UNIVERSITY OF CALIFORNIA,

IRVINE

# Correlation Between Depression and High Decipher Scores in men Diagnosed with Prostate Cancer.

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in Biomedical and Translational Sciences.

by

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## Dedication

This work is dedicated to my parents Canice and Juliana Ubbaonu for the sacrifice they put into giving me an education; to my beautiful wife Cecille who has supported me throughout the challenging but rewarding process of pursuing this master's degree and finally to my daughter Amara for being a constant source of joy and inspiration.

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## Abstract of Thesis

Correlation Between Depression and High Decipher Scores in men Diagnosed with Prostate Cancer.

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Interest has grown in understanding the connection between the state of mind of an individual and its effect on the body at the molecular level. Studies suggest that mental stress can alter the biochemical functions of the body (Juster, 2010). Depression is an example of a condition associated with mental stress. Exposure to environmental stresses is essential to the development of major depression, which in turn has been shown to affect the body systems in several ways. These effects are exerted at three main levels: genetic, molecular and neural. (Kupfer, 2016). Evidence is beginning to emerge that depression can lead to epigenetic changes in the human genome. Studies have also shown that epigenetic changes are critical to the development of many human malignancies including prostate cancer. It is therefore possible that these two processes may be linked, and that the presence of depression could predispose the individual to the development of cancers. Epigenetic mechanisms include DNA methylation, histone acetylation or methylation and post-translational effects of non-coding RNAs.

The Decipher genomic classifier (GC) uses coding and noncoding RNA to predict men with prostate cancer who are at risk for aggressive disease or disease progression. Using the Decipher genomic classifier as a surrogate for coding and noncoding RNA, we seek to demonstrate a possible association between depression and a tendency for developing aggressive prostate cancer. A multivariable logistic regression comparing Decipher scores in depressed subjects will first be done using depression as a continuous variable adjusting for age, SES and total illness burden index and again after dichotomizing subjects into depressed vs. non-depressed with the same adjustments. We will also seek to demonstrate that high levels of resilience attenuate this association.

A limitation of this study is that a correlation is not proof of direct causation. However, a positive association between depression and the 22 RNA components of the Decipher GC may suggest that at least one of these RNAs may be of interest in the epigenetic mechanisms connecting depression and prostate cancer which could be basis for further studies in the future.

Chapter 1

## Introduction

Interest has grown in understanding the connection between the state of mind of an individual and its effect on the body at the molecular level. These studies suggest that mental stress can alter the biochemical functions of the body through an increase in allostatic load (Juster, 2010). The internal physiologic balance of the human body is termed homeostasis. This definition focuses on the internal workings of the body independent of the effects of the environment. In contrast, the allostatic model describes a dynamic relationship between the human physiology and the environment. In the allostatic model the body's homeostatic baseline is constantly being reset to allow the individual to function within the prevailing environmental context. This reset process can create what is referred to as an allostatic load which ultimately leads to the emergence of physiologic dysfunction and disease (Sterling, 1988).

Stress is an abstract term which was originally described in the medical literature by Hans Selye as the mutual actions of forces that take place across any section of the body (Selye, 1956). Stress can be viewed as either internal to the organism or external. Internal stress may occur from derangements of physiological balance caused by diseases such as diabetes or infections. Here we refer specifically to the concept of stress as it arises from the individual's environment. A useful conceptual framework to understand external stress is that of formative versus reflective models (Kaplan, 2020). Life events such as poverty, bereavement and divorce can be described as stressors

that can contribute to the development of psychological disorders like anxiety and depression. Depression when it develops in an individual is the product of an interplay between the individual's genetic predisposition and these environmental stresses. Studies looking at the molecular effects of depression also show genetic changes at the level of transcription or translation which precede the pathophysiological manifestations of the disease. (Guan, 2007).

At the cellular level the introduction of internal or external stressors leads to translational changes which are necessary for the stress response. This response is mediated through the effects of proteins such as adrenaline, noradrenaline and corticosteroids which are produced in the endoplasmic reticulum. Persistent stress however, has been linked to endoplasmic reticulum dysfunction which is associated with the pathophysiology of several disease states like diabetes mellitus and cancer. (Guan, 2007)

The process of protein synthesis involves translation of genetic information encoded in a single stranded messenger RNA (mRNA) molecule. This process is closely regulated by the interaction of mRNA with several proteins including noncoding RNA like microRNA and long noncoding RNA (Rissland, 2017). The single stranded mRNA binds to proteins leading to formation of secondary and tertiary structures as the molecule folds and refolds on itself. This formation of secondary and tertiary structures is associated with base-pairing changes which allows for incredible versatility in the expression of the information encoded in a single mRNA (Bevilacqua, 2016). The unique structure and function of RNA molecules is being exploited in the development of biomarkers used in clinical prediction and prognostication in prostate and other

cancers. This was the basis for the development of the prostate cancer genomic classifier to predict early metastatic disease (Vergara, 2012) (Erho, 2013). The Decipher prostate cancer genomic classifier is a 22-RNA based test used for predicting the risk of metastatic disease in men who have had radical prostatectomy for prostate cancer. These RNAs have been associated with cell adhesion, cell cycle progression, tumor cell motility and immune system modulation. Higher scores correlate with an increased chance of poor outcomes after treatment (Erho, 2013).

The autonomic nervous system's fight or flight response is proof of the connection between the mind and body. This response is generated by the activity of the hypothalamic-pituitary-adrenal axis and its hormones adrenaline, noradrenaline and cortisol. The long-term effect of low-level stimulation over a long period of time is what is referred to as allostatic load and is theorized to lead to emergence of pathology. Genes for adrenergic receptors and corticosteroid receptors have been shown to be dysregulated in patients with prostate cancer, leading to speculation that several other pathways and genes including noncoding RNA are probably also affected (Flores, 2017). Interestingly, depression has also been linked with dysregulation of these same pathways (Kupfer, 2016). Here I intend to demonstrate an association between depression, and the genetic changes which are measured by the Decipher Genetic Classifier.

I hypothesize that men with high depression scores are more likely to have high risk prostate cancer biology as evidenced by high risk scores on the Decipher prostate

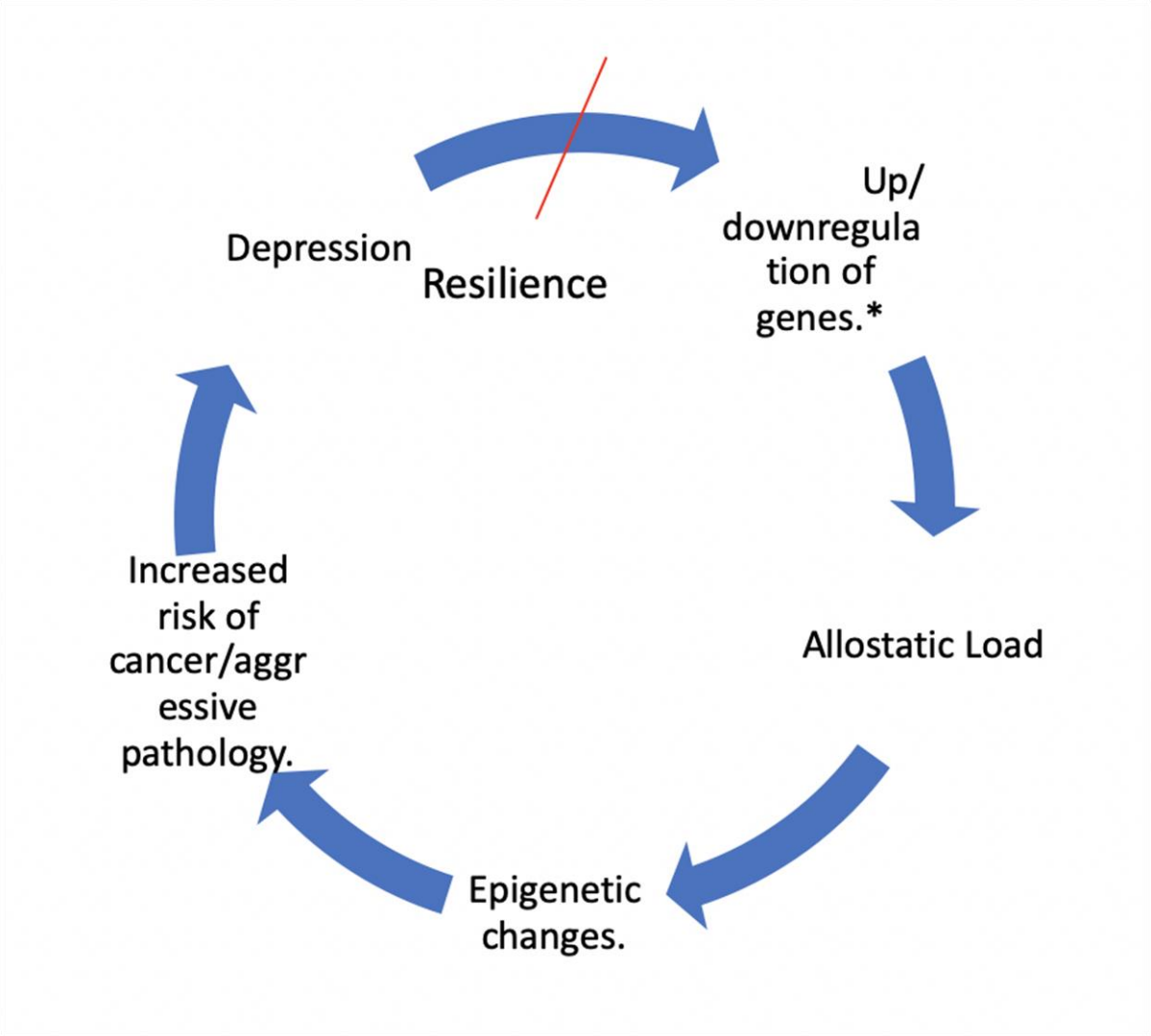
cancer test. Furthermore, when adjusted for levels of resilience, I believe that there might be a reduction in this association.

## Research Aims

The primary aim of this study is to demonstrate a correlation between depression and the emergence of molecular changes in noncoding and messenger RNA. The Decipher score is based on RNA changes and therefore is being used as a surrogate measure.

## Secondary aim:

To see if resilience confers a protective effect against the effects of depression on the Decipher Sore.




*Cortisol receptor, adrenaline receptor, growth factors.

Figure 1: Conceptual model representing the proposed process connecting depression, resilience and epigenetic changes.

Chapter 2.

## Background

How the human psyche might influence the development and propagation of cancers is the focus of this work. The mind can be defined as that “complex of faculties involved in perceiving, remembering, considering, evaluating and deciding” (Editors of Encyclopaedia Britannica, 2007). The connection between the mind and body has intrigued scientists and philosophers for centuries. Taoist and Confucian philosophers have held the view that the body resides in the mind and as such, is under the influence of the mind (Heihachiro, 2018). Hippocrates was one of the earliest philosopher-scientists to recognize the brain as the seat of human thought, sight, hearing, grief, anxiety and tears; an idea that was at the time at odds with the commonly held belief that the heart was the seat of human thought (Hippocrates, 2018). Scientists have since resolved the debate about the seat of the mind. However, what it means to have a mind or what the mind is, let alone what effects it might have on the body, remain topics of debate and research. Although the mind is domiciled in the brain, the brain is not the mind. The mind can be regarded as a latent construct, an abstract entity which cannot be physically seen or measured, but that manifests itself through various complex means -many of which are equally abstract - such as in thoughts, demands, decisions, and perception. Mind is also reflected in sensation, emotions, desires, reasoning and also the unconscious (Editors of Encyclopaedia Britannica, 2007).

The connection between the mind and physiological manifestations is easily demonstrated by such phenomena as salivating at the thought of food or a racing heart and dry mouth when sensing danger, which is part of the fight or flight response of the autonomic nervous system. This state of arousal required to fend off danger puts the body in a high stress state through the effects of the sympathetic nervous system. After the danger is perceived as over, the body returns to a resting state via the counter-effects of the parasympathetic system. What then happens if there is always a sense of impending danger? For instance, what effects does residence in an unsafe neighborhood have on the body? Is it possible that in such situations the fight or flight mechanism remains permanently activated albeit at a lower intensity? If this is the case, what implications might this have on the long-term health of the body systems?

**Allostasis**

The term allostasis was first coined by Peter Sterling and Joseph Eyer in 1988. It essentially is an extension of the concept of homeostasis. In physiology, homeostasis refers to the maintenance of nearly constant conditions in the internal environment of a living organism (Hall, 2011). Sterling and Eyer argued that this view supposes that the internal environment is more or less held in a constant condition, which in reality is not the case. Physiological parameters such as blood pressure, vary significantly throughout the day depending on the environmental context. Allostasis refers to the process by which “an organism must vary all the parameters of its internal milieu and match them appropriately to environmental demands. This view recognizes the need for

the internal balance to shift constantly in order to allow the organism to function within its unique environmental situation (Sterling, 1988).

Several control systems exist in the human body via which homeostasis or allostasis is maintained. The autonomic nervous system functions at a subconscious level and controls several organ functions such as heart rate, respiration and gastrointestinal motility. It also acts on the humoral system which produces several important hormones whose activities are regulated upwards or downwards during periods of stress (Hall, 2011). Over time, if the organism is subjected to constant environmental stress, a constant state of arousal may persist leading to pathological states like depression, anxiety and hypertension (Juster, 2010). The result of persistent stress on the internal milieu of the organism is what is referred to as allostatic load. The allostatic response appears to also be dependent on how the organism perceives or interprets its environment. The perception of stress is primarily a psychological phenomenon that is influenced by other factors such as the individual's genetic constitution, past experiences, coping mechanisms, health habits and historical exposure to traumatic events. Coping mechanisms allow individuals to deal with stressful situations and mitigate their effects. Others however, are not as capable and are therefore at greater risk of developing pathologies like depression (McEwen, 1998).

**Depression**

Depression is a medical syndrome that manifests with several symptoms, chief among which are a depressed mood and/or a loss of interest in activity or pleasure (DSM5, 2013). The development of the depressive phenotype is dependent on the interplay of

several factors. Generally, from an evolutionary and biological perspective, the development of any phenotype is dependent on the individual's genetic constitution, environment, specific triggers and chance (Mukherjee, 2016). Exposure to environmental stress is essential to the development of major depression, which in turn has been shown to affect the body systems in several ways.

**CES-D 9**

Several tools have been developed for the screening and diagnosis of depression. The Center for Epidemiologic Studies depression scale is one example of a tool which was designed for use in the community for depression screening. Originally published in 1977, it has been validated in several populations with high validity and reliability scores for this purpose (Radloff, 1977). The original CES-D scale is a twenty-item scale designed to elicit symptoms of current depression (within one week) in community dwelling adults. This means that it cannot be used to determine the chronicity of depression symptoms. Also, because it screens for symptoms within a one-week period, it fails to satisfy the diagnostic requirement of at least two weeks of symptoms stipulated in the Diagnostic and Statistical Manual (DSM-5). Although not designed for use as a clinical tool for formal diagnosis of depression, it has been shown to perform similarly to clinical screening tools like the Patient Health Questionnaire 9 (PHQ-9) (Amtmann, 2015). When compared to the Diagnostic and Statistical Manual 5 (DSM-5) the CES-D had a sensitivity of 91% for detecting depression but only a specificity of 55% (Yang, 2017). Shorter versions of the CES-D have been devised which are faster and easier to administer to subjects without sacrificing much in terms of validity,

reliability or sensitivity when compared to the twenty-item scale (Amtmann, 2015). In this study the CESD-9 which is a 9-item scale was used.

Instructions: Circle one for each item. During the past 4 weeks, how often were the following statements true:

| | None of the time | Some or a little | Occasionally | Most or all of the time. |
|---|---|---|---|---|
| a. I was bothered by things that usually don't bother me. | 1 | 2 | 3 | 4 |
| b. I felt that I could not shake off the blues even with the help from my family or friends | 1 | 2 | 3 | 4 |
| c. I had trouble keeping my mind on what I was doing. | 1 | 2 | 3 | 4 |
| d. I felt depressed. | 1 | 2 | 3 | 4 |
| e. I felt that everything I did was an effort. | 1 | 2 | 3 | 4 |
| f. My sleep was restless. | 1 | 2 | 3 | 4 |
| g. I was happy | 1 | 2 | 3 | 4 |
| h. I enjoyed life. | 1 | 2 | 3 | 4 |
| i. I felt sad. | 1 | 2 | 3 | 4 |

Table 1: 9 Item Center for Epidemiological Studies -Depressions Scale. (CES-D 9)

## Genetic and Epigenetic Mechanisms in Depression

The effects of depression are exerted at three main levels: genetic and epigenetic, molecular and neural (Kupfer, 2016).

Epigenetic mechanisms are emerging as important contributors to the pathophysiology of depression. Of particular interest is the epigenetic modification of FK506 binding protein 5 gene (FKBP5) which normally modulates the activity of the glucocorticoid receptor (GR). Specifically, FKBP5 down regulates the expression of the GR gene and

plays a key role in negative feedback mechanisms that return the GR to resting state after stimulation (Klengel, 2015). Epigenetic mechanisms involving microRNA (miR-34c) and DNA methyl transferases like dnmt1, dnmt3a and dnmt3b have been linked with demethylation of FKBP5 gene response elements, resulting in glucocorticoid receptor resistance. The ultimate effect of this is dysregulation of the glucocorticoid hormone response leading to multiple downstream effects on immune cells, neurons and possibly other cells (Klengel T. , 2013). Van Rossum and colleagues found in a study of patients with depression that polymorphisms in the *Bcl*I and ER22/23EK genes are associated with the development of depression. Polymorphisms in these genes have been shown to be associated with hypersensitivity to glucocorticoids and glucocorticoid resistance, respectively (van Rossum, 2006). These findings are important because the hypothalamic-pituitary-adrenal axis is known to be dysregulated in major depression with attendant elevation in cortisol levels (Malhi, 2018). There is also some evidence that persistently elevated cortisol may play a part in the development of malignancies. This is thought to be associated with the suppression of cellular immunity (Callewaert, 1991). Some prostate cancers have been shown to have mutations in the androgen receptor which leads to a switch from androgen to cortisol sensitivity. This switch confers androgen resistance making them resistant to androgen therapies with a tendency towards more aggressive behavior (Zhao, 2000). Though this association does not prove causality, it nonetheless suggests that depression might play an important role in the pathophysiology of prostate cancer through its effects on corticosteroid and catecholamine metabolism.

Equally interesting is the study by Lu et al which showed several genes involved in the adrenal pathway are strongly associated with more lethal prostate cancers. Eleven genes were evaluated in this study out of which 3 genes, ADRA1A, ADRA1D, and ADRBK1 showed very strong statistically significant association. When the authors adjusted their model for tumor stage and Gleason score however, the statistical significance was maintained in those patients with high Gleason scores and ki-67 expression (both of which are associated with poorer outcomes) (Fantony, 2018) (Lu, 2016).

ADRA1A and ADRA1D are two of three subtypes of alpha-1-adrenergic receptors (ARs). They are present in most organs and tissues, but are mainly expressed in prostate, fat, liver, brain, heart and spleen (ADRA1A, 2020). Adrenergic receptor stimulation predictably leads to peripheral vasoconstriction, increased heart rate, glycogenolysis, mydriasis and several other effects necessary for the fight or flight response of the sympathetic nervous system to stress. Interestingly these ARs have been linked to signaling molecules such as small G-proteins and MAP kinases which activate antiapoptotic pathways leading to tissue growth (Tank, 2015). The expression of ADRA1D has been shown in studies of human tissues to be involved in the proliferation of some prostate cancers; and this effect has been correlated with behavioral stress in some animal models (Morelli, 2014) (Antoni, 2006). Beta-2 and beta-3 receptors and cholinergic fibers of the parasympathetic nervous system have also been shown to play a role in the invasion, migration and metastasis of prostate cancer cells (Magnon, 2013).

Flores et al found significant upregulation of genes like Ets2 D4AAH4 and Skp2 B2GUZ0 known to be involved in cancer pathways in rats subjected to acute or repeated restraint stress. Interestingly, they also demonstrated that 14 days was sufficient time to ameliorate this effect and return the gene levels to prestress levels (Flores, 2017). Jung et al found that repeated social defeat induced a significantly decreased mRNA expression of FKBP52, a protein that has several functions including intracellular trafficking of steroid hormones and binding to immunosuppressants FK506 and rapamycin (Jung, 2014) (FKBP4, 2020). These findings suggest that, in rats at least, stress can upregulate genes associated with development of malignancies and that the removal or reduction of stress may help reduce this risk.

The implication being that measures aimed at controlling an individual's environment may be prophylactic against the development of certain malignancies.

Depression is recognized as a proinflammatory state that leads to increased circulating levels of inflammatory markers such as interleukin 6, tumor necrosis factor beta and C-reactive protein among others (Malhi, 2018). Chronic inflammation is a factor that is implicated in the development of many cancers. Admittedly what has been difficult to prove in cancer patients is which condition comes first. In other words, it may be that patients with conditions associated with increased inflammation such as cancers, tend to be more depressed. Therapy with interferon alpha, a cytokine, is associated with development of depression in up to 50% of patients; demonstrating the direct contribution of cytokines in the etiology of depression. That being said, many studies have demonstrated an association between depression and the development of many malignancies (Sotelo, 2014) (McGee, 1994).

## Resilience

The concept of resilience has been described as embodying "…the personal qualities that enable one to thrive in the face of adversity" (Connor, 2003). It has also been defined as the dynamic process though which an individual can adaptively overcome a stressful and/or traumatic event while maintaining relatively normal physical and psychological function over time (Osorio, 2017). It is therefore that attribute of an individual that allows them to maintain relatively normal physical, mental and emotional functioning in spite of a history of exposure to stressful circumstances. This ability to overcome adversity as an adaptive process is complex and involves genetic/biological, behavioral, social, spiritual, psychological and cognitive sub-domains (Osorio, 2017) (Connor, 2003). Resilience has been traditionally viewed as evidence of mental toughness. Studies have described psychosocial factors used by resilient individuals such as actively facing fears, optimism and positive emotions, cognitive reappraisal, positive reframing and acceptance, social support and a sense of purpose in life (Feder, 2009). What is interesting is that the ability to learn or use these coping skills are likely modulated by the individual's biology which influences how they physiologically respond to stressful events.

The role of the HPA axis in the pathophysiology of the stress response and development of depression was discussed earlier. Complex mechanisms involving several feedback pathways leading to fast onset and resolution of cortisol release in resilient animals and humans have been described. The value of this is to coordinate the onset and termination of the stress response. These effects are thought to be mediated via the actions of corticosteroids on mineralocorticoid (MR) and glucocorticoid

receptors (GR) respectively (Ron de Kloet, 2005). The GR especially acts to return the body to the pre-stress state. Interestingly, early childhood stress and depression have been shown to be associated with downregulation of GR. This may suggest that GR plays an important role in the development of depression as well as a lack of resilience in these individuals (Charney, 2004).

Corticosteroids are released into the blood stream and exert their effects remotely via interactions with the MR and GR. GR especially is expressed by most cells of the body and therefore, is responsible for much of physiological effects of steroids on the body. Recent DNA microarray and serial analysis of gene expression (SAGE) studies have demonstrated that activation of MR or GR led to altered expression of over 70 genes in the hippocampus of Wistar rats, 50% of which were up regulated (Datson, 2001). Several of the upregulated genes are involved in cell growth and cell adhesion (Sandi, 2004).

**Epigenetic Mechanisms**

The increase in cortisol and catecholamines seen in depression as well as their down-stream effects on their respective receptors are mediated by the genes that encode the proteins involved in the HPA axis. Several mechanisms play a role in this process, such as negative and positive feedback mechanisms from the receptors to the HPA axis. As mentioned earlier, the development of depression is dependent on the interplay between genetic predisposition, the environment and life stresses. Studies have shown that stress through multiple complex mechanisms which are still being elucidated, can alter DNA without changing its genetic sequence (Klengel, 2015).

The study of mechanisms that lead to the heritable silencing of genes without a change in their coding sequence is called epigenetics. For the sake of simplification, three main mechanisms for epigenetic modifications have been identified:

- Histone modifications which influence the coiling of DNA strands and formation of heterochromatin which is highly compact and less accessible to transcriptional factors hence essentially silencing the gene product.
- DNA methylation. This mainly occurs as methylation of the $C^5$ position of cytosine residues and has several effects on DNA, one of them being the transcriptional repression of the gene.
- Noncoding RNAs play an important role in post-transcriptional silencing by influencing the formation of heterochromatin (Egger, 2004).

There are several kinds of non-coding RNA such as microRNA (miRNA), long noncoding RNA (lncRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and piwi-interacting RNA (piRNA). miRNA and lncRNA especially have been studied extensively and have been shown to regulate translation and post translational modification via their actions on messenger RNA (mRNA). RNA can also act in concert with DNA methyl transferases leading to methylation and silencing of genes (Egger, 2004). Epigenetic mechanisms have been proposed as a versatile and fast mechanism by which organisms adapt to changes in their environment (Feinberg, 2006). These could however, come with increased risk for the development of cancers and other diseases. As an example, certain miRNAs referred to as onco-miRNA act to suppress the expression of tumor suppressor genes such as p53

and RB1 via epigenetic means and predispose to development of prostate and other cancers (Frame F. M., 2019).

The role of miRNA, lncRNA and others in the development and propagation of prostate cancer has generated a lot of interest for their potential use in the development of predictive and prognostication tools as well as novel therapeutic strategies (Frame, 2019). Examples of prognostication tools are the gene expression tests, which have emerged as adjuncts to clinical markers like Gleason score, cancer stage and prostate specific antigen (PSA), for predicting prostate cancer patients with the greatest risk of metastatic disease or relapse after radical prostatectomy (Vergara, 2012) (Catto, 2011).

**Gene Expression Tests.**

Prostate cancer gene expression tests are biopsy tissue-based tests which are used to predict those patients who are at the greatest risk of having metastatic disease. These tests are either DNA or RNA based. Examples are Decipher genomic classifier (GC) by GenomeDX, Myriad Polaris Cell Cycle Progression (CCP) and Oncotype DX Genomic Prostate Score (GPS) (Salami, 2018).

The Decipher genomic classifier is comprised of 22 coding and noncoding RNA found to be differentially expressed in metastatic prostate cancer when compared to non-metastatic. Results for the Decipher GC are reported on a scale of 0 to 1 with a cut off of greater than 0.5 consistent with higher risk of metastatic disease (Erho, 2013).

A previous study by Vergara et al showed an upregulation in RNA gene loci involved in cell proliferation and differentiation, cell cycle progression, cell structure, and motility; while RNA involved in cell adhesion, muscle contraction and neural development were

down-regulated. This study was the basis for the development of the Decipher GC (Vergara, 2012).

**Connecting Depression, Allostatic Load, Noncoding RNA and Poor Outcomes in Prostate Cancer.**

We have shown that the development of major depression arises from the interplay between the individual's genetic predisposition and environmental stresses. The response of the internal milieu to external stress is to adjust the internal homeostatic parameters such as blood pressure, heart rate, glycogenolysis and muscle tone in preparation for the flight or fight response. The persistence of this heightened state of physiological arousal is what is referred to as allostatic loading. At the molecular level, these physiological changes are mediated by up-regulation or down-regulation of genes that are responsible for the translation of proteins which play integral roles in the allostatic processes described above. Depression exerts its effects on the body partly by affecting genetic mechanisms involved in the HPA axis and potentially other areas of the body leading to its physical and psychological manifestations. Noncoding RNA such as miRNA and lncRNA are now recognized as important players in the regulation of some of these genes as well as in post translational modification of gene products (i.e. proteins).

Recently, noncoding RNA have been discovered to be useful biomarkers in the prediction and prognostication of metastatic disease in men with prostate cancer and studies have shown that cancer patients suffer disproportionately from major depression (Bortolato, 2017). It is therefore, not unreasonable to wonder if there might be a

correlation between the presence of depression and dysregulation of noncoding RNA in men with prostate cancer. To my knowledge, this association or correlation between major depression and high GC scores has not been studied and will therefore be the focus of this thesis.

Chapter 3

# Methods

This will be a retrospective cross-sectional study. Data from the California Initiative to Advance Precision Medicine in Early Prostate Cancer will be used. Data was collected from patients seen at five medical centers namely: University of California Irvine Medical Center, University of California Los Angeles Medical Center, Cedars Sinai Hospital, the West Los Angeles Veteran's Administration Hospital and the Long Beach Veteran's Administration Hospital.

The independent variable for this study is t depression. Analysis of the association between depression and the Decipher score will be done twice. The first analysis will be done with depression as a continuous variable while in the second analysis depression will be dichotomized into two groups: depressed (high risk for clinical depression) vs. not-depressed (low risk for depression). High risk for major depression is defined here as a CESD 9 score > 20 based on prior studies showing a sensitivity of 79% and specificity of 80% at this threshold (Blank, 2004). The terms high or low risk was adopted because the CESD 9 was developed for screening and not diagnosis of depression (Radloff, 1977).

A sample size of 282 (141 per group) is estimated to be needed to detect a difference in effect size of 10% or greater between groups at 80% power and alpha of 0.05. An effect

size of 10% was selected based on the assumption that a difference between groups of more than 0.10 or 1 in 10 subjects is clinically relevant.

Variables to be collected are: Decipher scores, depression score, age, race/ethnicity, marital status, employment status, resilience scores, socioeconomic status (SES), physical activity, healthy habits, overall health, total illness burden (TIBI) and prostate cancer specific variables: PSA, Gleason score and tumor stage.

Questionnaires were used to collect information on TIBI, physical activity, health habits, resilience and depression. The Centers for Epidemiological Studies Depression Scale (CESD-9) scale was used to screen for depression.

Resilience scores were calculated using the Connor-Davidson resilience scale (CD-RISC) (Connor, 2003). This will be treated as a continuous variable and the difference between mean scores of the independent variable groups will be analyzed using the independent samples t-test.

For this analysis, Depression is the independent variable. The dependent variable will be the Decipher score which will be treated as a continuous variable with scores recorded from 0 to 1.

The co-variables age, socioeconomic status and TIBI will be treated as potential confounders of the association between depression and higher decipher scores.

Age was chosen as a covariable because it is an established risk factor for the development of most malignancies (White, 2014). It has been described as the overall most important risk factor for the development of cancer (Institute., 2015).

Cancer and depression patients often times have several other comorbidities such as diabetes, pulmonary and cardiovascular disease which significantly impacts their quality of life (Sogaard, 2013) (Schaakxs, 2017). These comorbidities have also been linked with increased stress (Cummings, 2016). Since the premise of this study is that depression reflects a state of stress, it is possible that disease burden might be a confounder and for this reason, TIBI was added to the model for analysis.

Studies of contributors to allostatic load have shown that lower socioeconomic status individuals suffer from greater prevalence of cardiovascular and other diseases likely due to the contributing effect of their socioeconomic status (Ribeiro, 2019). As such, it is reasonable to include it in the multivariable analysis as a possible source of stress.

**Null Hypothesis**

There is no association between depression and Decipher scores in men diagnosed with prostate cancer.

**Alternative Hypothesis**

Depression is associated with higher decipher scores.

**Endpoint**

Statistically significant difference in decipher scores in depressed vs non-depressed patients using the beta coefficient of a multivariable linear regression.

**Secondary Endpoint**

Demonstrate an attenuation of the association between depression and Decipher scores when adjusted for resilience.

**Analysis**

Statistical analysis will be done using the SPSS statistical software version 26. (IBM Corp.) A multivariable linear regression will be performed to demonstrate association between the dependent and independent variables after correcting for the age, TIBI, socioeconomic status and resilience. This analysis will be done using depression as a continuous variable and again after dichotomizing the variable into depressed vs non-depressed groups. Below is an equation representing the model comparing decipher scores to depression in men with prostate cancer.

The general form of a regression equation is expressed as:

$y_i = \hat{y}_i + e_i$

Where yi= true observed value, $\hat{y}_i$ = predicted value and $e_i$= residual or error term.

The unadjusted linear regression equation is given as:

$\hat{y}_i = b0 + b1Dep_i + e_i$

and the multivariable regression is expressed as:

$\hat{y}_i = b0 + b1Dep_i + b2Age_i + b3SES_i + b4TIBI_i + b5Res_i \ldots e_i$

Where $\hat{y}_i$ is the dependent variable Decipher score.

Dep= Depression

SES=Socioeconomic status

TIBI= Total illness burden index.

Res= Resilience

b0 = y-intercept of the regression model.

b1, b2, b3 and b4 are the slope values for the respective regression models.

$e_i$= residual (or error term). Represents the difference between the predicted effect of the independent variable on the dependent variable, and the actual observed effect for the regression model.

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Chapter 4

# Results

## Results Tables

We are currently in the process of collating and cleaning up the data. Results of statistical analysis will be posted here once they are available. The tables below will contain the results of the analysis.

Table 2: Baseline characteristics.

| Patient Characteristics | Depression Likely (Total Number) | Not Depressed (Total Number) | 95% CI. |
|---|---|---|---|
| Age<br>Mean (SD) | | | |
| Non-Hispanic White (%) | | | |
| Non-Hispanic Black (%) | | | |
| Physical Activity.<br>Mean (SD) | | | |
| TIBI<br>Mean (SD) | | | |
| Resilience<br>Mean (SD) | | | |
| Healthy habits<br>Mean (SD) | | | |
| Overall Health.<br>(EVGFP) | | | |

| | | | |
|---|---|---|---|
| Mean (SD) | | | |
| Alcohol use (%) | | | |
| Marital Status (%)<br><br>Married | | | |
| Not Married | | | |
| Prostate cancer Specific | | | |
| PSA<br>Mean (SD) | | | |
| Gleason Score (%) | | | |
| Grade 1 (3+3=6) | | | |
| Grade 2 (3+4=7) | | | |
| Grade 3 (4+3=7) | | | |
| Grade 4 (4+4=8) | | | |
| Grade 5 (9 to 10) | | | |
| Decipher Score<br>Mean (SD) | | | |

Abbreviations: TIBI; Total illness burden index, PSA; Prostate specific antigen; EVGFP scale, Excellent-Very Good-Good-Fair-Poor.

Table 3: Result of simple linear regression and adjusted regression* with p value and 95% confidence interval.

Unadjusted and adjusted models for Decipher scores in men with prostate cancer.

| Variables | Unadjusted Effect (95%CI) | P-Value | Adjusted Effect (95%CI) | P-Value |
|---|---|---|---|---|
| Depression, Likely | | | | |

*Adjusted for presence of resilience.

Chapter 5.

## Discussion.

A number of possibilities could result from the study data. One possibility is that the unadjusted regression analysis reveals a significant association between depression and the Decipher score. Without adjustment, this association could still be considered to be the due to the effect of an unidentified confounder such as age and TIBI which have been selected for addition into the multivariable model. Therefore, it would be difficult to reach any conclusions as to a clear association between the variables in this event.

The multivariable model adjusting for the effect of total illness burden (TIBI) and age may show a retained association between depression and Decipher scores. This result will support the study hypothesis of a possible association between depression and Decipher scores. It has to be said though, that even in this event the association could still be the result of an unanalyzed confounder. Since the Decipher classifier is a composite of 22 coding and noncoding RNA, a positive correlation would raise the question of which of the individual RNAs are responsible for this association and why. These are questions which can then be further addressed in a separate study. This would be a multidisciplinary study involving clinicians, molecular biologists, psychometricians and statisticians. In such a study subjects would have to be diagnosed with clinical depression using a clinical tool like the Diagnostic and Statistical Manual 5. The depressed phenotype would have to be very meticulously documented. Also, important would be instruments designed to collect information about exposure to

childhood stress/abuse and other psychosocial variables of interest to the investigators (Klengel T. B., 2015). Using a case vs control design, depressed vs non-depressed patients with prostate cancer can be compared to see if this association can be re-demonstrated. If it is, then this would open the door for even further testing, this time looking at the expression of the RNA that comprise the Decipher genomic classifier. Such an analysis if it is positive would undoubtedly create even more questions. If some of these RNAs are indeed associated with clinical depression, then what is the nature of this association? Genetic association studies even when they yield results do not always, nor do they fully answer the question of causality. Another potential issue could be the magnitude of an association which could be so modest as to call into question the value of investing further time and effort into the subject (Uitterlinden, 2016). However, because both depression and resilience are modifiable conditions, it may prove of interest clinically in a prospective experiment to see if indeed preventing and treating depression may lead to better outcomes in patients with prostate and other kinds of cancers.

Assuming that the results of the multivariable regression shows that high resilience scores are associated with a reduction of the effect of depression on Decipher scores, then this could mean that indeed that people who are better able to cope with stress may have better health outcomes; such as less aggressive disease biology in prostate cancer. As discussed above, future studies could include resilience in a genetic association study seeking to re-demonstrate this association.

Finally, the results of this study could show no association between depression and Decipher scores nor any effect of resilience in a multivariable model. It has to be conceded that this is not an entirely unlikely outcome. The probability of finding an association between the depressed phenotype and a group of 22 RNA molecules is certainly small but is not zero. Therefore, even in the event of a negative study, the premise of the project cannot be discarded altogether since as discussed above evidence is growing that suggest this is an area of research that is very likely to yield results in more sophisticated multidisciplinary studies in the future.

**Limitations.**

There are several limitations to this study. This was a retrospective cross-sectional study which means causality cannot be attributed from the findings. A correlation between the independent and dependent variables may have been the effect of unidentified confounders. Another issue is that the CES-D 9 is not designed to diagnose depression. A cutoff score of >20 has a sensitivity of 79% and specificity of 80% (Blank, 2004), but is still likely to misclassify some subjects either as having or not having depression.

The reasoning behind the study hypothesis was that chronic psychological stressors such as depression, may cause epigenetic changes in patients. Since carrying out an actual genetic analysis would have been prohibitively expensive and time consuming, using the Decipher genomic classifier as a surrogate measure of potential epigenetic changes in RNA was a compromise. The use of surrogate end points however comes with potential limitations.

Testing for an association between a subject's genotype and disease phenotype requires carefully documenting the phenotype information (Uitterlinden, 2016). The phenotype of the independent variable, depression, was not done since the CESD-9 is not designed to actually diagnose depression. The outcome of interest here was the aggressive disease phenotype which remains incompletely defined, although studies have shown that a high Decipher score independently predicts metastasis (Spratt, 2018). An ideal surrogate measure is one that correlates perfectly with the actual outcome of interest (Grimes, 2005). It preferably should not only correlate but also be an intermediate to the outcome of interest (Johnston, 1999). The Decipher genomic classifier can be said to have a modest ability to predict a metastatic disease phenotype but it by no means has a perfect correlation. The implication of all of this is that the final outcomes of the study analysis cannot be assumed with certainty, to have demonstrated a direct link between depression and the Decipher score.

Very few human diseases can be traced back to a single genetic abnormality. More commonly, diseases such as depression and prostate cancer are the result of multiple polygenetic and environmental interactions. This makes it difficult to identify possible genetic influences in diseases outside of a full-scale genome-wide association study. However, a positive correlation in this study would be encouraging, and serve as a pointer towards trends that may require more rigorous testing possibly in a case-control genome-wide association study.

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# Appendix 1

**Center for Epidemiologic Studies Depression Scale (CES-D), NIMH**

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

| | During the Past Week | | | |
|---|---|---|---|---|
| | Rarely or none of the time (less than 1 day ) | Some or a little of the time (1-2 days) | Occasionally or a moderate amount of time (3-4 days) | Most or all of the time (5-7 days) |
| 1. I was bothered by things that usually don't bother me. | ☐ | ☐ | ☐ | ☐ |
| 2. I did not feel like eating; my appetite was poor. | ☐ | ☐ | ☐ | ☐ |
| 3. I felt that I could not shake off the blues even with help from my family or friends. | ☐ | ☐ | ☐ | ☐ |
| 4. I felt I was just as good as other people. | ☐ | ☐ | ☐ | ☐ |
| 5. I had trouble keeping my mind on what I was doing. | ☐ | ☐ | ☐ | ☐ |
| 6. I felt depressed. | ☐ | ☐ | ☐ | ☐ |
| 7. I felt that everything I did was an effort. | ☐ | ☐ | ☐ | ☐ |
| 8. I felt hopeful about the future. | ☐ | ☐ | ☐ | ☐ |
| 9. I thought my life had been a failure. | ☐ | ☐ | ☐ | ☐ |
| 10. I felt fearful. | ☐ | ☐ | ☐ | ☐ |
| 11. My sleep was restless. | ☐ | ☐ | ☐ | ☐ |
| 12. I was happy. | ☐ | ☐ | ☐ | ☐ |
| 13. I talked less than usual. | ☐ | ☐ | ☐ | ☐ |
| 14. I felt lonely. | ☐ | ☐ | ☐ | ☐ |
| 15. People were unfriendly. | ☐ | ☐ | ☐ | ☐ |
| 16. I enjoyed life. | ☐ | ☐ | ☐ | ☐ |
| 17. I had crying spells. | ☐ | ☐ | ☐ | ☐ |
| 18. I felt sad. | ☐ | ☐ | ☐ | ☐ |
| 19. I felt that people dislike me. | ☐ | ☐ | ☐ | ☐ |
| 20. I could not get "going." | ☐ | ☐ | ☐ | ☐ |

SCORING: zero for answers in the first column, 1 for answers in the second column, 2 for answers in the third column, 3 for answers in the fourth column. The scoring of positive items is reversed. Possible range of scores is zero to 60, with the higher scores indicating the presence of more symptomatology.