Research plans for the next term of Tapscott Chair tenure (2015-2020)

Despite the densely populated field of human morbid epigenetics, there are some very promising "niches" which have not been fully explored. During the next 5 year tenure of the Tapscott Chair I plan to focus on three projects, which, in my opinion, are particularly promising and directly relevant to understanding the causes and mechanisms of schizophrenia.

Epigenetic heritability of schizophrenia and other psychiatric diseases. The significant potential of epigenetics lies in the ability to challenge some fundamental principles in etiological studies of schizophrenia and other complex diseases, which traditionally focus on DNA variation and environmental hazards. Evidence is mounting, however, that epigenetic marks are only partially erased during meiosis and can be transmitted between generations, and therefore transgenerational epigenetic heritability is possible. Furthermore, our DNA methylome studies of MZ and DZ twins have provided further evidence for epigenetic heritability. These findings may partially explain the difficulties experienced in pinpointing specific DNA variants in diseases, sometimes called "missing heritability". In our laboratory we have collected several hundred germline samples from males affected with schizophrenia or bipolar disorder and controls. These samples will be subjected a very detailed DNA- and histone- modification analysis. Affected individuals will be compared to controls using comprehensive bioinformatics algorithms, and hopefully disease specific heritable epigenetic changes will be identified.

Epigenetically "flavored" DNA sequence variants as a new unit of disease risk factor. This effort will capitalize on the additive value of studying DNA sequence variants in their epigenetic contexts. Traditionally DNA markers have been investigated separately from the epigenetic ones. New and powerful heuristic developments may arise from the combined genetic – epigenetic applications, namely "allele- specific modification" (ASM). Some DNA sequence variants, in addition to their nucleotide differences, may also exhibit epigenetic differences. Nucleotide differences at some concrete position in the genome are categorical (e.g. A allele or T allele), however, their epigenetic marks can be continuous (i.e. both A and T are modified but A exhibits larger mean density of modification compared to the T allele). Such epigenetic asymmetry of DNA sequence variants are likely to reflect some fundamental principles of gene regulations, and such epigenetic "flavors" may provide new insights into the functional roles of DNA sequence variants in disease. We recently performed a study estimating the degrees of epigenetic variation and asymmetry (i.e. ASM effects) of about 500,000 genetic markers in *post-mortem* human brain samples. We then tested the most promising ones in the context of the largest genetic study of schizophrenia, which investigated DNA samples from more than 80 thousand patients and controls. The study detected ~100 genetic risk loci but, based on the lessons from other genetic studies, it is certain that large numbers of genetic risk factors were below detection threshold. Our addition of epigenetic information to the "subthreshold" DNA variants is a promising approach which helps to separate the "wheat from the chaff" in large datasets of traditional genomic studies of disease. Technically speaking, we detect evidence of significant enrichment of ASM- SNPs in the schizophrenia GWAS SNP $p < 0.1$ bin but not in any other bins with $p>0.1$. Schizophrenia had a much higher enrichment in brain ASM SNPs than non-psychiatric diseases and these ASM SNPs were significantly over-represented at regulatory elements such as enhancers. Our plan is to fully characterize the epigenetic asymmetry of DNA sequences variants in the brain and further explore their potential in mapping the genetic risk factors in schizophrenia and other psychiatric diseases.

Evidence for **cyclic epigenomic oscillations** is a recent and completely unexpected development in the lab. While the epigenome is often called dynamic, partially stochastic, and unstable, these features have been usually applied to the development-aging axis indicating mostly unidirectional and irreversible changes in the epigenomes. In our laboratory we serendipitously detected evidence that epigenomes in cells are subjected to rhythmic changes some of which are consistent with the 24 hr cycle. While additional work in the lab is necessary to finally prove the presence of an epigenomic

"clock", we have numerous lines of experimental evidence suggesting that such phenomenon is real and widely spread in living cells. Epigenomic oscillations make perfect sense in the context of well- known cyclic changes at transcriptomic (RNA) and translational (protein) level. The brain is a particularly interesting organ from the perspective of biological rhythmicity. Over the last decades, significant progress has been made in understanding how the central pacemaker, called the suprachiasmatic nucleus, works and how it synchronizes peripheral clocks in the muscle, liver, gut and other tissues. In addition to the central pacemaker, it has been discovered that different brain regions exhibit different types of 24 hour rhythmicity at the level of RNA transcripts and some proteins but hardly anything is known about epigenetic oscillations in the brain yet. Circadian cycles reach their peaks and troughs at different times in amygdala, hippocampus, striatum, cortex and other critically important parts of the brain. From these preliminary studies, we can conclude that different brain structures operate relatively independently but they also have to synchronize their activities for efficient functioning of brain cells and structures.

Based on our own preliminary findings, we can speculate that millions and millions of DNA bases in the nucleus of each cell are subjected to epigenomic "tides" every day and night. Given that different brain regions have their own circadian rhythm, it is evident that the overall organization of epigenomic "tides" in the brain is really very complex. In other words, the brain can be compared to a large orchestra consisting of violins, flutes, cellos, string basses, bassoons, trombones, drums and numerous other instruments. Each instrument plays its own melody and each melody contributes to a complex and sophisticated musical performance. However, if one instrument is off, the entire performance is ruined. Very likely, similar problems occur in the brain; if one out of numerous brain regional "clocks" starts ticking too fast or too slow, sooner or later it may have a detrimental effect on overall brain performance, resulting in psychiatric disease. Without a solid understanding of the basic organization of the brain "multi-clock" system, however, we cannot fully understand brain diseases.

Traditionally, epigenomic data have been collected at a single time point providing only epigenetic "snapshots". The evidence for epigenome oscillations adds a critically important component to the equation: time. The time dimension has been rarely investigated in molecular studies of the brain. However, incorporating the time dimension can yield insights into questions such as: why does schizophrenia exhibit remissions and relapses, where the same individual can look perfectly normal or very sick over relatively short periods of time (e.g. months)? Why is it so that psychotic symptoms in a substantial fraction of schizophrenia patients become less severe as they age, and partial recovery from schizophrenia is possible? Why can medications, which block dopamine receptors, be so efficient in treatment of schizophrenia symptoms? Why atypical neuroleptics can dramatically change metabolism and increase weight causing a series of major health problems in psychiatric patients?

In the next several years we are planning to perform a detailed analysis of basic epigenomic oscillatory features in the brain. For this we will be using mouse and human brains to generate a brain epigenome map, which will be indispensable in future studies of normal and abnormal brain activities. In parallel, we will investigate epigenomic "tides" across hundreds of millions of DNA bases in individuals affected with schizophrenia and other psychiatric diseases. Results of this study will document the intricate time-dependent and highly dynamic organization of the brain, and how this may be abnormal in psychiatric disease. In addition, it will provide a major resource, nationally and internationally, for the uncovering the molecular basis of schizophrenia and other psychiatric disease as well as the basic brain functions such as learning, memory, sleep, and cognition.

In summary, Tapscott Chair support for the next 5 years will significantly contribute to accomplishment of a series of innovative and multidisciplinary projects dedicated to uncovering the primary causes of schizophrenia. If successful, this effort will have a major impact on development of early diagnostics, development of new therapeutics and even cure.