Snf2h* as paternal effect genes, using the *Agouti viable yellow* (A^{vy}) allele as an epigenetically sensitive reporter. However, the full spectrum of factors involved in silencing of metastable epialleles remains elusive. Our current work focusses on the molecular and phenotypic characterization of a set of novel *MommeD* genes that when disrupted lead to defects in repeat silencing pathways. Intriguingly, we find that genome-wide DNA hypomethylation is compatible with life and does not lead to transposon de-repression in somatic tissues. The progress of this work will be presented.

Sperm RNAs are required for establishment but not long-term maintenance of paternal epigenetic inheritance of metabolic changes in mice

Valérie Grandjean - University of Nice Sophia Antipolis, FR

Recent studies have uncovered that paternal obesity induced by a high fat diet (HFD) affects metabolic health of offspring. Yet, these reports mainly employed one generation-exposure and the effects of paternal multigenerational HFD feeding on the metabolic health progenies remain largely unknown. Here, we show that maintaining paternal HFD feeding for five consecutive generations in mice induces a gradual enhancement in fat mass over generations and leads to the persistence of obesity across generations fed by regular diet. Sperm RNA-microinjection experiments into fertilized oocytes reveal that RNA-induced metabolic pathologies are not maintained for more than two generations, suggesting that sperm RNAs are required for establishment but not for the long-term maintenance of transgenerational epigenetic inheritance. Furthermore, sperm small RNAs profiling combined with metabolic and genetic analysis reveals that the abundance of RNAs such as sperm tRNA fragments or miRNAs, vectors of heritable metabolic disorders, does not correlate with long-term epigenetic transgenerational inheritance of dysfunctional metabolic phenotypes. These data suggest that long-term maintenance of transgenerational epigenetic inheritance of diet-induced metabolic pathologies is a multi-step process in which sperm RNA molecules would play a key role in the establishment of the alterations. The stabilisation of obesity observed upon successive multiple paternal HFD generations may contribute to the world-wide epidemy of metabolic diseases.

Sperm-mediated transgenerational inheritance: role of LINE-1 expression in the genesis and assimilation of epigenetic variations

Corrado Spadafora - Institute of Translational Pharmacology, National Research Council (CNR), IT

Spermatozoa of virtually all animal species can spontaneously take up exogenous DNA or RNA molecules, either naked or in cell-derived nanovesicles such as exosomes, and internalize them into nuclei. RNA-containing nanovesicles, containing predominantly small regulatory RNAs (miRNAs and tsRNAs), are released from somatic tissues in the bloodstream, can cross the Weismann barrier, reach the epididymis, and therein are taken up by spermatozoa, henceforth delivered to oocytes at fertilization and propagated in early embryos. This transgenerational flow of regulatory RNAs can progressively reshape the transcriptional landscape of early embryos and originate novel traits. Central to this process is LINE-1-encoded ORF2p, a protein endowed with reverse transcriptase (RT) and endonuclease (EN) activities, expressed in spermatozoa and early embryos. Novel evidence suggest that RT and EN are interplaying stress-responsive activities, originating a continuous reshaping of the nuclear architecture and inducing a chromatin conformation highly prone to variation. From this model a mechanistic key emerges, whereby LINE-1 ORF2p mediates the reformatting of chromatin organization in response to external stressors, which in turn affects cell fate determination and hence the emergence of novel traits. This phenomenon is compatible with a Lamarckian-type view and bears close resemblance to the Darwinian pangenesis theory.

Analysis of allele-specific methylation from bisulphite sequencing data

Mark D. Robinson - University of Zurich, CH

DNA methylation plays a role in several biological phenomena. Genomic imprinting, where genes are expressed in a parent-of-origin manner, is also regulated by DNA methylation. Imprinting occurs via allele-specific methylation (ASM), in which only the paternal or the maternal allele is methylated in all or most of the tissues of an individual. Imprinting and other forms of ASM are known to play crucial roles in embryonic and fetal development, placental formation, cell growth and differentiation. We sought to develop a simple yet effective method to screen for genomic regions that exhibit gain or loss of ASM between samples from distinct conditions, using bisulfite sequencing (BS-seq) data. We developed an ASM score and leverage well-known computational methods to perform a differential ASM analysis, within an R package called DAMEfinder. We cluster positions of persistent change of ASM into regions called DAMEs. We propose two ways to screen for ASM, one based on SNP information, and the other based only on BS-seq reads. We apply DAMEfinder to a real data set comparing normal colonic mucosa to cancerous colorectal lesions and find that colorectal cancer subtypes have distinct ASM signatures and verify previously reported genes that exhibit loss of imprinting.

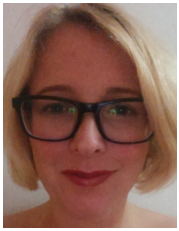
Transgenerational Epigenetics, Social Complexity, and Responsible Research

Michael Penkler - Munich Center for Technology in Society, Technical University of Munich, DE

Transgenerational epigenetics proposes an integrative perspective on how environmental experiences, including social factors, may influence biological processes and the development of health and disease across generations. This perspective promises to open up new avenues and possibilities for improving the health of current and future generations. At the same time, it also poses distinct challenges for a socially responsible translation of transgenerational epigenetics into society and policy. When researching how environmental factors influence the development of health and disease across generations, it is particularly important to reflect on how these environmental factors are understood and framed and to specify which aspects of life worlds are included and excluded. We suggest that research approaches that acknowledge and capture the complex ways in which environmental factors are socially structured can avoid simplistic attributions of responsibility to individual actors (such as mothers) as well as deterministic readings of transgenerational epigenetics. We suggest a number of strategies to promote socially responsible research practices that capture social complexity, such as engaging in co-creation practices with affected publics and communities and in interdisciplinary collaborations between life scientists and social scientists.

Transgenerational effects of age in the wild – patterns and consequences

Julia Schröder - Imperial College London, UK



The general idea of direct genetic inheritance is challenged by the notion of epigenetic effects – where experiences during a parent's lifetime can manifest in an offspring's phenotype. One problem in the study of transgenerational, potentially epigenetic inheritance in the field of evolutionary biology is that many studies use standard laboratory animal model organisms, which are often less genetically diverse – deliberately, to reduce noise in the signal – than their wild counterparts. The detected effects are thus mostly free from environmental influences. Thus, the scope as to which we can draw conclusions to the evolutionary impact and relevance of epigenetic inheritance on wild populations is limited. Yet, studies in the wild require long-term collection of transgenerational data on genetic relatedness, phenotypes, and biomarkers for potential pathways. All of these requirements are difficult to achieve in wild populations where it is often difficult to capture individuals multiple times throughout their lives, and where it is often impossible to distinguish mortality from dispersal events. Here, I present a comprehensive case study on the phenotypic transgenerational effects of parental age on life-history traits and telomere length and dynamics in an exceptional wild study population, the sparrows of Lundy Island. I will then then make an attempt at generalising by reviewing published effects of transgenerational effects in wild bird populations.

POSTERS

Posters in bold have been selected for short and flash talks

Anthonia Oluwatomisin Afenkhena An insight into epigenetic effects in cardiomyopathy

Camille Akemann et al. DNA methylation as a mechanism for transgenerational reproductive effects of TCDD in zebrafish

Valerie Amarger et al. Maternal malnutrition during gestation alters fetal hypothalamus development

Emil Andersen et al. Single-cell genome-wide bisulfite sequencing for assessing the role of DNA methylation in spermatozoa

Patrick Anglard Food/nutrition and drugs of abuse trigger different DNA methylation mechanisms in reward brain structures of self-administering rats

Stine Thorhauge Bak et al. Unravelling the role of low-grade chronic inflammation in epigenetic inheritance of paternal obesity

Ramji K. Bhandari et al. Epigenetic reprogramming of primordial germ cells and the reprogramming-resistant DNA methylation marks in medaka fish

Mélanie Blanc et al. Long-term and transgenerational neurobehavioral effects of the insecticide permethrin in zebrafish.

Sarah Cohen-Woods et al. Flinders environmental epigenetics through life study

Vincent Coustham et al. Transgenerational analysis of embryonic heat exposure in Japanese quail

Luís Crisóstomo et al. Paternal inheritance of metabolic syndrome traits and sperm anomalies induced by high-fat diet in mice

Patrice Delaney et al. Inorganic arsenic hepatoxic: short term exposure, long term effects

Ririn Rahmala Febri et al. Correlation between miRNA 135b expression and aneuploidy in IVF patients

Hiroto Fukushima et al. Reprogramming of histone modifications during early development of Japanese killifish (medaka).

Anne Gabory et al. Effect of maternal obesity and preconceptional weight loss on foeto-placental growth and offspring health in mice: expression of epigenetic modifiers at the interface with metabolism.

Srimonta Gayen et al. Single cell analysis reveals partial reactivation of X-chromosome instead of chromosome wide dampening in naïve human pluripotent stem cells

Pierre-Luc Germain et al. shortRNA: A flexible framework for the analysis of short RNA sequencing data applicable to studies on epigenetic inheritance

Pascal Giehr et al. Genome-wide efficiency profiling reveals modulation of maintenance and de novo methylation by Tet dioxygenases

Betina González et al. Cocaine alters mouse male germ cells epigenome with direct impact on histones post-translational modifications

Ivana Jaric Estrous cycle effects on neuronal chromatin organization and anxiety-like behavior

Oliver Kuchenbuch What can the term "epigenetic information" possibly mean?

POSTERS

Irina Lazar-Contes et al. Investigating the involvement of spermatogonial cells in epigenetic inheritance in a mouse model of early life trauma

Anne Le Goff The vulnerability of the germline: ethics in the time of epigenetics

Kyounghwa Lee Histone demethylase KDM7A regulates androgen receptor activity and cancer progression in bladder cancer

Tiina Lehtiniemi et al. The pivotal role of the endonuclease SMG6 in the control of male germ cell transcriptome

Konstantin Lepikhov et al. Forced modulation of 5hmC/5fC/5caC abundance in mouse zygotes

Robert Lyle et al. START: The STudy of Assisted Reproductive Technologies

Osam Mazda et al. Epigenetic reprogramming of human fibroblasts into other somatic cell lineages by means of gene transfer or chemical compounds

Danielle Meyer et al. Developmental exposure to TCDD disrupts gonadal transcriptome and methylome in adulthood

Karin Moelling et al. PIWI/RNase H-related proteins in transgenerational transmission

Katherine Moran et al. Genesis and propagation of epigenetic states underlying drug tolerance

Kresna Mutia et al. miRNA 135b and HOXA 10 expression in infertility patients: a preliminary study

Kresna Mutia et al. Lean polycystic ovary syndrome (PCOS): Epigenetic understanding of IRS-1

Miroslava Ondičová et al. A randomize controlled trial of folic acid intervention in pregnancy highlights a putative methylation-regulated control element at ZFP57

Ana Pereira et al. Learning about pathogenic bacteria in adult C. elegans bidirectionally regulates pathogen response in the progeny

Frédérique Pitel et al. Molecular characterization of transgenerational epigenetic phenomena after genistein exposure in quail

Julien Pontis Hominoid-specific regulatory sequences and their controllers shape human genome regulation

Rashmi Ramesh et al. The piRNA binding protein-Piwi interacts with the spliceosomal proteins in Drosophila embryogenesis

Valérie Robert et al. The C. elegans SET-2 histone methyltransferase maintains germline fate by preventing progressive transcriptomic deregulation across generations

Gregory Rompala et al. Adolescent THC exposure reduces microRNA levels in sperm

Nuria Sanchez-Baizan et al. Epigenetic inheritance and integration of masculinizing temperatures of the European sea bass (Dicentrarchus labrax)

Andres Sanz-Morejon et al. Epigenetic inheritance of the cardiac regenerative capacity in the zebrafish

Meret Schmidhauser et al. Deciphering epigenetic effects of estradiol exposure during preimplantation development

Yelyzaveta Shlyakhtina et al. Non-genetic heterogeneity underlies phenotypic divergence

POSTERS

Florian Uhle et al. The intergenerational burden of bacterial sepsis - evidence for an acquired and hereditary immunodeficiency

Alejandro Valdivieso et al. Persistent epigenetic changes due to elevated temperature in mature zebrafish gonads

Alexandra Weyrich et al. Paternal epigenetic effects: Do fathers transmit their experiences to their sons?

Akihito Yasuoka et al. Epigenetic inheritance of ethanol induced metabolic stress and its alleviation by dietary polyphenol

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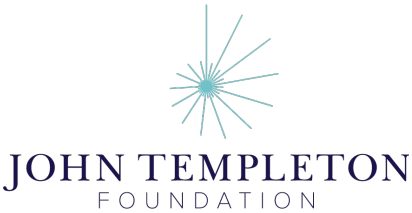
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