The Role of Single Nucleotide Polymorphisms Causing a Dysregulation of the HPA Axis on the Incidence of Depression

By

Elsa Bechu

****

Submitted in partial fulfillment
of the requirements for
Honors in the Department of Biology

UNION COLLEGE
June, 2021

Abstract

Bechu, Elsa The Role of Single Nucleotide Polymorphisms Causing a Dysregulation of the HPA Axis on the Incidence of Depression. Department of Biological Sciences, 2021.

Advisor: Brian Cohen

Depression is one of the most prevalent diseases worldwide, afflicting approximately 17 million adults in the US in 2019. A prominent causal factor of depression is dysregulation of our body's response to stress when exposed to continuous stressors over long periods of time. Stress is regulated by the Hypothalamic-Pituitary-Adrenal (HPA) axis, with cortisol as its effector hormone. The effects of cortisol are exerted through the glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) in the body and the brain, respectively. The HPA axis is regulated by a negative feedback loop where activation of the GR by circulating cortisol inhibits the production of additional cortisol.

Our lab investigated the role of single nucleotide polymorphisms (SNPs), or mutations, in the genes of the MR, GR, and associated regulatory proteins that are hypothesized to be involved in the dysregulation of function leading to hypersensitivity or resistance to cortisol in the HPA axis. Previous literature has demonstrated that prominent GR and MR SNPs correlate with higher incidence of depression and/or depressive symptoms. Our study continued this investigation into the relationship between mutations and depression in a clinical sample of psychiatric patients from Albany Medical College. We were especially interested in the interplay between mutations of the GR and MR influencing cortisol levels and response in our bodies. DNA from patients was collected with buccal swabs and genotypes were analyzed through allele specific and quantitative polymerase chain reactions. We compared genotypes to the Beck Depression Inventory (BDI) score of patients, as well as other scales measuring anxiety and mood. No significant two-tailed correlation between individual SNPs or GR/MR SNP combinations and

BDI scores was found. One SNP in the GR gene (rs33389) and one SNP on the 11ß-hydroxysteroid dehydrogenase type 1 (rs12086634), an enzyme that regulates peripheral cortisol levels, demonstrated significant one-tailed correlations with the Mindful Attention Awareness Scale and State Trait Anxiety scale, respectively. Obtaining a greater sample size would yield more representation of mutant alleles and potentially allow for more significance correlating genetic mutations to the incidence of depression.

The Role of Single Nucleotide Polymorphisms Causing a Dysregulation of the HPA Axis on the Incidence of Depression

In 2017, an estimated 262 million people, or 3.4% of the global population, were diagnosed with depression, making it one of the most prevalent diseases worldwide. In the United States alone, depression is the prominent causal factor of disability for people aged 15-44 (Ritchie and Roser, 2018). Depression manifests itself through a variety of different symptoms (figure 1), and the number, severity, and duration of symptoms can vary widely between patients. Multiple factors can contribute to the onset of depression, including personality traits and environmental factors, though most changes brought on by depression are a result of altered biochemical processes in the body (Torres, 2020). A prominent theory is that depressive symptoms are a result of our body's repeated response to stress; when stressors become continuous or consistent over long periods of time is when depressive symptoms tend to arise (Deak, 2016).

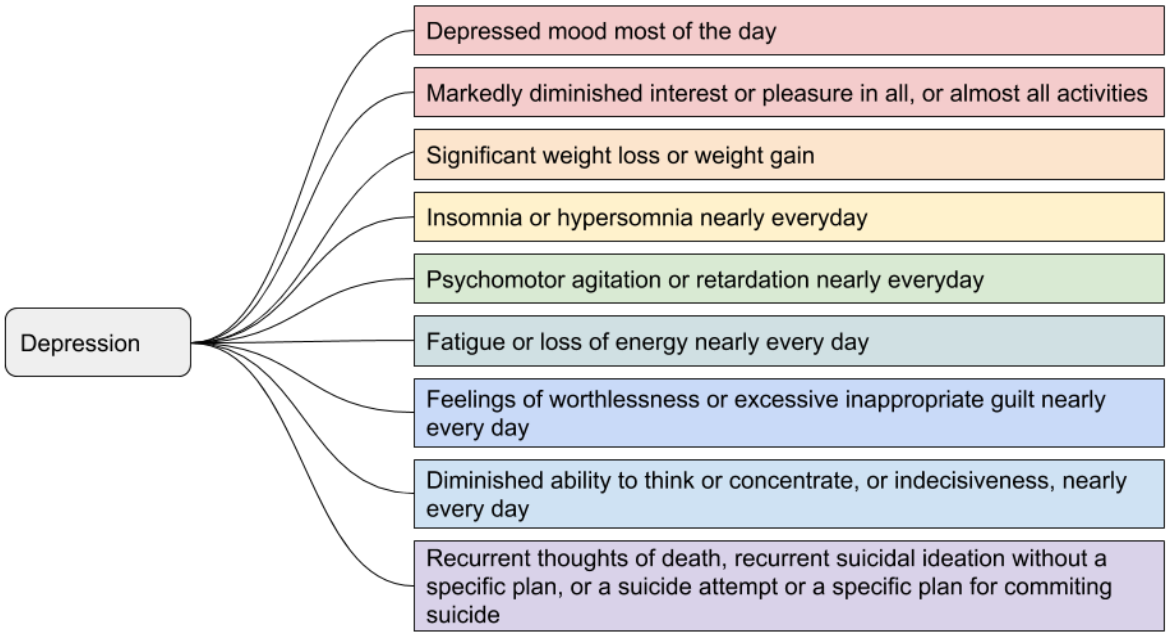

Figure 1. Symptoms of Major Depression. Taken from Cohen, 2020.

Stress is regulated in our bodies by the Hypothalamic Pituitary Adrenal (HPA) axis, and its effector hormone, cortisol, is commonly labeled as the stress hormone (Deak, 2016). The HPA axis is composed of three cell populations in the hypothalamus, pituitary, and adrenal glands that communicate through the release of hormones, ultimately having metabolic effects on the body (Figure 2). When an environmental stressor is present, corticotropin releasing factor (CRF) neurons in the hypothalamus receive neural input from multiple brain regions, causing them to increase activity and produce and secrete the CRF hormone. This CRF hormone then travels to the pituitary gland and induces activity in corticotroph endocrine cells in the anterior pituitary. These cells produce and release adrenocorticotropic hormone (ACTH). ACTH migrates to the adrenal cortex where it binds to the melanocortin 2 receptor, inducing the conversion of cholesterol to cortisol through a sequence of enzyme-mediated reactions. The HPA axis is regulated through a series of negative feedback loops (Deak, 2016).

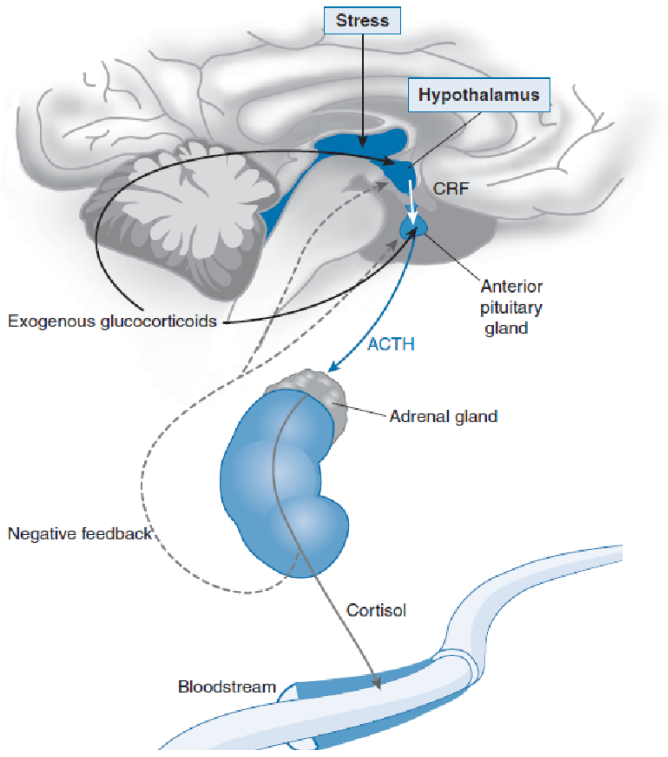

Figure 2. Simplified diagram of the HPA axis and its components. CRF neurons in the hypothalamic paraventricular nucleus receive neural input due to some kind of stress, triggering the production and release of the CRF hormone, which travels to the pituitary gland. Corticotrophs in the pituitary gland secrete ACTH, and endocrine cells in the adrenal gland secrete cortisol. Cortisol is released into systemic circulation, reaching cells throughout the body and brain. Cortisol also induces direct negative feedback inhibition to the pituitary gland and hypothalamus. Taken from Endocrine Physiology, Fourth Edition (Molina, 2013).

Cortisol is a glucocorticoid and the main effector hormone of the HPA axis. Glucocorticoids are steroid hormones that are involved in multiple physiological functioning's necessary for daily life. Glucocorticoids' main function is the regulation of metabolism, and secondary functions include the control of the inflammatory response and regulation of cognitive functions such as learning and memory (Gomez-Sanchez and Gomez-Sanchez, 2014; Timmermans et al., 2019). Cortisol release impacts metabolism by increasing blood pressure as well as increasing blood sugar by stimulating gluconeogenesis, and suppresses the immune response (Timmermans et al., 2019). Cortisol is the human glucocorticoid and exerts its effects through glucocorticoid and mineralocorticoid receptors (GR and MR receptors, respectively). The GR and MR are intracellular receptors that function as transcription factors after ligand binding. In their unbound form, the GR and MR are part of a multiprotein complex located in the cytoplasm, including chaperone proteins and immunophilins. Once cortisol binds, the receptors undergo conformational changes leading to dissociation from the protein complex and migration into the nucleus, where they regulate gene expression (Deak 2016; Moraitis et al., 2016). The GR is abundantly expressed in most cell types across the body, while the MR is expressed

predominantly in only the hippocampus, amygdala, prefrontal cortex, and kidneys (Koning et al., 2019; Deak, 2016). The MR has a high affinity for glucocorticoids and is normally bound at low basal levels of cortisol, whereas the GR has a low affinity for glucocorticoids and is normally occupied only under elevated cortisol levels (such as due to a stressor), and is therefore more responsive to small changes in glucocorticoid concentrations (DeRijk et al, 2006; Chen et al., 2016; Deak, 2016).

The activation of GR and MR are crucial for normal responses to stress; the receptors control processes regulating cognition, mood, and behavioral responses (Koning et al., 2019). When stressed, the activated MR aides in retrieving memory and appraisal processes, while the activated GR promotes consolidation of memory as well as energy redistribution to stop the stress response (Koning et al., 2019; Chen et al., 2016). Subsequently, proper functioning of the HPA axis is necessary for appropriate responses to stress. Dysregulations in the HPA axis, specifically in the activity of GR, MR, and circulating cortisol, have been theorized to contribute to improper responses to stress, and consequently, depression (Pariante, 2010; DeRijk et al, 2006; Chen et al., 2016; Plieger et al., 2017; Moraitis et al., 2016). This hypothesis is partially based on diseases of under- and over-production of cortisol, Addison's Disease and Cushing's Syndrome, respectively (Figure 3).

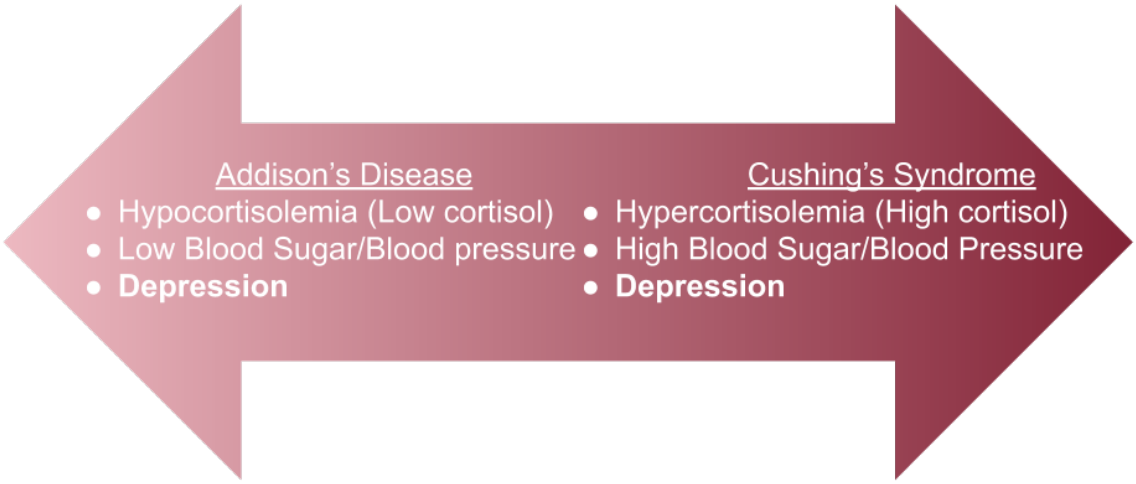

Figure 3. Common symptoms of Addison's and Cushing's syndrome.

Addison's Disease is characterized by insufficient production of aldosterone and cortisol, and symptoms can include irritability and depression (Rare Diseases, 2018). On the other end of the spectrum, Cushing's syndrome is characterized by excess cortisol production by the adrenal glands, and presents symptoms including mood dysregulation and depression (Pariante, 2010; Tang et al., 2013; Chen et al., 2016). These disease states demonstrate that differences in circulating cortisol, hypo or hypercortisolemia, can lead to depressive symptoms. Many patients with psychiatric disorders have been shown to express diminished HPA axis negative feedback regulation, demonstrated by elevated cortisol levels (Pariante, 2010). Unlike Cushing's and Addison's, in which elevated or reduced cortisol levels affect brain function through normally working GR/MR receptors, studies into depression exhibit altered cortisol responsiveness due to impaired functioning receptors (Pariante, 2010). Pariante and Miller measured GR function in vitro and in vivo and found that depressed patients have a diminished GR response when compared to healthy controls (2001). This diminished GR capacity is hypothesized to arise from either impaired function or decreased expression of GR in the brain (Pariante, 2010). Impaired

GR functioning is commonly referred to as glucocorticoid resistance, as the GR is unable to respond effectively to circulating cortisol, and this leads to elevated cortisol levels in the body. Other studies have also theorized that glucocorticoid resistance, stemming from GR impairment or lack of expression, is a key factor in the development of depression (Juruena et al., 2003; Rodriguez et al. 2016). Interestingly, both GR agonists and antagonists have displayed antidepressant effects, and some studies have also demonstrated overactivation of the GR as a risk factor in depression (Pariante, 2006; Koning et al., 2019). Lack of function and/or expression in the MR receptor has also been linked to depression; Medina et al. found that depressed patients had reduced MR expression in the anterior hippocampus, and Lopez et al. found reduced MR expression in the prefrontal cortex of depressed patients (2012; 1998).

Although often theorized to arise from chronic stress, depression does not impact all individuals who face chronic stress problems or traumatic events, nor do all depressed patients have impaired GR/MR receptor functioning. This has led to many theories on what increases an individual's chance at developing depression in the face of stress (Cohen, 2007). A prominent area of investigation is genetics based on the heritability of depression; if one of two identical twins has depression, there is up to a 70% chance the other will develop depression in their lifetime, and heritability of depression if a first degree relative was diagnosed is increased two to threefold (Torres, 2020; Lohoff, 2011). Mutations in the genes of the MR and GR, resulting in hypersensitivity or hyposensitivity to cortisol (depending on whether the mutation causes overstimulation or understimulation of the HPA axis in response to normal levels of cortisol, respectively) have been thoroughly investigated for possible links to depression. Such mutations result in a dysregulation of the HPA axis, and consequently, may demonstrate a higher likelihood for depressive symptoms. Mutations appear in the form of single nucleotide polymorphisms, or

SNPs. The NR3C1 gene codes for the GR receptor, and multiple polymorphisms along this gene have been associated with altered cortisol levels as well as depression (Plieger et al., 2017; Moraitis et al., 2016; Cohen et al., 2007; DE Rijk et al., 2006). Thoroughly investigated SNPs of the GR gene include the Bcl1 (rs41423247), the TthIII (rs10052957), the N363S (rs1800445), and the combined ER22/23EK (rs6189, rs6190) polymorphisms. The Bcl1 polymorphism major allele has been associated with increased responsiveness to cortisol (Van Rossum and Lamberts, 2004), and Plieger et al. found that minor allele carriers had significantly higher cortisol levels following acute stress (2017). The ER22/23EK polymorphism carriers displayed glucocorticoid resistance, and all carriers of this polymorphism also carried the TthIII minor allele (Moraitis et al. 2016). On the other hand, Van Rossum and Lamberts reported a GC resistance in ER22/23EK carriers, but no association with altered GC sensitivity in carriers of the TthIII polymorphism (2004). The N363S polymorphism was found to increase sensitivity to GCs (Moraitis et al., 2016). Thoroughly investigated SNPs of the MR gene include the MR I180V polymorphism (rs5522) and -2G/C (rs2070951) (van Leeuwen et al., 2011; Plieger et al., 2018; Klok et al., 2011). Klok et al. found that a haplotype consisting of the minor allele of -2G/C and the major allele of MR I180V caused an increase in MR activity, improving resilience to depression and fewer thoughts of hopelessness, especially in women (2011). We are interested in studying SNPs related to GR, MR, and regulatory protein functionality in order to determine hyper/hypo sensitivities to cortisol and potential predispositions to depression. SNPs of interest and their reported effects are listed in Table 1.

SNP	Common Name	Gene	Effect (of minor allele)
rs41423247	Bcl1	NR3C1	Hypersensitivity

rs10052957	TthIII	NR3C1	Hyposensitivity (resistance)
rs1800445	N363S	NR3C1	Hypersensitivity
rs5522	MR I180V	NR3C2	Hyposensitivity (resistance)
rs2070951	-2G/C	NR3C2	Hypersensitivity
rs33389	rs333	NR3C1	Hyposensitivity (resistance)
rs10515522	rs105	NR3C1	Hypersensitivity
rs12086634	rs120	11β-HSD1	Hyposensitivity (resistance)
rs1360780	FKBP51a	FKBP51a	Hypersensitivity

Table 1. SNPs of interest in our study and the gene on which they are located, as well as the effect of the minor allele.

Rs33389 and rs10515522, though less thoroughly investigated than the previously mentioned GR polymorphisms, were in a haplotype associated with elevated response to cortisol following acute stress by Plieger et al. (2017). The haplotype consisted of the minor allele of rs33389 and the major allele of rs10515522, among other SNPs (Plieger et al., 2017). Rs12086634 is a polymorphism along the gene coding for 11β-HSD1, which converts cortisone to the active form of cortisol. A reduction in the level and/or functioning of 11β-HSD1 has been found to protect hippocampal function from elevated cortisol levels (Deak, 2016). The rs12086634 polymorphism has been studied in relation to metabolic syndrome; Farag et al. found that in their sample of patients, with the skin diseases acne vulgaris (AV) and skin tags (STs), the GG genotype of rs12086634 was associated with elevated cortisol levels (2019). FKBP51 is a co-chaperone protein that regulates GR activity in its response to stress; it is a member of the

multiprotein complex that exists when the GR is in its unligated form (Deak. 2016). The T allele of rs1360780 has been found to increase FKBP5 induction by cortisol, allowing the GR to be more sensitive to cortisol (Binder et al., 2004).

We aimed to investigate these polymorphisms associated with hyper and hypo-sensitivities to cortisol on the HPA axis and their association with the incidence of depression. We collected DNA samples and Beck Depression Inventory scores from patients at the psychiatric division of Albany Medical college. Through genotyping of samples, we compared single nucleotide polymorphisms with the presence of depressive symptoms to determine associations between mutations and depression. Understanding the impact of certain SNPs will aid in better comprehending the mechanisms by which dysregulation of stress, and subsequently depression, is caused in the body. Consequently, this understanding will potentially lead to better solutions and medications for the treatment of depression.

Methods

Participants and Inventory Tests

Forty-three patients from the Albany Medical College Psychiatric unit provided their DNA for our study. To protect the anonymity of patients, Albany Medical College did not release the sex or age of our participants, although all patients are adults 18 or older who consented to provide their DNA. In addition to DNA collection, patient's scores on four inventory tests were provided to our lab: the Beck Depression Inventory (BDI), the State Trait Anxiety Inventory (STAI), the Adverse Childhood Experiences test (ACE), and the Mindful Attention Awareness Scale (MAAS). All 43 patients filled out the STAI, 40 filled out the BDI,

23 filled out the ACE test, and 29 filled out the MAAS. All inventories are self-reported measures of attitudes and symptoms associated with psychological disorders.

The BDI has 21 items and is a measure of depressive symptoms experienced in the last week. Each item provides four options associated with a number score, and the total score infers the severity (or lack thereof) of an individual's depression. Scores range from 1 to 63 (0-3 possible points per question), with any score over 40 signifying "extreme depression" (Beck, 1961).

The STAI measures both state and trait anxiety often used to diagnose anxiety and differentiate it from depression. There are 20 items measuring state anxiety and 20 items measuring trait anxiety, with each item being scored from 1 to 4 depending on the extremity at which the patient agrees with the statement (Spielberger, 1968). In our study, we used the combined STAI scores: the total of both the state and trait anxiety scales.

The MAAS measures a patient's mindfulness and awareness in the present moment and consists of 15 items. Each item is scored from 1 to 6, and higher scores indicate higher mindfulness and awareness (Brown and Ryan, 2003).

Finally, the ACE questionnaire measures different categories of adverse experiences in childhood in an attempt to predict future health and behavioral risks. The inventory has 17 questions split into seven categories with responses of 'yes' or 'no,' and an answer of yes to at least one question representing exposure to those types of adverse experiences (Felitti et al., 1998). Participants provided cortisol samples at the time of their visits to the psychiatric center, but the lack of uniformity in collection times and the likely cortisol fluctuations influenced by circadian rhythms prevented us from utilizing the samples and analyzing the data in an accurate manner.

DNA Collection and Extraction

DNA collection was achieved through self-administered buccal swabbing at Albany Medical College. Samples were collected between January and December of 2018. Once collected, DNA was sent to our lab at Union College for extraction. DNA was stored at 4 degrees C for 1 to 10 days before extraction. Extraction procedures are listed in appendix 1. Once extracted, DNA was stored at -20 degrees C and defrosted for genotype analysis.

The delay in DNA extraction for some samples may have been a contributing factor to the lack of results when undergoing genetic analysis. DNA samples that did not display results through allelic or quantitative PCR (polymerase chain reaction) went through a purification step using Chelex to remove non-genetic material from the samples. The Chelex prep method is listed in appendix 1.

Genotype Analysis

Genotype analysis was conducted through two methods: allelic polymerase chain reaction (allelic PCR) and quantitative polymerase chain reaction (qPCR). Allelic PCR was performed to genotype the Bcl1, TthIII, rs207, N363S, rs120, and FKBP51a polymorphisms, while qPCR was conducted to genotype rs5522, rs33389, and rs10515522.

The polymerase chain reaction is a DNA amplification method utilizing the DNA replication mechanism of DNA polymerase. Forward and reverse primers are used to identify and amplify selected regions of DNA, and billions of copies of the desired DNA are produced by the end of the reaction (NCBI, 2017). This reaction is done in vitro and the results are analyzed using gel electrophoresis. Gel electrophoresis is performed to separate DNA fragments according

to size. Negative electrical currents are pumped through the gel, causing the positively charged DNA fragments to repel the charge and move down the gel. The extent of the movement is governed by the size of the fragment. A dye is used to stain the DNA and make the bands visible. Because we selected forward and reverse primers, the approximate size of the expected bands for our product is known. Allelic PCR was run on two tubes per sample: one with a WT primer and another with a MU primer (both contain a common primer). Each PCR product was placed in a separate well and run on the gel; presence of a band in only the WT or MU column signified a WT or MU genotype (respectively), and a band in both columns signified a Heterozygote. An example of a gel is shown in appendix 1. Gels were visualized using the ImageLab application on a desktop computer.

qPCR, or quantitative PCR, measures DNA amplification in real time with fluorescence measurements (New England Biolabs, N.D). To run the reaction, a DNA template is mixed with DNA polymerase, free deoxynucleotides (dNTPs), and primer sets (similar to conventional PCR). In order to achieve these real time amplification measurements, qPCR has two elements that are not present in conventional allelic PCR: fluorescent dye and a fluorometer. Fluorescent reporter molecules in each reaction cause an increase in fluorescence with each amplification of DNA, and the increasing fluorescence is measured by the fluorometer. qPCR real time results are depicted on an amplification plot, measuring cycle number on the x axis and fluorescence on the y axis. Threshold for fluorescence is provided, and a positive result for that primer set is obtained once amplification exceeds the threshold (New England Biolabs, N.D). Protocols for all procedures are listed in appendix 1. Genotypic results for the qPCR were identified using allele amplification and discrimination plots on the ThermoFisher Quant Studio application. Allele amplification plots demonstrated the presence or lack of amplification for specific alleles, and

the allele discrimination plot utilized an automatic threshold value to deduce if amplification values were demonstrative of the presence of the desired allele. Examples of both plots are listed in appendix 1.

Data Analysis

Allelic and genotypic frequencies were determined on excel by calculating the number of WT and MU alleles per SNP. Each patient had two alleles: WT genotypes contained 2 WT alleles, heterozygous genotypes contained 1 WT and 1 MU allele, and MU genotypes contained 2 MU alleles. WT and MU alleles were divided by total alleles for each SNP to determine allelic frequency.

Data was analyzed using IBM SPSS and JMP Pro softwares. Inventory scores (BDI, STAI, MAAS, and ACE) were individually compared to genotypes of single SNPs as well as combinations of SNPs (haplotypes). Patient IDs, genotypes, and inventory scores were entered into SPSS and JMP softwares. Although three genotypes were present in the sample (WT, Hetero, and MU), SNPs were divided in two groups for statistical analysis: WT and Hetero/MU. The majority of SNPs did not have enough mutant and/or heterozygous samples to compare to WT groups in ANOVA statistical analyses without introducing a high probability of error. Rather, independent samples t-tests were conducted to compare inventory scores between the two genotype groups in SPSS. In JMP, the Fit Y by X function was used with the SNP genotypes as the X value and inventory scores as the Y value. Analyses were then performed using the Means/Anova/Pooled t function to determine significance.

In addition to the effects of individual mutations, we investigated the impact of haplotypes consisting of two SNPs. Each haplotype was composed of SNPs with opposing

effects on the GR and MR: one SNP causing hypersensitivity of a receptor (ex. GR) and the other causing hyposensitivity of the other receptor (ex. MR). The SNP combinations we studied are listed in Table 7. FKBP51a is not on the GR gene, but is on the gene of a protein regulating GR activity and was therefore included in the analysis. Two groups (labeled 1 and 2) were created for each combination of SNPs. Group 1 consisted of a WT allele in one or both genes. Group 2 consisted of a Hetero or MU allele in both genes. For example, a WT Bcl1 and Hetero or Mu rs55 haplotype would be in Group 1, as would a WT Bcl1 and WT rs55 haplotype, whereas a Hetero (or MU) Bcl1 and Hetero (or MU) rs55 haplotype (a total of 4 different possible haplotypes) would be in Group 2. These groupings were created to pair the presence of minor alleles across both SNPs and compare this to patients without a minor allele in both genes. The SNP combinations were analyzed in SPSS; independent samples t-tests were conducted between groups 1 and 2 for each SNP combination for each inventory in SPSS.

Results

Allelic Frequencies

Frequency percentages for each genotype in our sample population are recorded in Figure 7. Wildtype genotypes represented the majority of the study sample across all SNPs, ranging from 42.86% (Bcl1) to 92.68% (N363S) of patients. Homozygous mutant genotypes for the N363S and rs5522 alleles were not detected. In addition to genotype frequencies, the allelic frequencies for our population were calculated and compared to the general world population (Table 2). The World population was utilized because demographic data was not collected from the study participants and we therefore cannot make assumptions about ethnicity. World frequencies were reported from the NCBI dbSNP library, though N363S did not have frequency

data. All SNPs except Bcl1and rs33389 had larger WT frequencies than the world population data. Rs207 and rs105 had the largest disparities in allele frequencies, with the world populations having larger WT frequencies by 0.365 and 0.240, respectively. The rs33389 sample frequencies most closely matched that of the world population, with a 0.016 lower sample WT frequency when compared to world frequency. The method by which allelic frequencies are calculated did not allow for a simple t-test to be performed to determine if our sample was statistically significant from the world population.

Individual SNP analysis

Each SNP was analyzed for correlation between genotypes and scores on inventory scales that are associated with depression. The scales filled out by participants included the Beck Depression Inventory (BDI), the State Trait Anxiety Inventory (STAI), the Adverse Childhood Experiences (ACE) Scale, and the Mindful Attention Awareness Scale (MAAS).

BDI scores compared between genotypes are displayed in Table 3. No individual SNPs showed significant correlation with BDI scores. STAI sum scores compared between genotypes of each SNP are displayed in Table 4. No GR or MR SNPs were correlated with STAI-sum scores, but the minor allele of rs120 (the Hetero/MU group) was significantly correlated with lower STAI sum scores in a one-tailed t-test ($t(31)= -1.74$, $p=0.046$) (Figure 8). ACE sum scores compared between genotypes of each SNP (Table 5) demonstrated no significant correlations, though a one-tailed test revealed significantly higher ACE sum scores for Hetero/MU genotypes of rs33389 ($t(18)=2.04$, $p= 0.028$) (Figure 9). MAAS scores did not differ significantly between genotypes of any individual SNPs (Table 6), though rs120 neared two-tailed significance ($t(26)=$

2.02, p=0.053), and a one-tailed test demonstrated that Hetero/Mu genotypes displayed significantly higher MAAS scores (t(26)= 2.02, p=0.027) (Figure 10).

SNP Combination analysis

The investigated SNP combinations are listed in Table 7. SNPs were selected by pairing opposing effects of the different receptors, i.e. hypersensitive GR and hyposensitive MR and vice versa. The combinations theoretically have a more pronounced dysregulation of the HPA axis and cortisol regulation when compared to the actions of individual SNPs. For BDI, group 2 contained zero patients for Bcl1 and rs55, rs207 and rs333, N363S and rs55, and rs105 and rs55. The SNP combinations with patients in both group 1 and group 2 displayed no significant correlations of minor alleles and BDI score (Table 8). STAI sum scores had no significant correlation between groups of any SNP combination (Table 9), and rs207 and rs333, as well as N363S and rs55 did not have any patients in group 2. Bcl1 and rs55, Rs207 and rs333, N363S and rs55, and rs105 and rs55 did not have patients in group 2 for comparison of ACE scores, and no other minor allele group showed significant correlation with ACE sum scores (Table 10). Comparison of MAAS scores between haplotypes also did not demonstrate any significant correlation, and no data for group 2 was present in Bcl1 and rs55, Rs207 and rs333, N363S and rs55, and rs105 and rs55 (Table 11).

Discussion

The focus of this study was to assess the relationship between single nucleotide polymorphisms dysregulating HPA axis function and depressive symptoms. We investigated mutations of the GR and MR receptors, which regulate the activity of cortisol in our brains and

bodies. Our analysis was separated into two areas: the effect of individual SNPs, and combined effects of multiple SNPs (haplotypes) on different genes.

The GR SNPs studied were Bcl1 (rs41423247), TthIII (rs10052957), N363S (rs1800445), rs33389, and rs1051552. The mutations caused by these single nucleotide changes have previously been shown to demonstrate either reduced or overactivity of the GR, leading to elevated or decreased levels of cortisol in the body, respectively. Pariante and Miller noted diminished GR capacity leading to elevated cortisol levels in depressed patients versus healthy controls, whereas other studies have reported lower levels of cortisol in depressed patients when compared to healthy controls (Pariante and Miller, 2001; Huber et al., 2006). In addition, both GR agonists and antagonists have demonstrated antidepressant effects (Pariante, 2006). Due to the lack of consensus in previous studies, SNPs causing both an overactivation and under activation of the GR were selected for analysis. Although no two-tailed significance was found between polymorphisms and inventory scores, three instances of one tailed significance were observed: the rs33389 polymorphism was significantly correlated with higher ACE scores, and the rs120 polymorphism was significantly correlated to lower STAI sum scores as well as higher MAAS scores. No other significance was observed, though sample selection and a lack of a 'healthy' control group may have been contributing factors to our lack of findings.

The Bcl1 mutation is one of the most commonly investigated SNPs in relation to GR activity. The mutation at this location, a C to G substitution, is common among the population and therefore simplifies the ability to compare across genotypes. The G allele is associated with lower cortisol levels due to an overactive GR, whereas the C allele is correlated with elevated cortisol both before and after stressful tasks (Ising et al., 2008; Van Rossum et al., 2003). The allelic frequencies in our study were similar to those observed in the world population, and we

found no correlation between genotypes of the Bcl1 allele and any of the depression or anxiety inventories used in our study. Inventory scores between the WT and Hetero/MU groups did not approach significance for any scale, and standard deviations for genotype groups were large across scales.

The TthIII mutation on the GR gene has shown differing effects across studies. Moriaitis et al. found that carriers of the TthIII polymorphism in their study also carried the ER22/23EK mutations, and these individuals displayed elevated levels of cortisol due to GR resistance (2016). Contrastingly, a study by Van Rossum and Lamberts consisted of individuals who were carriers of ER22/23EK but not TthIII, and reported no correlation between the TthIII polymorphism alone and cortisol sensitivity, whereas the ER22/23EK polymorphism demonstrated an association with elevated cortisol levels (2004). Our study did not reveal a correlation between the presence of the TthIII mutation and inventory scores measuring depression or anxiety. This is seemingly in accordance with Van Rossum and Lamberts, as a lack of association with depressive symptoms may indicate a lack of abnormality in cortisol sensitivity by the GR. Because our study did not genotype for the ER22/23EK polymorphisms, it is impossible to deduce whether the patients in our study had these mutations as well. Further study into TthIII and its association with the ER22/23EK polymorphism and cortisol sensitivity is required to determine the effects of the mutation.

The N363S mutation has been predominantly recorded as causing increased sensitivity to cortisol, although some studies have demonstrated a resistance to cortisol or no effects on cortisol sensitivity (Moraitis et al., Ising et al., 2008). Our study found no effect of the mutation on inventory scores. Only three patients had a mutation in at least one allele in the N363S position, and this lack of genotypic diversity may have contributed to the lack of findings. Ising

et al. had no participants with a mutant genotype of N363S in their sample and only 14% of participants demonstrated a heterozygous genotype. They did not find any association between the mutation and cortisol sensitivity (2008). The world frequency for this SNP is not recorded, but it is likely that the mutation is very infrequent, creating difficulty in studying it.

Rs3389 and rs105 are the least studied polymorphisms of our study. Plieger et al. constructed a haplotype, which consisted of the minor allele of rs33389 and the major allele of rs10515522, and demonstrated that patients with this haplotype had elevated cortisol following a stress test (2017). Therefore, the rs33389 mutation likely has a hyposensitivity effect (elevated cortisol), while the minor allele of rs105 likely has a hypersensitivity effect (low cortisol levels). Although no significance was found correlating rs105 with any inventory, a one-tailed significance was found between the rs33389 minor allele and ACE sum scores. Resistance to cortisol was associated with higher ACE scores; patients with suspected elevated cortisol levels demonstrated more adverse childhood experiences. Prolonged exposure to stress has shown to alter HPA axis functioning, and past studies investigating the epigenetic effects of adverse childhood experiences on the cortisol response demonstrated evidence of blunted as well as elevated cortisol sensitivity (Tyrka et al., 2016). Tyrka et al. showed that adults who had experienced parental death or desertion as children exhibited elevated cortisol responses to pharmacological agents made to evaluate HPA function, while Carpenter et al. found that adult participants who had experienced childhood emotional abuse showed lower, or blunted, cortisol levels following a pharmacological agent (2009). The epigenetic mechanism of methylation has been linked to the alteration in HPA axis function, most notably methylation of the GR gene NR3C1 (Tyrka et al., 2016). The mutation is not a causal factor in epigenetic alteration, but the combination of both a mutation causing hyposensitivity and epigenetic changes from adverse

childhood experiences would suggest higher depressive symptoms, though this was not observed in our sample. Alternatively, if Carpenter et al.'s finding of lowered cortisol levels following emotional abuse in childhood were to be observed in our sample rather than Tyrka et al.'s elevated cortisol finding, the mutation and the epigenetic changes could theoretically cancel out any observed effect on HPA axis functioning.

Although not on the GR gene, the rs120 polymorphism, located on the 11β-HSD1 gene which regulates the conversion of the inactive form cortisone to active cortisol, was investigated due to previous literature demonstrating its effect on circulating cortisol levels. The major allele of the rs120 polymorphism has been associated with elevated cortisol levels (Farang et al. 2019). The minor allele has demonstrated a reduction in function and a protective effect against elevated cortisol levels (Deak, 2016). Therefore, the major allele mimics the impact of Cushing's syndrome: elevated cortisol without an alteration in GR functioning. The WT allele of rs120 was significantly correlated with higher STAI sum scores. Because the WT allele is associated with elevated cortisol, this result demonstrates that elevated cortisol levels with (seemingly) normal functioning GR and MR receptors is correlated with heightened anxiety. Adults with anxiety have been correlated with significantly higher cortisol levels, and drugs reducing cortisol production have shown to improve anxiety (Lenze et al., 2012). Our results support this claim of elevated cortisol levels as a risk factor for anxiety. Depression and anxiety are often observed to occur together and share many common symptoms and treatments (Sawchuk, 2017). Though seemingly similar, no correlation between rs120 genotypes and BDI scores was observed.

The rs120 polymorphism was also significantly correlated with higher MAAS scores. The MAAS scale measures mindfulness and awareness and multiple studies have demonstrated it is inversely correlated to the BDI and BDI-II scales (Brown and Ryan, 2003; Barajas and Garra,

2013). Our result suggests that individuals with a mutation leading to lower cortisol levels are associated with higher mindfulness and awareness. This is in agreement with our earlier finding of the rs120 major allele correlating with increased anxiety as the major allele was also associated with significantly lower mindfulness and awareness scores. Our result further supports elevated cortisol levels as risks for depression, and lowered cortisol as a potential protective factor (Deak, 2016; Lenze et al., 2012).

The rs207 and rs5522 polymorphisms have been demonstrated, within a larger haplotype consisting of the rs207 minor allele and rs5522 major allele, to cause an increase in MR activity (Klok et al. 2011). Studies investigating the role of MR function in the HPA axis have demonstrated an increase in MR activity as a protective factor against depression, showing evidence for improved resilience and fewer thoughts of hopelessness, especially in females (Klok et al. 2011). Contrastingly, other studies observed no difference in the cortisol stress response for carriers of the rs207 or rs5522 polymorphisms (Bouma et al., 2011; Ising et al., 2008). Our study did not demonstrate any significant correlation between either individual polymorphism and inventory scores. It is important to note that the rs5522 mutant frequency is very low in the world population (~14%) and was even lower in our sample (~5%), creating a difficulty in analyzing differences between groups. Other studies have also demonstrated this lack of genotypic diversity of rs5522, creating limited groups for comparison (Ising et al., 2008). Our results are therefore both in agreement and disagreement with past research, highlighting the importance of further research of the role of MR activity on cortisol levels and depressive symptoms.

The final individual SNP studied was rs1360780, or FKBP51a. This polymorphism is located on the FKBP5 gene, a protein regulating GR activity. The minor allele has shown effects

of impaired cortisol recovery as well as increased cortisol levels during recovery following the administration of trier social stress tests (Binder et al., 2004; Ising et al., 2008). Our study found no significant correlation between the FKBP51a and any inventory scores.

Cortisol levels in our participants were not measured at a consistent time over a period of sessions, and we therefore did not include cortisol levels into our analysis. Our basis of the altered sensitivities of cortisol due to the studied mutations arose from previous research that measured cortisol levels at distinct times throughout the day or before and following lab-administered stress tests. Future studies in our lab hope to conduct both genotype analysis and accurate cortisol measurements from participants to first compare the mutations with cortisol levels and determine if our sample shows significant correlations, and then investigate inventory scores of those participants with altered cortisol sensitivities.

Further studies should also focus on diversifying the sample of patients in terms of their psychiatric histories. A BDI score of 29-63 is considered severe, and although standard deviations were large, the lowest BDI mean in our sample for any genotype across SNPs was 28.28 (rs120 Hetero and MU group). Only seven of our 40 patients who filled out the BDI displayed minimal to moderate depression. Therefore, within all groups there were patients displaying high numbers of depressive symptoms. Further analysis including a larger subset of 'healthy' patients as a control group may yield a larger diversity of BDI scores and subsequently more difference between the genotype groups. The same pattern was observed with the STAI scale: STAI sum scores range from 40 to 160 and all our group averages were over 100. The ACE scores in our sample, on a scale from 1 to 10, had a low of 2.50 and most scores were in the 3-5 range. The CDC ACE study found only 20% of people had an ACE of 3 or more, demonstrating a skew in our study towards higher childhood abuse (CDC, 1998). MAAS scores

were more equally distributed and representative of other study populations (Carlson and Brown, 2004). A larger sample size would also provide better analysis by allowing genotype groups to be larger and introducing the possibility of comparing three genotype groups rather than two. This increased sample size and increased diversity mentioned above would improve our chances of identifying the possible effects of mutations.

Although many of our previous studies investigated the effects of individual SNPs on depressive symptoms, a plethora of academic studies have constructed haplotypes consisting of multiple SNPs, thereby studying the effects of a culmination of mutations. Because the GR and MR both respond to and translate the effects of cortisol in our bodies, we aimed to investigate if an interplay between malfunctioning of the receptors could be responsible for altered response to cortisol, and in turn, lead to depressive symptoms. The interaction of MR and GR is crucial for moderating the body's response to stress, and previous research has shown that alterations in the MR:GR ratio may have implications for depression and other cognitive functions, though the interaction between the receptors has not been fully characterized (Gomez-Sanchez and Gomez-Sanchez, 2015; Rivers et al., 2019). By characterizing the apparent effect of hypo and hyper sensitivity for each receptor, we theorized outcomes that would occur when combining polymorphisms for the receptors (Figure 11).

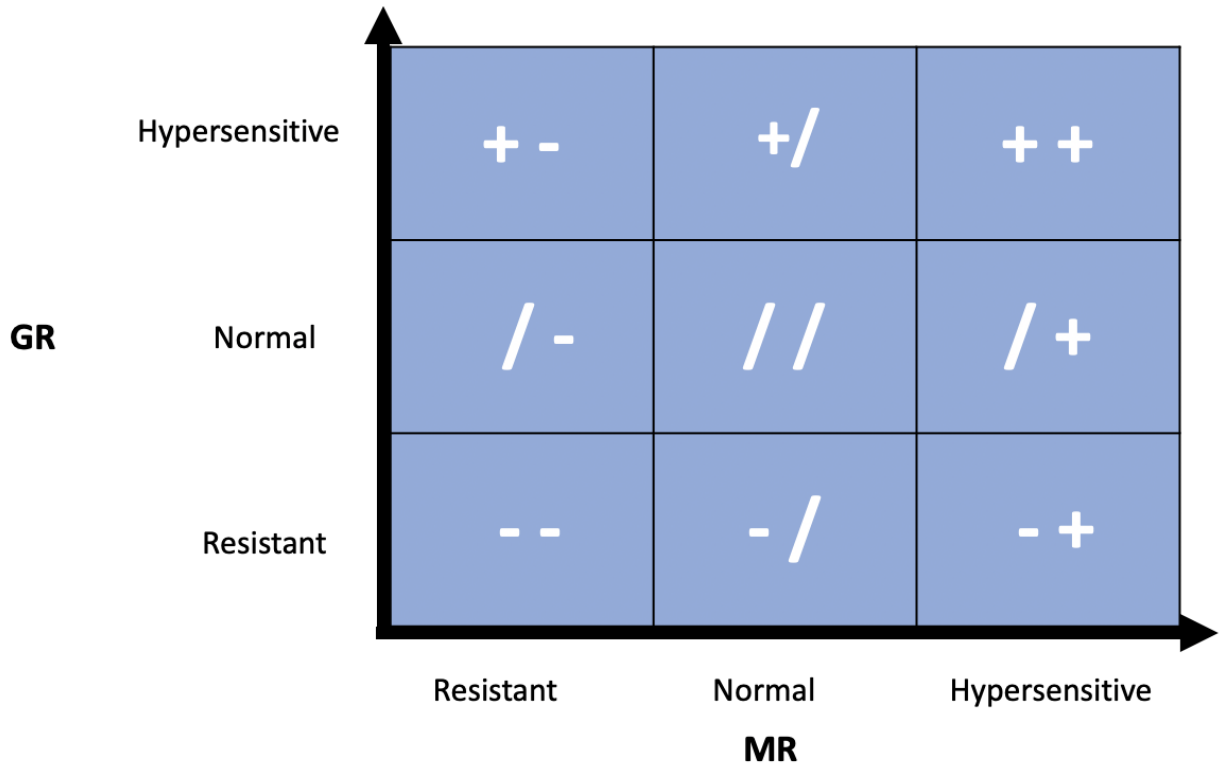

Figure 11. Activity of the GR and MR and combined effects. The first symbol in each box represents GR activity, the second represents MR activity. A "/" represents normal activity, whereas a "+" is hyperactivity and a "-" is hypoactivity (resistance).

Boxes including normal activity for one receptor are represented by individual SNPs of the other receptor mentioned earlier in the study (for example, normal MR and resistant GR is demonstrative of the TthIII or rs33389 polymorphism). Of interest are the boxes representing mutations in both receptors. If both receptors are hyperactive (top right box), there would be low cortisol levels due to increased activity of the GR and more negative feedback, but an overactive MR would theoretically 'compensate' for low levels by binding more cortisol. In addition, the MR is normally occupied at low, basal levels of cortisol so lower circulating levels would likely not have strong negative effects. On the other end, resistance (hypoactivity) in both receptors

(bottom left box) would create elevated levels of circulating cortisol because of less negative feedback from the GR, but the MR resistance would prevent it from being overwhelmed by high circulating levels, essentially 'canceling out' any negative effect. Because of the theoretical compensating interaction between receptors, we did not investigate these combinations. Rather, we studied combinations of hypersensitivity and resistance. A resistant GR leading to elevated cortisol levels paired with a hypersensitive MR (bottom right box) would create significantly increased activity at the MR due to the increased availability of cortisol. On the other end, an overactive GR leading to low cortisol levels paired with a resistant MR (top left box) would potentially restrict the MR's ability to bind to cortisol, and be less occupied even at basal levels when cortisol is normally bound. Therefore, the GR feedback mechanisms influencing MR activity, in addition to the effects observed in the GR when feedback is altered, cause a dysregulation in both receptors.

Of the six SNP combinations studied, we did not observe any correlation between genotype groups and inventory scores. Our small sample size and limited genetic diversity for many SNPs prevented us from comparing many SNP combinations because the mutant haplotype groups contained no patients. Of the mutant haplotype groups that did have participants, their sample size of one to two patients also limited our comparison analysis and introduced high probabilities of error.

The combinations of SNPs developed in this study were purely theoretical based on an integration of results obtained from previous research. The interaction between the GR and MR has not been investigated to the same extent as individual polymorphisms and further physiological research is required to grasp the nature of the interaction between the receptors. The differing binding affinities of the receptors, as well as the potential protective effects of

certain MR mutations likely play integral roles in this interaction. Understanding the interaction will better allow us to discern how mutations in one or both receptors alters function in the other, and ultimately aid in creating functional haplotypes for further research.

In conclusion, our study demonstrated one-tailed significance for two mutations in which elevated cortisol levels correlated to higher anxiety and childhood abuse, as well as lower mindfulness. No significance was found between haplotypes and inventory scores. Further research with larger and more diversified samples, as well as a deeper mechanistic understanding of receptor interactions, are the necessary next steps to understanding the role of genetic mutations in HPA axis functioning. A greater grasp on causes of dysregulation of the HPA axis and mishandling of stress will better aid in research for therapeutic drugs aimed at alleviating symptoms of depression and anxiety.

Appendix 1. Methods

Protocol 1. DNA Collection and Extraction, from Team Cohen Master Lab Manual

DNA Collection

1) Rub the brush on the inside of the mouth for 15-30 sec.
2) Remove the swab handle and put the brush section of the swab inside a 1.5 mL microcentrifuge tube.
3) If not proceeding directly to DNA extraction, store swabs at 4ºC.

DNA Extraction

1) Add 600 µL of 50 mM NaOH.
2) Close the tube containing the brush and vortex for 10 min.
3) Heat the tube at 95°C for 10 min.
4) Add 120 µL 1 M Tris (pH 8.0) to the tube and remove and discard the brush.
5) Centrifuge 1 min at max speed and transfer the supernatant to a new tube.
6) Store the DNA at 4°C.
7) Between 2 and 10 µL of this biosample is used in a typical PCR reaction.

Protocol 2. Chelex Cleanup Prep

1) Pipette 200 µL of DNA into a microfuge tube.
2) Resuspend Chelex beads by vortexing for 10 seconds.
3) pipette 500 µL of Chelex solution into the microfuge tube containing the DNA.
4) Mix DNA with the Chelex by vortexing for 10 seconds.
5) Incubate the microfuge tube containing the DNA/Chelex mixture in the boiling water bath for 10 minutes.
6) Incubate it on ice for two minutes.
7) Spin the tube in a microfuge (max. speed) for 1 minute to pellet the Chelex and debris to the bottom of the tube.
8) Transfer supernatant to a new microfuge tube.

Protocol 3. Allelic PCR, adapted from Qiagen Master Mix sheet

1) Collect 2 PCR tubes per DNA sample.
2) Label PCR tubes by numbering them with sharpie.
3) In your Qiagen Master Mix sheet, input the number of reactions (2 per sample) plus two under mix 1.
4) Record your templates according to their number label in the google sheet
5) Vortex each DNA sample and pipette 2ul of template into each PCR tube (2 tubes/reactions per sample)
6) Collect three microfuge tubes. In two separate tubes, pipette the WT and MU primers
7) In the other microfuge tube, make mix 1 based on the measurements on the Qiagen master mix sheet, adding Taq last. Vortex after each addition.
8) Equally split the master mix into the WT and MU microfuge tubes. Vortex microfuge tubes
9) Pipette 13ul of WT mix into odd-numbered PCR tubes
10) Pipette 13ul MU mix into even-numbered PCR tubes
11) Place tubes in PCR machine and lock the lid

12) Select appropriate PCR protocol from Team Cohen folder according to correct annealing temperature
13) Once the PCR reaction is complete, samples can be stored in the fridge if a gel is not run directly afterwards.

Protocol 4. Gel Electrophoresis, from Team Cohen Master Lab Manual

Assembling the gel box

1) Turn the gel box so the electrodes are facing you.
2) Place the gel tray into the box perpendicular to the box so that the rubber gaskets on the side seal along the edge of the box.
3) There are 2 notches for the combs- make sure one set of notches is on the far side of the gel box and one set is closer to you in the middle.

Making the gel

1) Measure 100ml 1X PE with a graduated cylinder and place in a 500ml Erlenmeyer flask.
2) Add 2g of agarose.
3) Swirl, then microwave (for the microwave in WLDC201 microwave for 1 minute and 15 seconds).
4) Remove from microwave with a hot glove, hold up to the light and swirl to make sure everything is dissolved.

Pouring the gel

1) When the gel has cooled slightly, pour into the gel tray.
2) Place two 20-well combs into the tray- one comb should be on the far side of the gel and the other comb in the middle. Both should be pointing to 1.5mm.
3) Allow the gel to solidify.
4) Immediately wash the flask out with hot water.

Running the gel

1) When the gel has solidified, turn the gel perpendicularly so that the comb that was on the far side is now on the left.
2) Add sufficient 1XPE to the gel box so that the gel is completely submerged.
3) Pipette 3ul of dye into each sample.
4) Load samples onto the gel and slide the lid on.
5) Connect the electrodes to the power supply.
6) Press the set button and check the current settings. Set volts to 100V by pressing Set button on right side of the power box and adjusting the volts dial.
7) Press the start button and the light labeled DC should come on.
8) Let gel run until dye has moved to the end of the lanes.

Visualizing the gel

1) Turn off the voltage and remove the gel from the box.
2) Bring the gel with the container to the imaging instrument.
3) Open the drawer and place the gel on the glass. Close the drawer and use the door to reposition the gel as needed.
4) On the computer next to the imager, go to image lab then click the file tab, click on recent protocols and choose Ethidium Bromide.
5) Click the yellow position gel button and position your gel so that all 20 lanes are visible.
6) Once the gel is in place, shut the door and click run protocol.

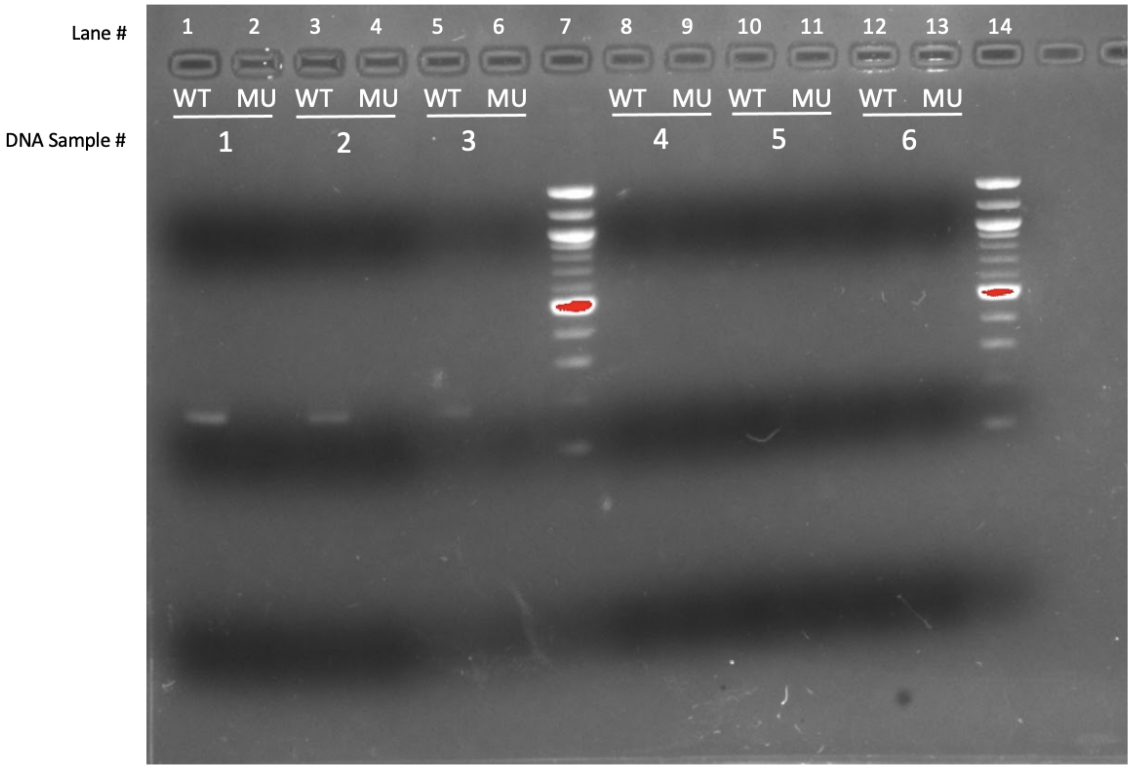

Figure 4. Imaged Gel Feb 2, 2021 for the rs207 SNP, with lanes labeled 1-14. Pairs of two lanes represent one sample (ex. Lanes 1 and 2 represent the same DNA of sample 1). Lanes 7 and 14 are 100 base pair ladders. The expected product size for the rs207 bands are 180bp. Presence of a band in lanes 1, 3, and 5 signify a WT genotype for samples 1, 2, and 3.

Protocol 5. qPCR, adapted from qPCR Master Mix sheet

Preparing the reaction tubes

1. Ideally, run reactions with 7, 15, or 8n-1 ≤ 96 number of samples (with n being any whole integer)
2. Collect appropriate number of qPCR tube racks
3. Open qPCR master mix sheet and input the number of reactions under mix 1 (one reaction per sample)
4. In a centrifuge tube, make mix 1 according to the measurements on the Master mix sheet
5. Pipette 2ul template into each reaction tube, and pipette 2ul water into last tube (to serve as negative control)
6. Pipette 8ul mix into each tube, including negative control
7. Centrifuge tubes in centrifuge machine next to qPCR instrument, making sure to use another empty tube holder as a balance

Running the qPCR

1. Open quantstudio
2. Open existing experiment > desktop > experiments > Cohen lab > templates > choose Cohen standard
3. Change experiment name (date, snp)

4. Fill in plate layout (plate tab)
    a. Drag and select
    b. Snp assay > one
    c. Double click negative control, mark SNP as no template control
    d. Hit next
5. Turn instrument on (power switch on the back)
6. Open instrument drawer (top right button)
7. Place tubes where you assigned on plate layout
8. Click start run on quantstudio (computer)
9. Save result outside of template folder (in Cohen lab folder)
    a. Date, name, snp
10. When run is complete, Login to account on screen of instrument and save to your cloud

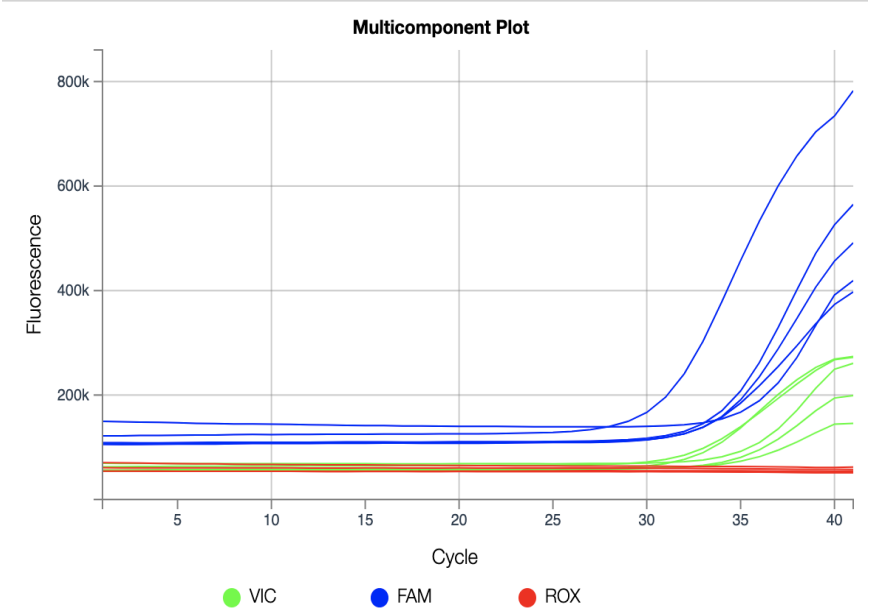

Figure 5. Allele Amplification Multicomponent Plot from ThermoFisher QuantStudio for 5 DNA samples. Blue and Green lines represent allele amplifications based on fluorescence values (blue: WT, green: MU). Each sample has one blue and one green line. The red line represents the negative control (no amplification) value. Ratios of WT:MU fluorescence were utilized to determine the sample genotype, pictured in Figure 6 below.

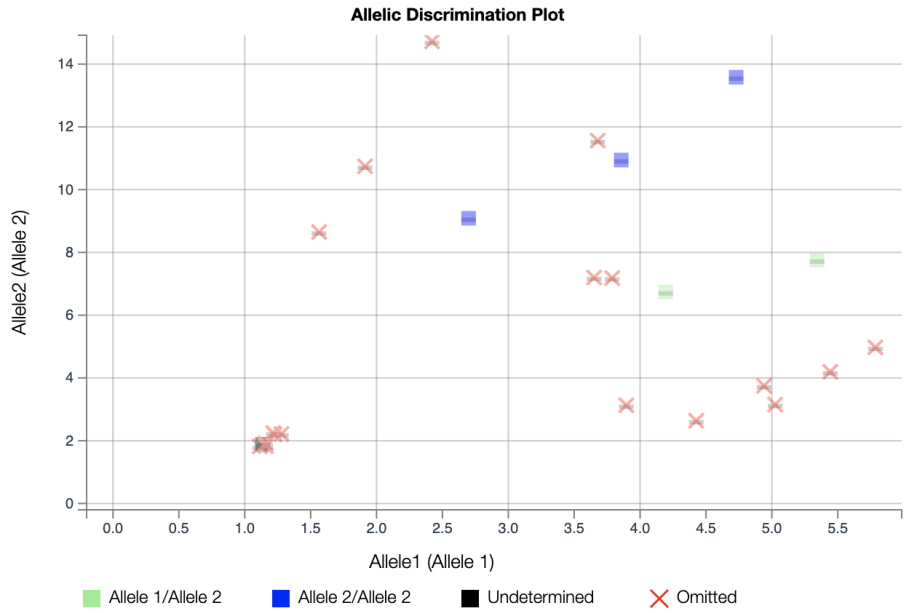

Figure 6. Allele Discrimination Plot corresponding to the above Multicomponent Plot. The sample consisted of two heterozygous samples (green) and two wildtype samples (blue). Omitted samples are those that were not pictured in the amplification plot and are therefore not correlated to Figure 5.

Appendix 2. Results Tables/Figures

Allelic Frequencies

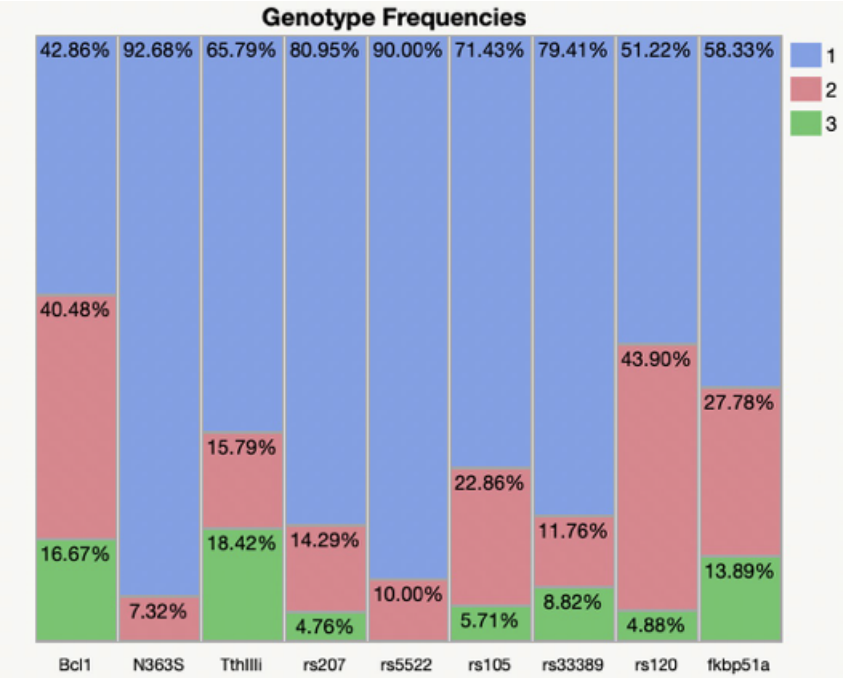

Figure 7. Genotypic Frequencies by percentage for investigated SNPs in the sample population. 1: WT, 2: Hetero, 3: MU

SNP	Allele	Sample Frequency	World Frequency
Bcl1	WT	0.643	0.661
	MU	0.357	0.339
N363S	WT	0.963	N/A
	MU	0.037	N/A
TthIIIi	WT	0.724	0.692
	MU	0.276	0.308
Rs207	WT	0.881	0.516
	MU	0.119	0.484
Rs5522	WT	0.950	0.886
	MU	0.050	0.114
Rs105	WT	0.829	0.589
	MU	0.171	0.411
Rs33389	WT	0.853	0.869
	MU	0.147	0.131
RS120	WT	0.793	0.583
	MU	0.207	0.417
Fkbp51a	WT	0.722	0.686
	MU	0.278	0.315

Table 2. Allelic Frequencies of the sample population compared to allelic frequencies of world and European populations for the investigated SNPs. World Frequency data was collected from the NCBI dbSNP library.

Individual SNP Analysis

SNP	Groups	N	Mean BDI	P value

Bcl1	WT	16	29.13 ± 16.370	0.418
	Hetero + Mu	23	33.26 ± 14.906	
N363S	WT	35	30.89 ± 15.480	0.457
	Hetero + Mu	3	38.00 ± 19.519	
TthIIIi	WT	23	34.04 ± 15.850	0.496
	Hetero + Mu	12	30.17 ± 15.736	
Rs207	WT	16	32.88 ± 16.820	0.853
	Hetero + Mu	3	31.00 ± 5.000	
Rs5522	WT	26	31.31 ± 14.664	0.141
	Hetero + Mu	1	54	
Rs10515522	WT	23	32.78 ± 16.382	0.999
	Hetero + Mu	9	32.78 ± 15.514	
Rs33389	WT	25	29.64 ± 16.817	0.410
	Hetero + Mu	7	35.29 ± 10.858	
RS120	WT	20	33.25 ± 17.935	0.316
	Hetero + Mu	18	28.28 ± 11.007	
FKBP51a	WT	19	29.84 ± 14.815	0.913
	Hetero + Mu	14	30.43 ± 15.535	

Table 3. Comparison of genetic associations with BDI scores between WT and MU alleles. Hetero and MU genotypes combined due to small N and the presence of a MU allele in both genotypes. No significant correlation between any SNP and BDI score was found in the study sample.

SNP	Groups	N	Mean STAI sum	P value
Bcl1	WT	15	106.47 ± 20.511	0.405

	Hetero + Mu	19	113.11 ± 24.433	
N363S	WT	31	109.19 ± 22.699	0.425
	Hetero + Mu	3	120.33 ± 24.583	
TthIIIi	WT	19	112.68 ± 22.770	0.622
	Hetero + Mu	12	108.42 ± 24.028	
Rs207	WT	16	111.38 ± 27.264	0.979
	Hetero + Mu	4	111.75 ± 7.274	
Rs5522	WT	23	108.39 ± 24.666	0.473
	Hetero + Mu	3	119.00 ± 7.00	
Rs10515522	WT	22	107.15 ± 22.346	0.209
	Hetero + Mu	8	120.00 ± 22.399	
Rs33389	WT	22	105.77 ± 24.999	0.146
	Hetero + Mu	6	122.00 ± 15.761	
RS120	WT	17	115.41 ± 21.754	0.091
	Hetero + Mu	16	102.38 ± 21.150	
Fkbp51a	WT	16	107.25 ± 23.605	0.715
	Hetero + Mu	15	110.33 ± 22.896	

Table 4. Comparison of genetic associations with summed STAI scores between WT and MU alleles. Hetero and MU genotypes combined due to small N and the presence of a MU allele in both genotypes. No significant correlation between any SNP and STAI sum score was found in the study sample.

SNP	Groups	N	Mean ACE sum	P value
Bcl1	WT	13	4 ± 3.109	0.691
	Hetero + Mu	10	4.50 ± 2.718	

N363S	WT	20	3.95 ± 2.665	0.262
	Hetero + Mu	3	6.00 ± 4.359	
TthIIIi	WT	13	3.85 ± 2.940	0.495
	Hetero + Mu	10	4.70 ± 2.908	
Rs207	WT	12	3.101 ± 0.869	0.564
	Hetero + Mu	2	2.50 ± 2.12	
Rs5522	WT	16	4.31 ± 3.049	0.893
	Hetero + Mu	2	4.00 ± 2.828	
Rs10515522	WT	17	4.06 ± 2.989	0.972
	Hetero + Mu	4	4.00 ± 2.944	
Rs33389	WT	16	3.81 ± 3.038	0.056
	Hetero + Mu	4	7.00 ± 0.816	
Rs120	WT	13	4.31 ± 2.780	0.543
	Hetero + Mu	9	3.56 ± 2.833	
Fkbp51a	WT	13	3.38 ± 2.663	0.261
	Hetero + Mu	8	4.88 ± 3.182	

Table 5. Comparison of genetic associations with summed ACE scores between WT and MU alleles. Hetero and MU genotypes combined due to small N and the presence of a MU allele in both genotypes. No significant correlation between any SNP and ACE sum score was found in the study sample.

SNP	Groups	N	Mean MAAS	P value
Bcl1	WT	14	48.93 ± 22.293	0.921
	Hetero + Mu	15	48.27 ± 12.435	
N363S	WT	25	50.20 ± 15.927	0.401

	Hetero + Mu	3	41.00 ± 31.575	
TthIIIi	WT	16	46.69 ± 15.589	0.598
	Hetero + Mu	11	50.55 ± 21.993	
Rs207	WT	12	50.75 ± 21.541	0.84
	Hetero + Mu	2	47.50 ± 2.121	
Rs5522	WT	20	49.15 ± 16.762	0.098
	Hetero + Mu	2	28.00 ± 7.071	
Rs10515522	WT	20	49.15 ± 18.639	0.887
	Hetero + Mu	7	50.29 ± 16.183	
Rs33389	WT	20	51.40 ± 19.478	0.392
	Hetero + Mu	6	44.00 ± 12.442	
Rs120	WT	15	44.00 ± 13.711	0.053
	Hetero + Mu	13	56.23 ± 18.226	
Fkbp51a	WT	16	52.06 ± 18.068	0.577
	Hetero + Mu	9	47.78 ± 18.397	

Table 6. Comparison of genetic associations with MAAS scores between WT and MU alleles. Hetero and MU genotypes combined due to small N and the presence of a MU allele in both genotypes. No significant correlation between any SNP and MAAS score was found in the study sample.

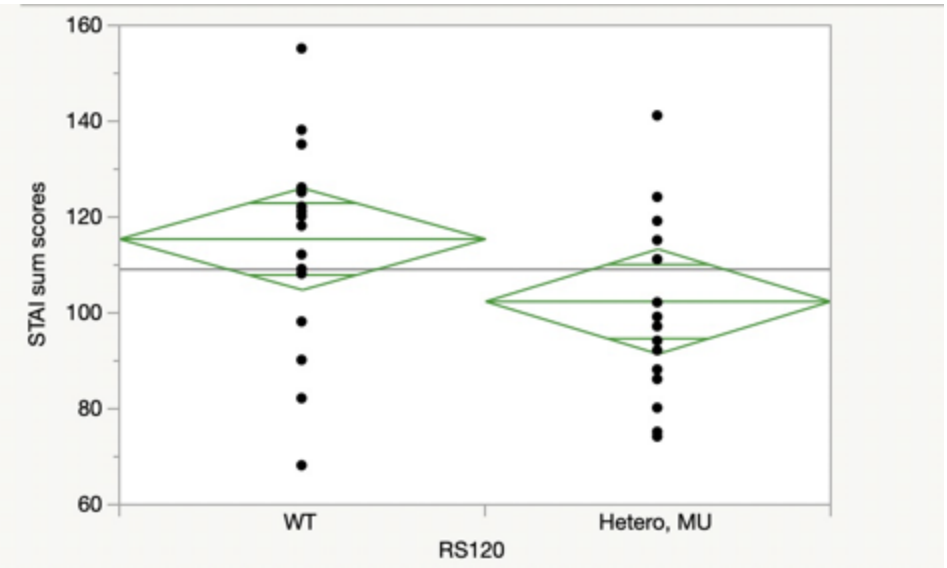

Figure 8. Comparison of STAI sum scores between WT and MU alleles of rs120. WT allele was significantly correlated with higher STAI sum scores in a one-tailed test (t(31)= -1.74, p=0.046).

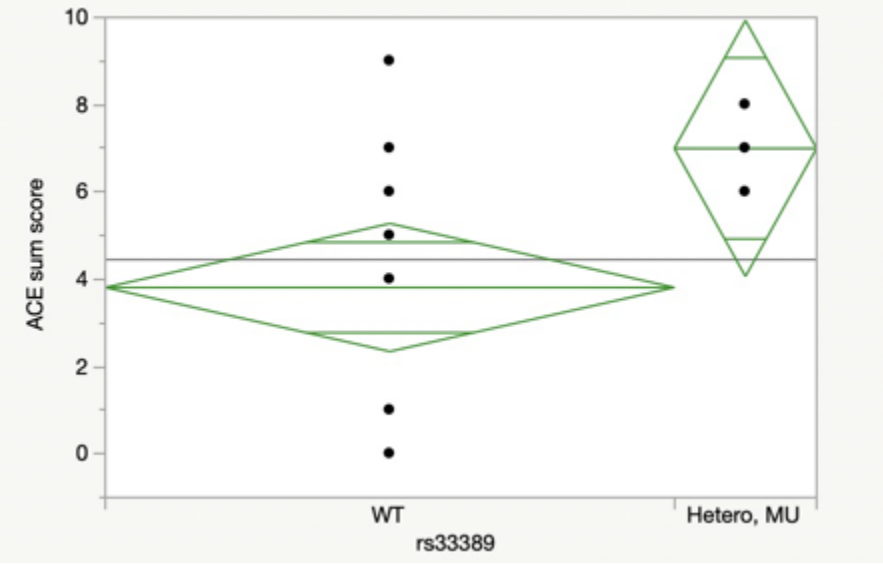

Figure 9. Comparison of ACE sum scores between WT and MU alleles of rs33389. WT allele was significantly correlated with lower ACE sum scores in a one-tailed test (t(18)= 2.04 , p= 0.0281).

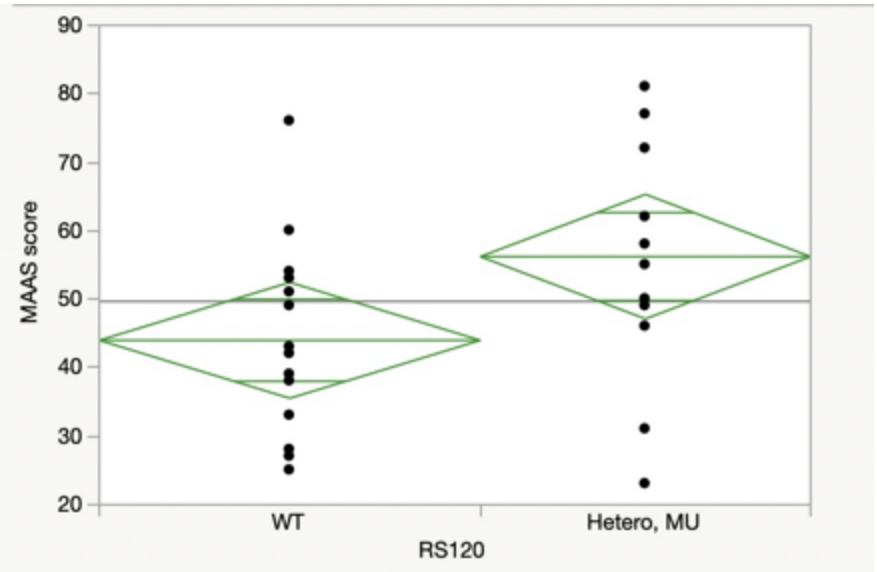

Figure 10. Comparison of MAAS scores between WT and MU alleles of rs120. WT allele was significantly correlated with lower MAAS scores in a one-tailed test ($t(31)= 2.02$, $p=0.027$).

SNP Combination Analysis

Combination	Effect of GR SNP	Effect of MR SNP
Bcl1 and rs55	Hypersensitivity	Hyposensitivity
TthIII and rs207	Hyposensitivity	Hypersensitivity
rs333 and rs207	Hyposensitivity	Hypersensitivity
N363S and rs55	Hypersensitivity	Hyposensitivity
rs105 and rs55	Hypersensitivity	Hyposensitivity
FKBP51a and rs55	Hypersensitivity	Hyposensitivity

Table 7. Investigated SNP combinations and associated (opposing) effects on the GR and MR proteins of the HPA axis.

Combination	Group	N	Mean BDI	Sig
Bcl and rs55	1	27	32.15 ± 15.027	N/A
	2	0	N/A	
TthIII and rs207	1	17	33.06 ± 16.304	0.705
	2	2	28.50 ± 3.536	
rs207 and rs333	1	16	30.38 ± 15.331	N/A
	2	0	N/A	
N363S and rs55	1	26	32.00 ± 15.305	N/A
	2	0	N/A	
rs55 and rs105	1	23	33.22 ± 15.338	N/A
	2	0	N/A	
FKBP51a and rs55	1	24	30.71 ± 15.098	0.144
	2	1	54	

Table 8. Comparison of BDI scores between haplotypes of investigated SNP combinations. Group 1 represents at least one WT genotype of both SNPs (i.e. WT for both Bcl1 and rs55, or WT for Bcl1 and Hetero or Mu for rs55 and vice versa), whereas Group 2 represents both SNPs having a Hetero or Mu genotype.

Combinations	Group	N	Mean STAI sum	Sig
Bcl and rs55	1	25	109.56 +/- 23.962	0.953
	2	1	111	
TthIII and rs207	1	18	111.56 +/- 25.624	0.956
	2	2	110.50 +/- 12.021	
rs207 and rs333	1	16	109.94 +/- 26.479	N/A
	2	0	N/A	

N363S and rs55	1	26	109.62 +/- 23.479	N/A
	2	0	N/A	
rs55 and rs105	1	22	110.36 +/- 24.242	0.98
	2	1	111	
FKBP51a and rs55	1	23	108.35 ± 24.637	
	2	2	116.50 ± 7.778	0.651

Table 9. Comparison of summed STAI scores between haplotypes of investigated SNP combinations. Group 1 represents at least one WT genotype of both SNPs, whereas Group 2 represents both SNPs having a Hetero or Mu genotype.

Combinations	**Group**	**N**	**Mean ACE sum**	**P value**
Bcl and rs55	1	18	4.28 ± 2.947	N/A
	2	0	N/A	
TthIII and rs207	1	13	3.62 ± 2.987	0.903
	2	1	4	
rs207 and rs333	1	11	3.91 ± 3.177	N/A
	2	0	N/A	
N363S and rs55	1	18	4.28 ± 2.947	N/A
	2	0	N/A	
rs55 and rs105	1	17	4.06 ± 2.883	N/A
	2	0	N/A	
FKBP51a and rs55	1	17	4.41 ± 2.980	0.786
	2	1	2	

Table 10. Comparison of summed ACE scores between haplotypes of investigated SNP combinations. Group 1 represents at least one WT genotype of both SNPs, whereas Group 2 represents both SNPs having a Hetero or Mu genotype.

Combinations	Group	N	Mean MAAS	P value
Bcl and rs55	1	22	47.23 ± 17.185	N/A
	2	0	N/A	
TthIII and rs207	1	13	50.62 ± 20.630	0.833
	2	1	46	
rs207 and rs333	1	11	53.36 ± 21.341	N/A
	2	0	N/A	
N363S and rs55	1	21	48.00 ± 17.213	N/A
	2	0	N/A	
rs55 and rs105	1	21	48.14 ± 17.051	N/A
	2	2	N/A	
FKBP51a and rs55	1	19	48.68 ± 17.777	0.86
	2	1	33	

Table 11. Comparison of MAAS scores between haplotypes of investigated SNP combinations. Group 1 represents at least one WT genotype of both SNPs, whereas Group 2 represents both SNPs having a Hetero or Mu genotype.

References

About the CDC-kaiser ACE study. (2021). cdc.gov. https://www.cdc.gov/violenceprevention/aces/about.html

Barajas, S., & Garra, L. (2014). Mindfulness and psychopathology: Adaptation of the mindful attention awareness scale (MAAS) in a spanish sample. *Clínica Y Salud, 25*, 49-56. https://doi.org/10.1016/S1130-5274(14)70026-X

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry, 4*, 561-571. https://doi.org/10.1001/archpsyc.1961.01710120031004

Binder, E. B., Salyakina, D., Lichtner, P., Wochnik, G. M., Ising, M., Pütz, B., Papiol, S., Seaman, S., Lucae, S., Kohli, M. A., Nickel, T., Künzel, H. E., Fuchs, B., Majer, M., Pfennig, A., Kern, N., Brunner, J., Modell, S., Baghai, T., . . . Muller-Myhsok, B. (2004). Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature Genetics, 36*(12), 1319-1325. https://doi.org/ng1479 [pii]

Brown, K. W., & Ryan, R. M. (2003a). The benefits of being present: Mindfulness and its role in psychological well-being. *Journal of Personality and Social Psychology, 84*(4), 822-848. https://doi.org/10.1037/0022-3514.84.4.822

Brown, K. W., & Ryan, R. M. (2003b). The benefits of being present: Mindfulness and its role in psychological well-being. *Journal of Personality and Social Psychology, 84*(4), 822-848. https://doi.org/10.1037/0022-3514.84.4.822

Carlson, L. E., & Brown, K. W. (2005). Validation of the mindful attention awareness scale in a cancer population. *Journal of Psychosomatic Research, 58*(1), 29-33. https://doi.org/10.1016/j.jpsychores.2004.04.366

Carpenter, L. L., Tyrka, A. R., Ross, N. S., Khoury, L., Anderson, G. M., & Price, L. H. (2009). Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. *Biological Psychiatry, 66*(1), 69-75. https://doi.org/10.1016/j.biopsych.2009.02.030

Chen, J., Wang, Z., Zhang, S., Zuo, W., & Chen, N. (2016a). Does mineralocorticoid receptor play a vital role in the development of depressive disorder? *Life Sciences, 152*, 76-81. https://doi.org/https://doi.org/10.1016/j.lfs.2016.03.022

Chen, J., Wang, Z., Zhang, S., Zuo, W., & Chen, N. (2016b). Does mineralocorticoid receptor play a vital role in the development of depressive disorder? *Life Sciences (1973), 152*, 76-81. https://doi.org/10.1016/j.lfs.2016.03.022

Cohen, S., Janicki-Deverts, D., & Miller, G. E. (2007). Psychological stress and disease. *Jama, 298*(14), 1685-1687. https://doi.org/10.1001/jama.298.14.1685

DeRijk, R. H., Wüst, S., Meijer, O. C., Zennaro, M., Federenko, I. S., Hellhammer, D. H., Giacchetti, G., Vreugdenhil, E., Zitman, F. G., & de Kloet, E. R. (2006). A common polymorphism in the mineralocorticoid receptor modulates stress responsiveness. *The Journal of Clinical Endocrinology & Metabolism, 91*(12), 5083-5089. https://doi.org/10.1210/jc.2006-0915

Dienes, K. A., Hazel, N. A., & Hammen, C. L. (2013a). Cortisol secretion in depressed, and at-risk adults. *Psychoneuroendocrinology, 38*(6), 927-940. https://doi.org/10.1016/j.psyneuen.2012.09.019

Dienes, K. A., Hazel, N. A., & Hammen, C. L. (2013b). Cortisol secretion in depressed, and at-risk adults. *Psychoneuroendocrinology, 38*(6), 927-940. https://doi.org/10.1016/j.psyneuen.2012.09.019

Farag, A. G. A., Badr, E. A., Eltorgoman, A. M. A., Assar, M. F., Elshafey, E. N., Tayel, N. R., & Aboutaleb, H. E. (2019). Role of 11β HSD 1, rs12086634, and rs846910 single-nucleotide polymorphisms in metabolic-related skin diseases: A clinical, biochemical, and genetic study. *Clinical, Cosmetic and Investigational Dermatology, 12*, 91-102. https://doi.org/10.2147/CCID.S193156

Fawne Hansen, & Eric Wood. (2014). *The adrenal fatigue solution: HPA axis dysfunction.* Adrenal Fatigue Solution. https://adrenalfatiguesolution.com/hpa-axis/

Felitti, V. J., Anda, R. F., Nordenberg, D., Williamson, D. F., Spitz, A. M., Edwards, V., Koss, M. P., & Marks, J. S. (1998). Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The adverse childhood experiences (ACE) study. *American Journal of Preventive Medicine, 14*(4), 245-258. https://doi.org/10.1016/S0749-3797(98)00017-8

Gomez-Sanchez, E., & Gomez-Sanchez, C. E. (2014). The multifaceted mineralocorticoid receptor. *Comprehensive Physiology, 4*(3), 965-994. https://doi.org/10.1002/cphy.c130044 [doi]

Hardeveld, F., Spijker, J., Peyrot, W. J., de Graaf, R., Hendriks, S. M., Nolen, W. A., Penninx, Brenda W. J. H, & Beekman, A. T. F. (2015). Glucocorticoid and mineralocorticoid receptor polymorphisms and recurrence of major depressive disorder. *Psychoneuroendocrinology, 55*, 154-163. https://doi.org/10.1016/j.psyneuen.2015.02.013

Huber, T. J., Issa, K., Schik, G., & Wolf, O. T. (2006). The cortisol awakening response is blunted in psychotherapy inpatients suffering from depression. *Psychoneuroendocrinology, 31*(7), 900-904. https://doi.org/10.1016/j.psyneuen.2006.03.005

Ising, M., Depping, A., Siebertz, A., Lucae, S., Unschuld, P. G., Kloiber, S., Horstmann, S., Uhr, M., Müller-Myhsok, B., & Holsboer, F. (2008a). Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. *European Journal of Neuroscience, 28*(2), 389-398. https://doi.org/https://doi.org/10.1111/j.1460-9568.2008.06332.x

Ising, M., Depping, A., Siebertz, A., Lucae, S., Unschuld, P. G., Kloiber, S., Horstmann, S., Uhr, M., Müller-Myhsok, B., & Holsboer, F. (2008b). Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. *European Journal of Neuroscience, 28*(2), 389-398. https://doi.org/https://doi.org/10.1111/j.1460-9568.2008.06332.x

Juruena, M. F., Cleare, A. J., Bauer, M. E., & Pariante, C. M. (2003). Molecular mechanisms of glucocorticoid receptor sensitivity and relevance to affective disorders.

Acta Neuropsychiatrica, 15(6), 354-367. https://doi.org/https://doi.org/10.1046/j.1601-5215.2003.00051.x

Klok, M. D., Giltay, E. J., Van der Does, A. J. W., Geleijnse, J. M., Antypa, N., Penninx, B W J H., de Geus, E. J. C., Willemsen, G., Boomsma, D. I., van Leeuwen, N., Zitman, F. G., de Kloet, E. R., & DeRijk, R. H. (2011). A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Translational Psychiatry, 1*(12), e62. https://doi.org/10.1038/tp.2011.59

Koning, Anne-Sophie C A M, Buurstede, J. C., van Weert, Lisa T C M, & Meijer, O. C. (2019). Glucocorticoid and mineralocorticoid receptors in the brain: A transcriptional perspective. *Journal of the Endocrine Society, 3*(10), 1917-1930. https://doi.org/10.1210/js.2019-00158

Lenze, E. J., Mantella, R. C., Shi, P., Goate, A. M., Nowotny, P., Butters, M. A., Andreescu, C., Thompson, P. A., & Rollman, B. L. (2011). Elevated cortisol in older adults with generalized anxiety disorder is reduced by treatment: A placebo-controlled evaluation of escitalopram. *The American Journal of Geriatric Psychiatry : Official Journal of the American Association for Geriatric Psychiatry, 19*(5), 482-490. https://doi.org/10.1097/JGP.0b013e3181ec806c

Lohoff, F. W. (2010). Overview of the genetics of major depressive disorder. *Current Psychiatry Reports, 12*(6), 539-546. https://doi.org/10.1007/s11920-010-0150-6

López, J. F., Chalmers, D. T., Little, K. Y., & Watson, S. J. (1998). Regulation of Serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human

hippocampus: Implications for the neurobiology of depression. *Biological Psychiatry, 43*(8), 547-573. https://doi.org/https://doi.org/10.1016/S0006-3223(97)00484-8

Mason, D. J., Glickstein, B., & Westphaln, K. (2018). Original research. *The American Journal of Nursing, 118*(10), 42-50. https://doi.org/10.1097/01.NAJ.0000546380.66303.a2

Medina, A., Seasholtz, A. F., Sharma, V., Burke, S., Bunney, W., Myers, R. M., Schatzberg, A., Akil, H., & Watson, S. J. (2013). Glucocorticoid and mineralocorticoid receptor expression in the human hippocampus in major depressive disorder. *Journal of Psychiatric Research, 47*(3), 307-314. https://doi.org/https://doi.org/10.1016/j.jpsychires.2012.11.002

Moraitis, A. G., Block, T., Nguyen, D., & Belanoff, J. K. (2017). The role of glucocorticoid receptors in metabolic syndrome and psychiatric illness. *The Journal of Steroid Biochemistry and Molecular Biology, 165*, 114-120. https://doi.org/https://doi.org/10.1016/j.jsbmb.2016.03.023

Pariante, C. M. (2009). Risk factors for development of depression and psychosis: Glucocorticoid receptors and pituitary implications for treatment with antidepressant and glucocorticoids. *Annals of the New York Academy of Sciences, 1179*(1), 144-152. https://doi.org/10.1111/j.1749-6632.2009.04978.x

Pariante, C. M., & Miller, A. H. (2001). Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. *Biological Psychiatry, 49*(5), 391-404. https://doi.org/https://doi.org/10.1016/S0006-3223(00)01088-X

Plieger, T., Felten, A., Splittgerber, H., Duke, É, & Reuter, M. (2018). The role of genetic variation in the glucocorticoid receptor (NR3C1) and mineralocorticoid receptor (NR3C2) in the association between cortisol response and cognition under acute stress. *Psychoneuroendocrinology, 87*, 173-180. https://doi.org/https://doi.org/10.1016/j.psyneuen.2017.10.020

Ritchie, H., & Roser, M. (2018). *Mental health.* ().OurWorldInData.org. https://ourworldindata.org/mental-health#citation

Rivers, C. A., Rogers, M. F., Stubbs, F. E., Conway-Campbell, B. L., Lightman, S. L., & Pooley, J. R. (2019). Glucocorticoid Receptor–Tethered mineralocorticoid receptors increase glucocorticoid-induced transcriptional responses. *Endocrinology, 160*(5), 1044-1056. https://doi.org/10.1210/en.2018-00819

Rodriguez, J. M., Monsalves-Alvarez, M., Henriquez, S., Llanos, M. N., & Troncoso, R. (2016). Glucocorticoid resistance in chronic diseases. *Steroids, 115*, 182-192. https://doi.org/https://doi.org/10.1016/j.steroids.2016.09.010

Sawchuk, C. P. D., L.P. (2017). *Depression and anxiety can occur together. read about the connection.* Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/depression/expert-answers/depression-and-anxiety/faq-20057989

Spencer, R. L., & Deak, T. (2016). *A users guide to hpa axis research1*https://doi.org/10.1016/j.physbeh.2016.11.014

Starecheski, L. (2015). *Take the ACE quiz — and learn what it does and doesn't mean.* NPR.org. https://www.npr.org/sections/health-shots/2015/03/02/387007941/take-the-ace-quiz-and-learn-what-it-does-and-doesnt-mean

Tang, A., O'Sullivan, A.,J., Diamond, T., Gerard, A., & Campbell, P. (2013). Psychiatric symptoms as a clinical presentation of cushing's syndrome. *Annals of General Psychiatry, 12*(1), 23. https://doi.org/10.1186/1744-859X-12-23

Tyrka, A. R., Ridout, K. K., & Parade, S. H. (2016). Childhood adversity and epigenetic regulation of glucocorticoid signaling genes: Associations in children and adults. *Development and Psychopathology, 28*(4), 1319-1331. https://doi.org/10.1017/S0954579416000870

Tyrka, A. R., Wier, L., Price, L. H., Ross, N., Anderson, G. M., Wilkinson, C. W., & Carpenter, L. L. (2008). Childhood parental loss and adult hypothalamic-pituitary-adrenal function. *Biological Psychiatry, 63*(12), 1147-1154. https://doi.org/10.1016/j.biopsych.2008.01.011

van Leeuwen, N., Bellingrath, S., de Kloet, E. R., Zitman, F. G., DeRijk, R. H., Kudielka, B. M., & Wüst, S. (2011). Human mineralocorticoid receptor (MR) gene haplotypes modulate MR expression and transactivation: Implication for the stress response. *Psychoneuroendocrinology, 36*(5), 699-709. https://doi.org/https://doi.org/10.1016/j.psyneuen.2010.10.003

van Rossum, E. F. C. (2004). Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. *Recent Progress in Hormone Research, 59*(1), 333-357. https://doi.org/10.1210/rp.59.1.333

van Rossum, Elisabeth F. C., Koper, J. W., van den Beld, Annewieke W., Uitterlinden, A. G., Arp, P., Ester, W., Janssen, Joop A. M. J. L., Brinkmann, A. O., de Jong, F. H., Grobbee, D. E., Pols, H. A. P., & Lamberts, S. W. J. (2003). Identification of the BclI

polymorphism in the glucocorticoid receptor gene: Association with sensitivity to glucocorticoids in vivo and body mass index. *Clinical Endocrinology, 59*(5), 585-592. https://doi.org/10.1046/j.1365-2265.2003.01888.x