Title: Genetic trade-offs between resilience and susceptibility to stress at the cellular level.

Summary

Resilience and susceptibility to stress and diseases exert pervasive impacts on human well-being. Such impacts have typically been studied in psychological and sociological contexts, whereby genetic and ecological factors mediate levels and directions of responses to diverse stressors during development. Ultimately, however, such responses are determined at the cellular level, through gene-by-environment and epigenetic interactions that involve trade-offs between different cellular pathways and functions. The purpose of this project is to develop the first cellular-level models of human resilience and susceptibility to external stimuli. These models will permit the molecular-genetic, epigenetic and computational dissection of the mechanisms involved in differential susceptibility to both negative and positive environmental impacts.

Mechanistically, CRISPR/cas9 gene editing technology will be used to engineer human neuronal cell lines to express a set of alleles, for known loci, that are associated with extreme high to extreme low susceptibility to negative and positive environments. These differential susceptibility cell lines will be subjected to stress- and benefit-associated perturbations, with outcomes quantified in terms of (a) epigenomic profiles of methylation and histone modifications, (b) gene expression, and (c) cellular-level phenotypes such as growth and survival to identify relevant gene co-regulation networks underlying genetic susceptibility. This experimental paradigm will allow molecular elucidation of the mechanisms of differential susceptibility to stimuli, based on a detailed analysis of the nature and roles of molecular and cellular level trade-offs in physiological function. This project will develop new computational and statistical methods to address the challenges posed by the complex gene-by-environment interactions in these data sets.

This interdisciplinary work is novel and high risk because no previous studies have sought to recapitulate differential susceptibility effects combining multiple genetic modifications in a cellular system. It is potentially transformative in that it aims to open up a new experimental paradigm for analyzing the impact of external stimuli, though psychologically and disease-informed genetic modifications that can be subject to multilevel omics analyses in the context of the relevant ecological-evolutionary theory regarding trade-offs.

Keywords

genetic susceptibility and resilience loci, neuronal models, epigenomics, stressors, genome editing, diathesis stress

Proposed Research Project (5 pages)

Resilience and sensitivity to stresses exert pervasive impacts on human wellbeing. Such impacts derive from a broad array of external stressors, including maternal prenatal stressors, childhood experiences such as institutionalization, trauma and abuse, victimization, nutrition, and many others. But some individuals are much more susceptible than others to the effects of deleterious experiences and environments whereas others are resilient to such adversive exposures. The exact causes for such differential susceptibility and resilience to these external factors are unknown but research points to the complex interaction between genes and environments. Differential sensitivities to negative versus positive environments and experiences have typically been studied in psychological and sociological contexts and have been useful in identifying genetic variants mediating these effects.[1] However, the underlying molecular mechanisms, which are of key importance to understanding resilience and sensitivity, remain virtually unknown, and cannot be determined under current research frameworks. The goal of this project is to develop a completely new type of research system for studying sensitivity and resilience. The system is based at the level of cells because, ultimately, it is cellular traits that determine organismal ones. If successful, it will allow the first experimental analyses of the mechanistic bases of differential sensitivity and resilience, in the context of tradeoffs between key aspects of development and cellular function.

SENSITIVITY AND RESILIENCE

Under the *diathesis stress* model of sensitivity and resilience, individuals with a 'sensitive' genetic makeup suffer costs in deleterious, high-stress environments, but not in typical ones. By contrast, 'resilient' individuals avoid the costs of poor environments.[1] This model was developed from observations, in human subjects, that some individuals were measurably more sensitive than others to environmental conditions, as a result of having different alleles at specific loci. The rationale behind developing new cellular models for these effects is that (1) CRISPR-Cas9 gene-editing technology allows the creation of replicated cell lines that show the same genetic differences as those found in human subjects; (2) the cell lines can be subjected to rigorous experimental control of favourable and adverse conditions; (3) the cells exhibit phenotypes, involving growth rates, differentiation rates, survival, and physiological functions, that can be measured precisely; (4) the cells can be analyzed genetically and epigenetically, at multiple interacting omics levels, to deconstruct the molecular bases of sensitivity and resilience; and (5) evolutionary-ecological hypotheses based on tradeoffs can be experimentally analyzed, such that the impacts and mechanisms of tradeoffs can be studied much more directly than in human subjects.

As such, in principle, human genetic variation in these centrally-important traits can be recapitulated under laboratory conditions, and models regarding sensitivity and resilience can be tested with regard to their applicability. Our research has three main, specific, goals:

(1) Identification of exposures which generate a robust measurable cellular response relevant to the targeted genes. We will test multiple stimuli at different doses and differentiation stages to select exposures which alter non-genetically modified cells' phenotype that can be measured precisely and give a biological readout. Cellular phenotype used for this objective includes growth rates, differentiation stages and rates, survival, physiological function and transcriptomic profiling. These phenotypic responses will be combined using a flexible statistical model to generate an integrative cellular stress response score. Both cell lines will also be sequenced to determine their genotype. Experiments and analyses will be conducted in year 1.

(2) Proof of principle: recapitulation of differential sensitivity genotypes and effects in experimental cellular systems. We will generate neuronal cell lines with extreme-high, versus extreme-low, susceptibility, with regard to sets of alleles mediating sensitivity from the literature and genetic linkage disequilibrium of these alleles in human population. We will then validate and select successfully edited clones to use for objective 3. Experiments and analyses will be conducted in year 1.

(3) Mechanisms of differential sensitivity: testing for epigenetic signatures of sensitivity. We will determine whether or not the cell lines generated with high versus low genetic susceptibility alleles created in (2) exhibit differential cellular responses to stimuli. This will be achieved by characterization of cellular phenotype and transcriptome as done in objective (1) generating a cellular stress response score for each cell line, and then comparing these scores to the baseline one generated in objective (1), in cells without genetic modification. In addition, we will generate and combine epigenomic profiles, including DNA methylation, histone modifications and chromatin accessibility data, with the cellular stress response score to create a comprehensive epigenomic stress response score indicative of differential susceptibility. Experiments and analyses will be conducted in year 2.

DIFFERENTIAL SENSITIVITY GENOTYPES

Previously identified genetic variants associated with differential susceptibility and resilience to environmental adversities in human cohorts will be used to generate cell lines with a combination of risk versus protective genotypes. CRISPR-Cas9 tools using homology-directed repair (HDR) for polymorphic repeats[2,3] and single nucleotide polymorphism (SNP)[4] will be used. We will combine risk and protective alleles to create cell lines with an index of risk (from low to high) according to haplotypes observed in the human population.

The selection of specific gene variants is based on the following criteria: 1) gene variants associated with differential phenotypic outcomes to adversities replicated in multiple studies and/or meta-analyses (behavioral phenotype), 2) gene variants related to genes with a biological role in the nervous system development and physiology (biological phenotype), and 3) their associated genes play a key role in the stress system, dopaminergic and serotonergic transmission and pathways. These include: the dopamine D4 and D2 receptors (*DRD4*, *DRD2*) and dopamine transporter (*DAT1*) polymorphic repeats[5]; the serotonin transporter (*SLC6A4*) single nucleotide polymorphism (SNP, rs25531) and repeat polymorphic region (5HTTLPR)[6–10]; the monoamine oxidase A (*MAOA*) SNP (rs6609257) and repeat polymorphic region[11,12]; the Val66Met SNP (rs6265) in the brain-derived neurotrophic factor (*BDNF*) gene[13]; the catechol-O-methyltransferase (*COMT*) SNPs (val158met (rs4680), rs737865, and rs165599)[14]; the Sirtuin 1 (*SIRT1*) SNP (rs3758391)[15]; the FK506 binding protein 51 (FKBP5) SNP (rs1360780)[16,17].

In addition to conferring differential susceptibility to external stressors, these variants were selected because they impact gene expression[18-22], protein function[23,24], as well as brain volume and function.[14,25-3225,26] A number of these variants also associate or directly regulate epigenetic modifications[1], such as DNA methylation and histone modification, as well as alter chromatin conformation.[22] Moreover, by combining two or more of these polymorphisms into an index of risk alleles, studies have shown cumulative effects of differential susceptibility to environmental adversities on phenotypic outcomes (e.g. SIRT1-COMT-BDNF[15], DAT1-DRD2-DRD4-5HTTLPR-MAOA[33] and 5HTTLPR-DRD4[34]). These results strongly suggest that these risk alleles act in concert rather than having unique individual effects. However, no studies to date have performed a comprehensive analysis combining multiple molecular and cellular readouts to decipher their interactive or cumulative impacts on cell function in response to relevant stimuli.[35]

CELLULAR MODELS

We will use two types of human cell lines that exhibit different properties making them ideally suited for the development of novel, cellular systems to study sensitivity and resilience. **First**, we will utilize immortalized human mesencephalic neuronal precursor cells (LUHMES) that can be differentiated into homogeneous and fully postmitotic dopaminergic neurons that are electrically active and express functional dopamine transporter.[36,37] These cells are a key model in understanding the impact of genotype interaction with external stimuli in the context of dopaminergic neurogenesis. **Second**, we will use hippocampal progenitor cells (HPCs, HPC03A/07) that are vital models for understanding neuronal physiology and brain development, especially in the context of stress reactivity, serotonin and dopamine effects on cellular and organismal functions.[38,39] As such, and given the strong links of hippocampal-cell

neurogenesis patterns with human mental illnesses, these cells represent highly-relevant cellular models for resilience and sensitivity effects.

In addition, both of the selected neuronal cell lines offer a unique experimental design to test external stimuli on neurogenesis and differentiated neurons as they are inducible progenitor cells. The LUHMES cells proliferate with bFGF containing media and are differentiated into mature dopaminergic neurons by using differentiation media containing dibutyryl cAMP, tetracycline and GDNF. Differentiation can be observed in 2-4 days. The HPCs were conditionally immortalized with the c-myc-ER^{TM} transgene. HPCs proliferation is maintained by growth factors (epidermal growth factor (EGF), fibroblast growth factor (bFGF)) and 4-OHT where differentiation into neurons and glial cells is allowed by the removal of these factors and is observed after 5-7 days [38].

CELLULAR STIMULI

The cell lines will be subjected to two highly relevant stimuli to assess differential genetic effects: cortisol and oxygen gradients. The effects of both stimuli will be tested during proliferation stage, differentiation stage and then removed for a period of 5-7 days to assess lasting effects. Different doses of cortisol from high (100uM) to low (100nM), will be applied to mimic deleterious or beneficial stress exposures. Such high and low cortisol concentrations have been shown to either induce a decrease or an increase in cell proliferation in HPCs and alter differentiation, respectively.[38] In parallel experiments, cells will be exposed to different oxygen concentrations, from hypoxia (0 - 2% O_2) to normative O_2 levels (20%), as an independent exposure. In both experiments, cells will be harvested directly after exposure as well as following a 5 days period of washout to assess both the acute and the lasting effects of the stimuli and will be normalized against vehicle for cortisol treatment. Both exposures have been shown by us and others to induce a strong measurable cellular readout in terms of number of proliferating and differentiating cells as well as gene expression and epigenetic changes during neurogenesis.[38,40,41]

CELLULAR PHENOTYPES AND EPIGENETICS READOUTS

The impact of genetic modifications on exposure will be quantified at two levels: 1) cellular phenotypes and transcriptomic responses (under objective 1), and 2) epigenomic profiling (under objective 3). Cellular growth, survival, proliferation and differentiation will be assessed via cell counts and immunostaining using proliferation marker, BrdU, and differentiation markers, MAP-2, Tuj1 and synaptophysin, directed antibodies. Transcriptomic response will be quantified using RNA-sequencing. The epigenomic response will be profiled for the DNA methylome using reduce representation bisulfite sequencing (RBSS), histone modifications, specifically H3K27ac (localized at active enhancers) and H3K4me3 (localized at active promoter) using ChIP-seq, and chromatin accessibility using ATAC-seq.

INTEGRATIVE CELLULAR STRESS RESPONSE SCORE

We will develop a comprehensive definition of stress through a computational model that outputs integrative stress response score for each individual. Our score will integrate all assayed data types (phenotype, expression and epigenetic) to produce a single score that captures the strength of deleterious to beneficial perturbations on a cell's state. We will define this score by comparing the sensitive and resilient genotypes. We hypothesize that a resilient response is a subset of the normal stress response. That is, the stress has the same direct influence, but the resilient genotype buffers cellular pathways such that some downstream effects are mitigated.

Developing a statistically robust stress score is challenging because of the large number of data types and the inconsistent levels of variability between them. To handle this variability, we will use a negative binomial distribution model for read count data; such a model can learn the nonlinear mean-variance relationship present in sequencing-based assays. In addition, we will use prior information from existing gene expression and epigenome data sets to give a higher weight to genes with a plausible causal link to resilience loci.

A SYSTEMS-LEVEL UNDERSTANDING OF DIFFERENTIAL SENSITIVITY

Cellular systems occur in the context of a gene network: a stressor directly influences a small number of genes, which cause a cascade of reactions through connections between genes such as protein-protein interactions and gene regulation. A link between stress and response occurs through a path in this network. We hypothesize that a protective genetic variant affects a gene on this path such that the link between stress and phenotype is broken. We will evaluate this hypothesis by building a gene network computational model of stress response by integrating phenotypic, expression and epigenetic responses. We will build the network using the graphical lasso method. This method is preferred to the more commonly-used gene correlation network, which reports a connection between every correlated pair of genes. The problem is that this creates spurious relationships due to the property of transitivity: causal links between gene pairs (A,B) and (B,C) induces an indirect correlation between pair (A,C). The graphical lasso removes these indirect correlations.

High risk and feasibility

The work is fundamentally novel and high risk because no previous studies have sought to recapitulate differential susceptibility effects in cellular systems. Current research in genetic and social determinants of health focuses on organism-level phenotypes using single polymorphisms and/or exposures, which give incomplete understanding of the underlying mechanisms and possible causal effects of such factors. The main challenge of studying such complex interactions using a cellular model is that the effects of the risk vs protective genotypes on cellular phenotypes and epigenomic profiles in response to stimuli remain unknown, until such data are collected, it is difficult to predict their effects. Although the selected polymorphisms have been shown to alter cellular functions, predominantly gene expression, protein function and DNA methylation (mentioned above), these might not be substantial enough to generate impactful phenotypic alterations observable at the cellular level. Such effects might require higher level models, such as organoid and animal models, more representative of the complexities of organs and systems to observe organism-level phenotypes. Despite these considerations, such a study is needed to determine if the identified genetic polymorphisms give rise to observable cellular phenotypes or not, in response to external triggers. To answer this important question, we will assess the main effects of genotype (comparison of susceptible vs. resilient cell lines) on cellular profiles at baseline (without stimuli) as well as in response to the stimuli (after stimuli, GxE). Even if substantial effects are not observed at the genotype level, our study design will also generate a comprehensive -omics profiling of the cellular responses to a gradient of oxygen and cortisol exposures (main effect of stimuli). Such a comprehensive assessment using cutting-edge -omics profiling combined with computational modeling is lacking and will provide novel insights into stress responses quite generally.

Most studies to date have used CRISPR technology to alter single gene expression and/or translation, although more challenging studies targeting the expression of multiple genes have been successful.[42–44] To understand the complexity of human genetic variation, we need to move away from single gene alterations and generate more representative models of human genetic make-up to study their effects. Such models, by characterizing multiple layers of cellular profiles, will help to disentangle the nuances of human genetic variation on cellular as well as organismal function.

Most importantly, this research draws together for the first time investigators from the health sciences, cell biology, evolutionary biology, and computational modelling, to generate a new, highly-integrative approach to a central question: the biological bases of sensitivity and resilience. As such, the work defies several discipline-centric paradigms, and sets out to open up completely new avenues for progress in this key area. This makes the studies inherently risky in its extension of scientific frontiers, but that is how breakthroughs typically occur.

High reward

Major breakthroughs in science are commonly due to invention of new technologies and systems for study; we believe that this research represents an outstanding opportunity for just this level of advance. This research is potentially transformative in that it aims to open up a new experimental paradigm to analyze both the beneficial and negative cellular responses to stressors, in the context of psychiatric

and disease-informed genetic modifications that can be subject to multilevel computational, -omic analyses in the context of relevant ecological-evolutionary theory regarding tradeoffs.
The main specific rewards include: (1) ability to mechanistically understand causes of resilience and susceptibility, and trade-offs between them, that will enhance our understanding of all systems, from cellular to societal, to which these core properties apply; (2) insights into the many health-related problems, such as depression, anxiety, PTSD, and others, that are known to be mediated in part by differential genetic sensitivities and G by E interactions; (3) development of novel systems that would represent a major methodological breakthrough, opening up new research possibilities and creating whole new research area at interfaces of experimental cell biology, genomics and epigenomics, ecology, evolution, and health; (4) a multi-omics profile of neuronal developmental stages at baseline and in response to highly relevant stimuli; (5) development of an integrative cellular stress response score by building a comprehensive gene network integrating phenotypic, expression and epigenetic responses.

Interdisciplinarity

This proposed research is inherently highly interdisciplinary because it integrates cell biology and molecular biology with evolutionary ecology and computational modeling. Moreover, it does so in the contexts of understanding the genetic basis of phenotypic plasticity and its implications for human health and wellbeing. The work has clear health impacts in that it is aimed at determining the genetic, epigenetic, molecular and cellular bases of human resilience and sensitivity, in the contexts of poor compared to good, environments. The mechanisms of resilience and sensitivity effects should represent novel targets for the maintenance and recovery of health, in both psychological and physical contexts. This study also has strong implications for evolutionary and ecological research because it addresses the core unresolved question of when phenotypic plasticity is favored, relative to genetic mediation or resilience to environmental changes. This is a classic question in biology, with implications for virtually all organisms. The work impacts upon aspects of humanities in that it may lead to the discovery of biological mechanisms mediating stress sensitivity that impact research and policy directions in the areas of social resilience, with knowledge transfer to clinical psychologists, social workers, and educators. In this context, the connections of biology to the humanities and social sciences centre on the nature of complex systems dynamics, and adaptation from genes to organisms to societies.

Equity, Diversity and Inclusion (EDI)

During the course of this work we will seek to maximize the societal, individual, and scientific benefits that derive from application of the most-recently developed best practices of EDI. In choice of trainees, we will (a) include clear and flexible position criteria that recognize non-traditional career backgrounds, (b) use inclusive, unbiased and non-gendered language, (c) have our position postings reviewed by an EDI expert, and (d) show and demonstrate a commitment to EDI on our laboratory websites and in our research endeavors and publications. In mentoring, we will provide equitable training- and mentor-access opportunities, recognizing the variety of forms of mentoring that differentially benefit different individuals. In our work environments, we will provide and encourage access to EDI-relevant training for all individuals (e.g., in implicit bias, GBA+, and peer review), and make clear that a supportive and inclusive work environment is central to the lab community.

Sex and gender considerations

Biological sex and gender are relevant factors at the genetic, epigenetic, cellular, and organismal levels, especially with regards to aspects of differential sensitivities to environmental factors. The genotype variants selected for this study have sex and gender specific effects that will be analyzed in statistical models using multiway interaction effects. The discovery of their impacts at the cellular levels will thus be relevant for sex and gender, with applications for interpretation and knowledge transfer.

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Research Team's Biographical Information (2 pages)

The proposed work is novel and highly ambitious in terms of the multidisciplinary expertise required to answer the central questions. To ensure its success, we have assembled a solid international team of researchers including experts in epigenetic, genetic, cellular and molecular biology, neurosciences, bioinformatics, and evolution biology, with diverse methodological strengths (lab, analytic methods, machine learning, theory).

Nominated Principal applicant *Dr. Nadine Provencal*, an epigeneticist and Assistant Professor in the Faculty of Health Sciences at Simon Fraser University (SFU) and Investigator at BC Children's Hospital Research Institute. Dr. Provencal is an ECR with expertise in epigenetic profiling of cellular (1) and animal models (2) as well as human cohorts (3, 4). Her research focuses on the molecular mechanisms involved in the long-lasting effects of early life adversities in the development of behavioral and psychiatric disorders. She uses a translational approach combining state of the art epigenomics (5) and bioinformatics methods (1). Dr. Provencal will oversee the proceeding of the project, insure exchange and communication between team members by organising monthly meetings as well as directly supervise the trainees and technician. She has extensive experience working on multidisciplinary research projects with experts from different fields. Her lab will be responsible for the epigenomic profiling of the cells using next-generation sequencing (RNA-seq, RBSS, ChIP-seq and ATAC-seq). Because of her expertise working with neuronal cellular models of stress and their impact on the genome, her lab will generate the experimental design for the cortisol exposure in the cell lines.

Co-Principal applicant *Dr. Maxwell Libbrecht* is an Assistant Professor in Computing Science at Simon Fraser University. His research focuses on developing machine learning methods applied to high-throughput genomics data sets. Dr. Libbrecht is an ECR with extensive experience developing statistical and machine learning models for data from sequencing-based genomics assays such as ATAC-seq and RNA-seq, including having produced integrative epigenome annotations of hundreds of human tissues (6-8). For this project, he will be responsible for the computational analysis including developing a stress response score and learning a genetic network as well as directly supervise the trainees.

Co-applicant *Dr. Bernard Crespi*, a Canada Research Chair Tier 1 in Evolutionary Genetics and Member of the Royal Society of Canada, contributes to this project through his expertise at the interfaces of evolutionary biology, the health sciences, and the humanities, with his specific proficiencies in the analysis of human development and child health, sensitivity and resilience,human sociality, and cognitive tradeoffs (9-11). For this proposal, evolutionary theory regarding phenotypic plasticity and tradeoffs is of key importance for analyzing and understanding resilience and sensitivity, at the levels of both cells and organisms.

Co-applicant *Dr. Tim Beischlag*, a molecular and cellular biologist and Professor in the Faculty of Health Sciences at SFU. The primary focus of his research program is elucidation of the molecular mechanisms underlying the metastatic transformation of solid tumour cells. Dr. Beischlag has expertise in the area of the loss of tumor suppressor genes in relation to the hypoxia-inducible activation of cancer cell transformation (12-14). In particular, his lab has expertise in gene-editing, gene expression analysis, signal transduction, cellular physiology and behaviour. His lab will be responsible for the development and validation of genetically modified cell lines using the CRISPR-Cas9 gene editing system, particularly applications involving template-directed repair. Dr. Beischlag's expertise is with cellular models of hypoxia. He will be responsible for the experimental design of oxygen exposure in the cell lines and will contribute to the characterization of these lines.

Co-applicant *Dr. Frank Lee*, a neurobiologist and Associate Professor in the Faculty of Health Sciences at SFU. Dr. Lee's primary focus is investigating the machinations of the dopamine systems in the brain with specific focus on the dopamine transporter and the dopamine D2 receptor (15,16). The co-applicant has extensive experience with primary cultures of neurons and specifically the LUHMES cells in his lab (17). Dr. Lee lab will be responsible for the culture and differentiation of LUHMES cells. Dr. Lee also has extensive experience utilizing the CRISPR-Cas9 gene editing system and will also assist in the generation of the different edited cell lines.

Collaborator *Dr. Annamaria Cattaneo*, an expert in biological psychiatry and early career researcher heads the Biological Psychiatry Unit at the IRCCS Centre Fatebenefratelli Institute, Italy, and is Senior Researcher in the Dept of Psychological Medicine at the Institute of Psychiatry, King's College London,

UK. Dr. Cattaneo main research interests and expertise relate to the investigation of the role of stress hormones and its related pathways, including inflammation and neuroplasticity, in the vulnerability for psychiatric disorders (18, 19) and in the mechanisms of action of psychotropic drugs, in *in vitro* neuronal models (20) as well as in clinical samples. For the current project she will provide her expertise and facilities in performing the planned experiments in HPCs, a cell lines for which her lab have extensive expertise for the culture and differentiation of these cells as well as using them to model stress exposure impacts on neurogenesis.
Collaborator *Dr. Till Andlauer*, a geneticist and senior postdoctoral researcher at the Max Planck Institute of Psychiatry and the Technical University of Munich, Germany. Dr. Andlauer's research focuses on the contribution of common genetic variants to the risk for psychiatric and neurological disorders. His expertise lies in statistical methods to the analyses of large *omics* datasets of patients with multiple sclerosis (21), depression (22), and bipolar disorder (23). Therefore, he has a profound knowledge of genetic susceptibility factors and the necessary experience to assess polygenic risk for psychiatric disorders. I will employ statistical genetics methods to support the project in generating combinations of common risk variants suitable to generate cell lines with distinct risk profiles.

Relevant published work by the applicants and collaborators

Provencal, N: [1] Provençal, N., Arloth, J. et al. Glucocorticoid Exposure During Hippocampal Neurogenesis Primes Future Stress Response by Inducing Changes in DNA Methylation. *Direct submission to a PNAS special volume as invited paper, Jan. 2019.* **[2]** Provençal, N., Suderman, M.J. et al. (2012) The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. Journal of Neurosciences, 32(44):15626-42. **[3]** Provençal, N., Suderman, M.J. et al. (2014) Association of childhood chronic physical aggression with a DNA methylation signature in adult human T cells. PLoS One, 9(4): e89839. **[4]** Cecil, A.M.C., et al. (2018) DRD4 methylation as a potential biomarker for physical aggression: An epigenome-wide, cross-tissue investigation. American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics, 177(8):746-764. **[5]** Roeh S. et al. (2018) HAM-TBS: High accuracy methylation measurements via targeted bisulfite sequencing. Epigenetics and Chromatin. 11(1):39.
Libbrecht, M: [6] Libbrecht, M.W. et al. (2015) Joint annotation of chromatin state and chromatin conformation reveals relationships among domain types and identifies domains of cell-type-specific expression. Genome Research, 25: 544-557. **[7]** Libbrecht, M.W. et al. (2015). Entropic graph-based posterior regularization. Proceedings of the International Conference on Machine Learning (ICML). **[8]** Libbrecht, M.W. and Noble W.S. (2015) Machine learning applications in genetics and genomics. Nature Reviews Genetics, 16: 321-332.
Crespi, B: [9] Crespi, B. (2011) The evolutionary biology of child health. Proceedings of the Royal Society of London B 278: 1441-1449. **[10]** Crespi, B. J. (2015) Cognitive tradeoffs and the costs of resilience. Behavioral and Brain Sciences 38. **[11]** Del Giudice M, Crespi BJ (2018) Basic functional trade-offs in cognition: An integrative framework. Cognition 179:56-70.
Beischlag, T: [12] Khakshour S. et al. (2017) Retinoblastoma protein (Rb) links hypoxia to altered mechanical properties in cancer cells as measured by an optical tweezer. Sci Rep. 7(1):7833. **[13]** Labrecque M.P. et al.. (2016). The retinoblastoma protein regulates HIF1-mediated genetic programs, tumor cell invasiveness and neuroendocrine differentiation in prostate cancer cells. Oncotarget. 7(17). **[14]** Labrecque MP et al (2014) A TRIP230-retinoblastoma protein complex regulates hypoxia-inducible factor-1α-mediated transcription and cancer cell invasion. PLOS One, e99214.
Lee, F: [15] Khan, S and LEE, F.J.S (2014) Delineation of domains within CB1 and D2R that mediate the formation of the heterodimer CB1-D2R complex. J Mol. Neurosci 53(1):10-21. **[16]** Han, J. et al. (2017) Neuroprotective effects of extracellular DJ-1 on reperfusion injury in SH-SY5Y cells. Synapse 71:e21963. **[17]** Luk, B., et al. (2015) A Physical Interaction between the Dopamine Transporter and DJ-1 Facilitates Increased Dopamine Reuptake. PLOS One, e0136641**.**
Cattaneo, A: [18] Cattaneo, A. et al. (2016) Absolute Measurements of Macrophage Migration Inhibitory Factor and Interleukin-1-β mRNA Levels Accurately Predict Treatment Response in Depressed Patients. Int J Neuropsychopharmacol. **[19]** Cattaneo, A. et al. (2018) FoxO1, A2M, and TGF-β1: three novel genes predicting depression in gene X environment interactions are identified using cross-species and cross-tissues transcriptomic and miRNomic analyses. Mol Psychiatry. 23(11):2192-2208. **[20]** Anacker, C. et al. (2013) Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. Neuropsychopharmacology 38, 872–883.
Andlauer, T: [21] Andlauer, T.M. et al. (2016) Novel multiple sclerosis susceptibility loci implicated in epigenetic regulation. Science Advances 2, e1501678. **[22]** Wray N.R. et al. (2018) Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nature Genetics 50(5):668-681. **[24]** Andlauer T.M. et al. (2018) Bipolar multiplex families have an increased burden of common risk variants for psychiatric disorders. https://www.biorxiv.org/content/early/2018/11/12/468975.

Budget Justification (1 page)

Items	Year 1	Year 2
Research Associate Salary (one at 10%)	5000	5000
PhD students Salary (two trainees, one wet lab and one bioinformatics for two years at $11,000/year per trainee, 50%)	22000	22000
Total salaries	**27000**	**27000**
Cell culture and genetic editing costs: -Genotyping of LUHMES and HPC cells via sequencing -Characterisation of the effects of exposures on cell lines (establishment of a robust experimental approach) using immunostaining and RNA-seq. -Combined Crispr-Cas9 gene editing for all selected variants in both cell lines and validation via sequencing	73000	
Epigenomic profiling of edited cells with high and low risk alleles: - mRNA sequencing - ChIP-seq for H3K27ac and H3K4me3 - DNA Methylation using RBSS - Chromatin accessibility using ATAC-seq		73000
Total direct costs	**100000**	**100000**
Total indirect costs (overheads)	**25000**	**25000**
Total	**125000**	**125000**

Salary for research associate, Dr. Andressa Coope, based at SFU in Provencal's lab (Year 1 and Year 2 @ 10%/year). She will help trainees perform cell culture, DNA/RNA extractions, Crispr-Cas9 gene editing and preparation of libraries for epigenomic profiling using next-generation sequencing (NGS). Dr. Coope has the expertise and experience required to perform these high-throughput experiments. She will also be responsible for organizing and biobanking all the data and samples in the lab as well as work closely with the trainees to provide guidance and knowledge transfer.

Salary for two PhD students to be trained, one in molecular and cellular biology to perform the experiments in the lab and one in bioinformatics to perform computational analyses (salary for two years at $11,000/year per trainee, 50%). The first student will develop skills in advance methods for Crispr-Cas9 gene editing in neuronal cells and epigenomic profiling using NGS technology and will be directly supervised by Dr. Provencal and co-supervised by Drs. Beischlag and Lee. The second student will develop skills in advanced computational modeling integrating multi-omics data from NGS as well as statistics and will be directly supervised by Dr. Libbrecht and co-supervise by Dr. Provencal. In addition, both trainees will acquire a unique multidisciplinary training combining genetic, biological, evolution, statistical and bioinformatics knowledge through the expertise and guidance of our multidisciplinary international team. Students will be asked to report on the advancement of the project every month in front of the team. Trainees will also have opportunities to work in other team members' labs, including visiting Dr. Cattaneo's lab in Italy to facilitate the experiments in HPCs.

Drs. Provencal, Beischlag and Lee share open lab space at SFU, which has all the required equipment including, MiSeq, TapeStation and cell culture facilities to perform the experiments. The project will also utilize the Genome BC Genomics Platforms at the Genome Sciences Centre for NGS and secure servers for data storage and analysis at SFU. SFU offers dynamic interdisciplinary learning environments with regular seminars from international experts and graduate student meetings.