Dr. Art Petronis
Tapscott Chair in Schizophrenia Studies
Department of Psychiatry
University of Toronto, and
Centre for Addiction and Mental Health

Report 2015-2020

SUMMARY

- Published 13 peer reviewed articles, of which more than half (8) in the journals with an impact factor 10 or higher.
- Received numerous new grants for the total of $6.14 M from CIHR, NIH, and Brain Canada.
- Gave 15 national and international presentations.
- Graduated two PhD and two MSc students. Three graduate students are being trained in the lab.
- Since 2015, my papers have been cited over 1,800 times.
- Reviewed grant proposals for CIHR, NIH, and several European funding agencies.
- Launched the Psychiatric Chronoepigenomics program, the first in the world.

Progress report

My second 5-year term as the Tapscott Chair marked a turning point in the scientific evolution of our CAMH Epigenetics team. We discovered a new epigenetic phenomenon, and we re-conceptualized the epigenetic basis of mental disease. Our experimental and theoretical developments launched a series of innovative approaches for uncovering the fundamental mechanisms driving brain dysfunction. The following paragraphs will elaborate on our achievements in the last five years.

Background: epigenetics as a newcomer in psychiatric disease studies. Contemporary technologies offer numerous opportunities in biomedical research. However, progress in uncovering disease etiology also depends on how well we understand the key principles of cell biology. Traditionally, disease causal factors have been divided into two groups: genetic risk variants and environmental hazards. Growing evidence for the importance of epigenetic factors prompted us to suggest that epigenetics could be a third “pillar” in pathology. Nearly 20 years ago, we postulated that epigenetic misregulation (i.e. “broken switches” on genes), in combination with genetic and environmental risk factors, may play fundamental roles in human diseases.

Putative epigenetic misregulation is particularly relevant in explaining disease features that are impossible to resolve through DNA-based interpretations. For example, monozygotic twins have essentially identical genomes but their epigenomes can be quite different; this may account for their differential predisposition to schizophrenia, bipolar disorder, or some other psychiatric diseases. However, despite significant effort over the last decade, identification of disease-specific epigenetic risk factors with translational value has proven to be a difficult task.

Over the last 5 years, we have accumulated significant evidence that epigenetic factors, namely DNA modifications, are not as static as it has been generally accepted, and the dynamic nature of epigenetic regulation may dramatically change our epigenetic perspectives of psychiatric disease studies.

Discovery of circadian oscillations in the epigenomes. We recently discovered a new phenomenon pertaining to dynamic epigenetic regulation: epigenetic “attachments” to cytosines (one of four DNA nucleotides) exhibit periodic circadian oscillations (Oh, Ebrahimi et al. *Nature Communications* 2018; Oh, Koncevicius et al., *Genome Biology* 2019). Circadian rhythmicity is one of

the oldest evolutionary adaptations to Earth's rotation around its axis. The internal circadian "clock" in the cells best exemplifies how a perpetual environmental factor (i.e., day and night cycle) can get "under the skin" and become an indispensable part of cell's life. A broad spectrum of biological phenomena is regulated in a circadian fashion, from temperature-dependent fluctuations in biochemical reactions of prokaryotes, to higher-order behaviours (i.e., sleep-wake cycles) in multicellular organisms. Numerous clinical and epidemiological studies have shown that disruptions of circadian rhythms are linked to human morbidities including psychiatric diseases, however, molecular and mechanistic circadian studies of disease have been scarce.

On this note, human and mouse studies conducted in our lab in 2015-19 have shown that circadian epigenomes allow for a new epigenetic interpretation and perspective of psychiatric diseases. We showed that such "circadian" cytosines are more common in the regulatory parts of genes. This is an important observation because many psychiatric illnesses including schizophrenia do not behave like classical genetic diseases (e.g., cystic fibrosis and hemophilia) in that they are likely caused by *misregulation* of genes rather than irreversible mutations in genes themselves. Furthermore, we found that such daily oscillations in cytosines status were involved in long term epigenetic changes that occur with aging. This finding may shed a new light on understanding of a fundamental question: why do we age? Finally, and most importantly, the oscillating genomic regions overlap with some regions detected in traditional epigenetic studies of human disease. Traditional epigenetic studies deal with a major problem: the detected signals are so weak that it is hard to differentiate disease- causing epigenetic factors from meaningless epigenetic noise. There are good reasons to think that our new circadian epigenomic strategies can differentiate between the two options, and this would be an enormous step forward in uncovering the molecular basis of major psychiatric diseases.

Our discovery of circadian epigenomes significantly enriched the epigenetic theory of psychiatric disease. The principle of epigenetic oscillations offers simple and intuitive explanation for the origin of epigenetic misregulation in disease. We believe that daily circadian reprogramming is likely to be prone to errors, and imperfect cycling of epigenetic factors is the actual source of pathogenic processes. Parts of epigenome which do not oscillate, and therefore are stable, are less likely to cause any problems. We also predict that different people may oscillate their epigenomes in somewhat different ways. This may explain why our predispositions to diseases differs widely among individuals. Some of us get affected by psychiatric disease, while others with cancer or Alzheimer's.

The Psychiatric Chronomics program at CAMH. Based on these discoveries, our epigenetics team has launched the world's first research program dedicated to Psychiatric Chronomics. Chronomics (*Greek "chronos"* means time and "-omics" refers to large scale cell biology studies) is an innovative multidimensional analysis of temporal dynamics of cellular processes in health and disease. Chronomics includes epigenomics, transcriptomics, proteomics, metabolomics, and various other -omics investigated with *a temporal dimension*.

Chronomic strategies can open a new major chapter in psychiatric research by tackling a series of most prominent, but poorly understood, features of schizophrenia, bipolar disorder, major depression and other brain diseases. For example, it is a big mystery why individuals with predisposing genetic risk to schizophrenia or bipolar disorder do not become sick until they reach early adulthood. Why are disease risk genes relatively harmless for the first two decades of life? Equally intriguing and unclear is why a substantial fraction of psychiatric patients get better when they become older and some may even enjoy full recovery. Such common disease features indicate that performance of genes can vary significantly over different time points, despite the fact that genes are made of DNA which is very stable and does not change during individual's lifetime. Deviations from normal temporal trajectories of epigenomes, transcriptomes, proteomes, and metabolomes can play critical roles in *every* psychiatric disease, and chronomics is relevant to *every* patient.

Chronomics may uncover the molecular mechanisms that are critical for precision psychiatry including disease remissions and relapses, changes in psychotropic drug response over extended periods of time, and resistance to treatment. Despite its obvious importance, there has been a lack of

systematic investigation into temporal aspects of disease, primarily due to dominance of genetics research. Since DNA sequence is stable and hardly changes over time (within an individual), temporal dimension remained in the "blind spot" of traditional genetics. Not surprisingly, there is no dedicated disease chronomics research program in the world. In early 20th century, fundamental laws of physics were revolutionized by rethinking the concept of time. In the 21st century, rethinking time in biology may revolutionize biomedical research and transform our understanding of brain illness.

The CAMH Epigenetics team is uniquely positioned to launch this innovative program for the following reasons. We were the first to develop the guidelines for chronomics research using epigenomics as the proof of principle. Our effort in one field of chronomics, namely chrono-epigenomics, has already led to two high quality papers (Oh, Ebrahimi et al. *Nature Communications* 2018; Oh, Koncevicius et al., *Genome Biology* 2019). We have also actively explored funding opportunities and already received over $5M from Brain Canada and CIHR to perform chrono-epigenomic research We have recently launched a collaboration study with the CAMH Brain Imaging (MRI) centre and are now analyzing temporal changes in brain structures *in vivo*. In addition to epigenomics and brain imaging, our strategies developed for chrono-epigenomics can be productively applied to transcriptomics, lipidomics, proteomics, glycomics, and other -omic approaches. Numerous laboratories at CAMH and the world will significantly benefit from this program and become *bona fide* members contributing to its further success and uniqueness.

In summary, Tapscott Chair support over the 5-year term significantly contributed to the accomplishments of a series of innovative experiments and led to the development of new principles in large scale chronomics research of schizophrenia and other psychiatric diseases.

PUBLICATIONS (2015-20)

Most important refereed papers:

1. Oh G, Koncevičius K, Ebrahimi S, Carlucci M, Groot DE, Nair A, Zhang A, Kriščiūnas A, Oh SE, Labrie V, Wong AHC, Gordevičius J, Jia P, Susic M, **Petronis A.** Circadian oscillations of cytosine modification in humans contribute to epigenetic variability, aging, and complex disease. *Genome Biology*, 20(2): 2457; 2019.
2. Oh G, Ebrahimi S, Carlucci M, Zhang A, Nair A, Groot DE, Labrie V, Jia P, Oh ES, Jeremian RH, Susic M, Shrestha TC, Ralph MR, Gordevičius J, Koncevičius K, **Petronis A**. Cytosine modifications exhibit circadian oscillations that are involved in epigenetic diversity and aging. *Nature Communications* 2018 Feb 13;9(1):644.
3. **Petronis A**, Labrie V. The crossroads of psychiatric epigenomics. *World Psychiatry*. 2019; 18(3):353-354.
4. Oh G, Wang SC, Pal M, Chen ZF, Khare T, Tochigi M, Ng C, Yang YA, Kwan A, Kaminsky ZA, Mill J, Gunasinghe C, Tackett JL, Gottesman II, Willemsen G, de Geus EJ, Vink JM, Slagboom PE, Wray NR, Heath AC, Montgomery GW, Turecki G, Martin NG, Boomsma DI, McGuffin P, Kustra R, **Petronis A**. DNA modification study of major depressive disorder: beyond locus-by-locus comparisons. *Biological Psychiatry*. 2015; 77(3):246-55.
5. Oh G, Ebrahimi S, Wang SC, Cortese R, Kaminsky ZA, Gottesman II, Burke JR, Plassman BL, **Petronis A**. Epigenetic assimilation in the aging human brain. *Genome Biol*. 2016; 17(1):76.
6. Labrie V, Buske OJ, Oh E, Jeremian R, Ptak C, Gasiūnas G, Maleckas A, Petereit R, Žvirbliene A, Adamonis K, Kriukienė E, Koncevičius K, Gordevičius J, Nair A, Zhang A, Ebrahimi S, Oh G, Šikšnys V, Kupčinskas L, Brudno M, **Petronis A**. Lactase nonpersistence is directed by DNA-variation-dependent epigenetic aging. *Nat Struct Mol Biol*. 2016; 23(6):566-73.
7. Gagliano SA, Ptak C, Mak DY, Shamsi M, Oh G, Knight J, Boutros PC, **Petronis A**. Allele-Skewed DNA Modification in the Brain: Relevance to a Schizophrenia GWAS. *Am J Hum Genet.* 2016; 98(5):956-62.

8. Gordevičius J, Kriščiūnas A, Groot DE, Yip SM, Susic M, Kwan A, Kustra R, Joshua AM, Chi KN, **Petronis A,** Oh G. Cell-free DNA modification dynamics in abiraterone acetate- treated prostate cancer patients. *Clinical Cancer Research* 2018; 24(14):3317-3324.

Other refereed papers:

9. Pal M, Ebrahimi S, Oh G, Khare T, Zhang A, Kaminsky ZA, Wang SC, **Petronis A**. High precision DNA modification analysis of HCG9 in major psychosis. *Schizophr Bull.* 2016 Jan;42(1):170-7.
10. Oh E, Jeremian R, Oh G, Groot D, Susic M, Lee K, Foy K, Laird PW, **Petronis A**, Labrie V. Transcriptional heterogeneity in the lactase gene within cell-type is linked to the epigenome. *Scientific Reports* 2017; 7:41843.
11. Zhang M, Xi Z, Ghani M, Jia P, Pal M, Werynska K, Moreno D, Sato C, Liang Y, Robertson J, **Petronis A**, Zinman L, Rogaeva E. Genetic and epigenetic study of ALS-discordant identical twins with double mutations in SOD1 and ARHGEF28. *J Neurol Neurosurg Psychiatry.* 2016 May 6. pii: jnnp-2016-313592.
12. Pollock RA, Thavaneswaran A, Pellett F, Chandran V, **Petronis A**, Rahman P, Gladman DD. Further Evidence Supporting a Parent-of-Origin Effect in Psoriatic Disease. *Arthritis Care Res* (Hoboken). 2015 Nov;67(11):1586-90.
13. Wang DY, Kosowan J, Samsom J, Leung L, Zhang KL, Li YX, Xiong Y, Jin J, **Petronis A**, Oh G, Wong AHC. Inhibition of the G9a/GLP histone methyltransferase complex modulates anxiety-related behavior in mice. *Acta Pharmacologic Sinica*. 2018 Feb 8. doi: 10.1038/aps.2017.190.

Grants (received in 2015-19)

Title of the grant	Agency	Amount awarded	Start	End
The Ontario Brain Epigenomics Platform	Brain Canada	$832,500	04/01/2015	03/31/2018
The Aging Brain: Circadian, Transcriptomic and Epigenomic Dimensions	CIHR and Brain Canada	$2,407,350	07/01/2016	06/30/2020
Spatiotemporal Dynamics of Chromatin: the Circadian and Aging Connection	CIHR	$800,000	05/01/2017	03/31/2019
Genetic-Epigenetic Interactions in Lactase Non-Persistence	CIHR	$598,891	10/01/2017	09/30/2019
Circadian Epigenomic Biomarkers for Predicting Bipolar Disorder in High-Risk Youth	CIHR	$1,499,850	04/01/2018	03/31/2023
	Total	$6,138,591		