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The Linkage Between HPA Axis Dysregulation and Metabolic Syndrome

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The Linkage Between HPA Axis
Dysregulation and Metabolic Syndrome

By

Brianna Godlewski

Submitted in partial fulfillment
of the requirements for
Honors in the Department of Biological Sciences

UNION COLLEGE

June, 2017

ABSTRACT

BRIANNA GODLEWSKI The linkage between HPA axis dysregulation and metabolic syndrome. Department of Biological Sciences, June 2017.

ADVISOR: Brian Cohen

A diagnosis of Metabolic Syndrome (MSX) requires patients to present with three or more of the following symptoms: elevated fasting blood glucose levels, elevated serum triglyceride levels, low serum HDL levels, elevated blood pressure, and truncal obesity. This pathology shares many similarities with Cushing's Syndrome (CS) but a diagnosis of CS requires hypercortisolemia. This similarity has led our lab and others to hypothesize that MSX may be a Cushingoid-like state caused by hypersensitivity of the glucocorticoid receptor (GR), a nuclear hormone receptor that is activated when cortisol binds, and or hyperactivity of the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 1, the cortisol reductase enzyme. The 11 β -HSD1 enzyme is responsible for the conversion of inactive cortisone to cortisol, and works primarily in the adipose tissue. Using an allele specific polymerase chain reaction (PCR) protocol, previous research in our lab has found significant correlations between the BclI and N363S polymorphisms of the glucocorticoid receptor and altered patient metabolic profiles in a population of patients seeking bariatric surgery, suggesting their possible contribution to diseases such as obesity and Metabolic Syndrome.

Recent work within our lab has found that the frequency of the single nucleotide polymorphism rs12086634, the hyperactivity polymorphism of interest within the 11 β -HSD1 enzyme, is 23.9% for our obese population and only 14.1% for our random college population. This suggests that this polymorphism may contribute to increased obesity and the MSX metabolic profile by increasing the localized cortisol in adipose tissue.

Additionally, our lab has expanded our study to include the cortisol resistance polymorphism TthIIIi (rs10052957) to test the hypothesis that there would be a decreased prevalence of this polymorphism in our subject population. The TthIIIi allele is present at 1.7% frequency in an obese population and at 6.94% frequency in a random college population.

Greater understanding of the interplay between these single nucleotide polymorphisms can help physicians and patients make more informed decisions about treatment options for obesity and metabolic syndrome.

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is a highly controlled central component of the neuroendocrine system¹. When activated, primarily by stress, the HPA axis is responsible for restoring homeostasis to the body through a signaling pathway that begins in the brain¹. When stress is detected, corticotropin releasing hormone (CRH) is released from the hypothalamus and travel to the anterior pituitary where it binds to the adrenocorticotrophic hormone (ACTH) receptor¹. ACTH is then released from the anterior pituitary and targets the glucocorticoid receptor in the adrenal cortex, specifically the zona fasciculata, signaling for the release of glucocorticoids (GCs), which in humans is mainly cortisol¹.

GCs are extremely diverse molecules with different functions in each part of the body. Cortisol, the main GC in humans, plays an important role in immunosuppression, anti-inflammatory action, growth, brain functioning, and most importantly glucose and lipid metabolism².

Cortisol's activity is mediated by

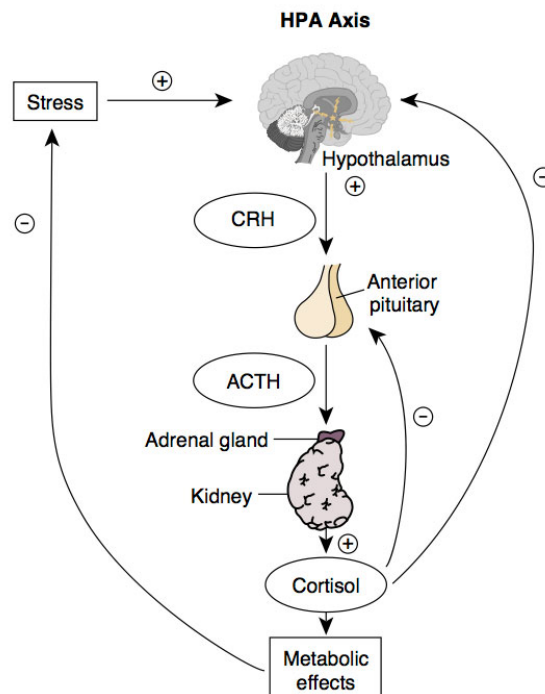


Figure 1 HPA Axis.

the GC receptor. As a steroid hormone derived from cholesterol, cortisol is able to cross the plasma membrane and bind to its receptor in the cytoplasm of almost all cells in the body. The receptor is part of the nuclear receptor family due to its ability to enter the

nucleus when cortisol is bound and the receptor is activated². Once in the nucleus the activated complex acts as a transcription factor to control gene expression².

Cortisol's control of gene expression plays a role in important metabolic, cardiovascular, immune, and psychological functioning³. Cortisol has been shown to increase low-density lipoprotein (LDL) circulation, but not high-density lipoprotein (HDL) circulation, thus resulting in an increase in triglycerides³. Cortisol is also known for its role in fat accumulation, specifically in the abdominal region³. Additionally, cortisol has been indicated to raise blood pressure³. These functions, all mediated by cortisol, present an interesting avenue of study: cortisol's effect on obesity.

Obesity is an epidemic affecting more than 34.9% of the population, which translates to more than 78.6 million Americans⁴. Obesity is defined in adults as an individual with a body mass index (BMI) greater than or equal to 30, with BMI being calculated by dividing weight in kilograms (kg) by height in meters squared (m^2). This large proportion of the population has an increased risk of heart disease, stroke, type 2 diabetes, and certain types of cancer⁴. Because of the higher prevalence of these conditions within this population, we, as a nation, spend \$1,429 more on healthcare per obese person compared to those of normal weight, which totals to \$147 billion in obesity related medical expenses⁴. The alarming number of obese, and the costs that are associated with them, makes obesity an important public health problem to investigate. Parallel to the increase in obesity has been an increase in bariatric surgery⁵. There are three main types of bariatric surgery: Roux-en- Y gastric bypass, the sleeve gastrectomy, and the adjustable gastric band. Bariatric surgery is a known and highly recommended solution for those suffering from morbid obesity and its associated comorbidities.

A subset of the obese population suffers from Cushing's Syndrome. Cushing's Syndrome is characterized by high levels of cortisol, whether endogenous or exogenous⁶. Typical physical representations of the syndrome include obesity, moon face, buffalo hump, thin skin, poor wound healing, purple striae, effects on libido and menstruation, hirsutism, and a range of psychiatric symptoms, including depression⁶. This range of symptoms is a result of the vast number of tissues that contain the GR.

An additional subset of the obese population suffers from a syndrome extremely similar to Cushing's Syndrome, but lack the corresponding hormone levels. Metabolic Syndrome (MSX) is known as a syndrome of elimination. It is characterized by a complex group of symptoms, that together make up a MSX diagnosis. A diagnosis requires a patient to present with at least three of these five symptoms: high fasting blood glucose, high blood pressure, abdominal obesity, high triglyceride levels, and low HDL

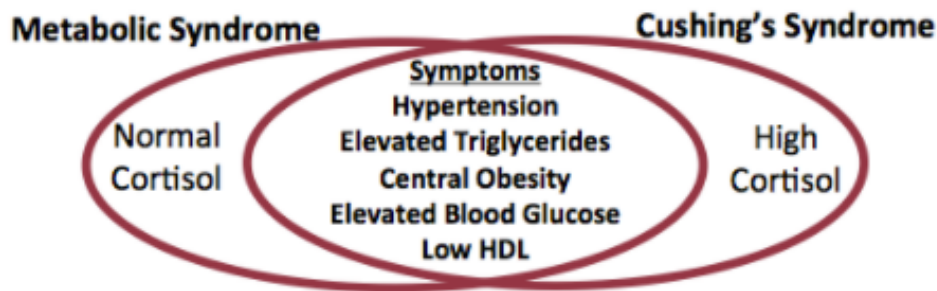


Figure 2 Comparisons between MSX and Cushing's Syndrome.

cholesterol levels⁷. How is it possible that these two syndromes can so closely resemble one another but have such different hormone panels?

Single-nucleotide polymorphisms (variations in the DNA sequence) (SNPs) within the gene for the cortisol receptor (GR) and/or within the genes that encode the 11beta-hydroxysteroid dehydrogenase type 1 (11 β -HSD1) enzyme, may contribute to

obesity in general, and the similarities observed between MSX and Cushing's. By increasing cortisol sensitivity within the GR, identical symptoms to Cushing's Syndrome can be observed without the elevated levels of cortisol. BclI (located within the intron 2, 647 base pairs from the exon/intron junction)⁸, and N363S (where the amino acid asparagine normally found at position 363 in the protein sequence is changed to the

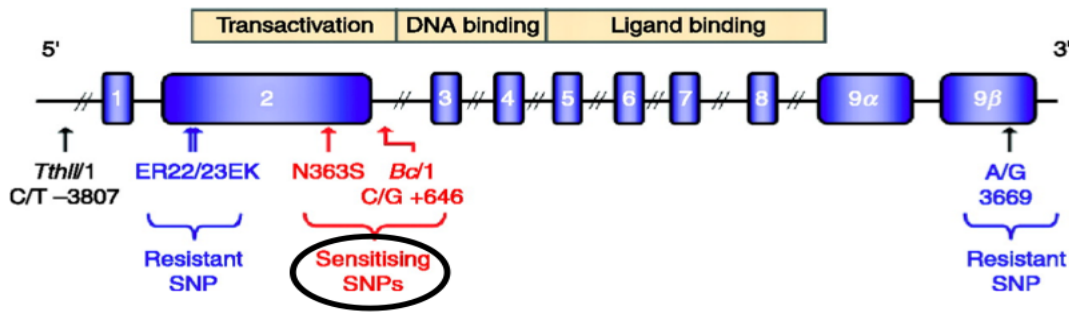


Figure 3 GR with noteworthy SNPs.

amino acid serine)⁹ are two of the most studied SNPs causing hypersensitivity of the GR. Both are known to cause the GR to over respond to normal, or even low, levels of cortisol, thus producing the appearance of symptoms that coincide with high levels of cortisol. Additionally, Tthll1 (a C to T change 6305 base pairs upstream of the first initiation codon), a known resistance polymorphism, has made its way into several studies examining the effects of SNPs on obesity³. Although a resistance polymorphism does not seem to make sense in an obesity study investigating how low to normal levels of cortisol can produce a phenotype similar to that produced by high levels of cortisol, several studies have seen interesting results when combinations of SNPs are present.

In addition to SNPs within the GR, SNPs within the enzyme 11 β -HSD1, cause an increase in localized cortisol concentrations. The 11 β -HSD enzyme is found in two forms throughout the body and is responsible for the conversion of inactive cortisone and

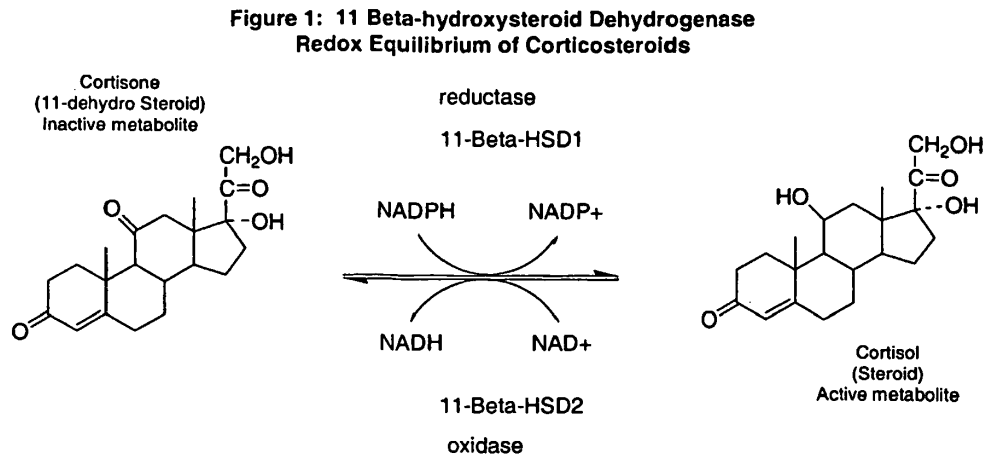


Figure 4 Conversion of cortisol to cortisone and vice versa via the 11 β -HSD1 enzyme.

cortisol¹⁰. The type 2 enzyme converts active cortisol to inactive cortisone, while the type 1 enzyme converts inactive cortisone to active cortisol¹⁰. The type 1 enzyme is of particular interest when studying obesity due to its expression within adipose tissue. Two SNPs of particular interest are rs12086634^{11,12} and rs846910¹¹. Both are polymorphisms within the genes encoding for 11 β -HSD1 enzyme that result in over-activity^{11,12}. Over-activity of the 11 β -HSD1 enzyme results in over-conversion of inactive cortisone to active cortisol, thus causing increased localized cortisol concentrations. Specifically, within adipose tissue, this is detrimental and can contribute to the physical manifestation of truncal obesity.

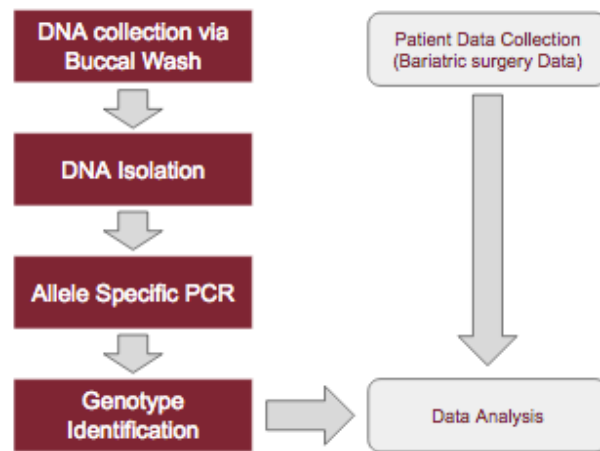
The purpose of our study was to compare the prevalence of these SNPs within the GR and the genes that encode the enzyme 11 β -HSD1 within an obese, bariatric population compared to a normal weight population comprised of college students. Additionally, we are interested in how these SNPs and/or combination of these SNPs

correspond with metabolic parameters of our obese population. Finally, we wish to investigate the correlation between these SNPs and/or combination of these SNPs and success of bariatric surgery, as defined by weight lost and weight re-gained. By studying these SNPs and their influence on a bariatric population, personalized decisions following genotyping can be made to ensure greater success in the treatment of the ever-expanding obese population.

Methods

Data Collection

Buccal samples were collected from 35 patients at the Ellis Bariatric Center, Schenectady, NY, along with medical information and several metabolic



parameters: height, original **Figure 5 General overview of the procedure.**

weight, pre-surgery weight, type of surgery and date completed, lowest weight, current weight, blood pressure, serum triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol, and blood glucose levels. Ellis Hospital's Institutional Review Board approved sample collection for two years (Appendix A). Patients were given an informed consent that iterated the purpose of the study and what information we would be collecting from the online record system (Appendix B). Buccal samples were also collected from 44 undergraduate students at Union College, Schenectady, NY.

Students were given an informed consent and were asked to provide their height, weight, and whether they had ever been diagnosed with depression (Appendix C). Following the informed consent, both sets of participants were given a 10mL centrifuge tube with sterile saline. Participants were asked to swish the liquid around their mouths for approximately ten seconds and then empty their mouth into a small plastic cup. The contents were then transferred from the plastic cup back to the centrifuge tube carefully and were labeled. Both the Ellis patients and undergraduate students were known by their medical record number or their sample number for the remainder of the study in order to maintain patient privacy and anonymity. The tubes were then stored in the lab freezer until DNA extraction.

DNA Isolation

DNA was isolated using a chelex bead protocol that is detailed in Appendix D.

Allele Specific PCR

In order to complete polymerase chain reaction on the DNA that had been isolated from our patient samples, allele specific primers were needed.

Sequences for our target SNP's were gathered from the

BclI - hypersensitivity of the GR	TthIII1 - resistance of the GR
WT up 5': AGTAGACAAGTTATGCTCTGCTGACC	WT r Primer 5': AGATCAGGTCCTTGCCCTTGT
MT up 5': AGTAGACAAGTTATGCTCTGCTGACG	MT r Primer 5': AGATCAGGTCCTTGCCCTTGT
Com down 5': AAATCAAACGAAAGCTGAAA	Com Forward 5': GAACTCCAGTGTGCCAGAAA
N363 - hypersensitivity of the GR	Rs12086634 - hyperactivity of 11β-HSD 1
WT r Primer 5': AGATCCTTGGCACCTATTCCATT	Rs12086634.wt.r: AAGAGATGGCTATATTAAGAAATTC
MT r Primer 5': AGATCCTTGGCACCTATTCCATC	Rs12086634.mut.r: AAGAGATGGCTATATTAAGAAATTA
Com forward 5': CAGTTTACTGTCAGGCAAGC	Rs12086634.com.f: TCTGCTGTATCACTGCAGGT

Figure 6 Functioning primer sets.

literature. Then, using a Web-Based Allele Specific Primer (WASP) program, we designed allele specific primers for each SNP in question. The WASP program gave us several different primer options for each SNP, and based upon melting temperatures we

were able to select what we thought would be the most successful primer set. Each primer set included a common primer, a wild type primer, and a mutant primer. The wild type primer would only bind if the wild type sequence was present, but not if the mutant sequence was present, and vice versa with the mutant primer. This allowed us to genotype patients. Based upon the melting temperatures provided by the WASP program, we were able to begin the process of primer optimization for the new SNPs our lab was investigating. This involved using gradients to determine the temperature that yielded the most specific banding. In some cases Q solution was used or changing of the magnesium concentrations. Ultimately two additional primer sets were optimized for rs12086634 and TthIII1. Optimal conditions for our functioning primer sets can be seen in Table 1. PCR was run on participant samples using these optimized conditions.

Table 1 Optimized conditions for functioning primer sets.

Polymorphism	PCR contents	Template Concentration	Annealing Temp	With or without Q
Bcl1	1 µl buffer, 1 µl common primer, 1 µl WT or MU primer, 0.5 µl dNTPs, 0.5 µl taq, 34 µl H ₂ O	8 µl	52°	w/o
N363	1 µl buffer, 1 µl common primer, 1 µl WT or MU primer, 0.5 µl dNTPs, 0.5 µl taq, 34 µl H ₂ O	8 µl	55°	w/o
RS12086634	1 µl buffer, 1 µl common primer, 1 µl WT or MU primer, 0.5 µl dNTPs, 0.5 µl taq, 34 µl H ₂ O	8 µl	51°	w/o
TthIII1	1 µl buffer, 1 µl common primer, 1 µl WT or MU primer, 0.5 µl dNTPs, 0.5 µl taq, 34 µl H ₂ O	8 µl	61°	w/o

Gel Electrophoresis

PCR products were analyzed by electrophoresis on a 2.5% agarose gel at approximately 130 V for 30 to 40 minutes. Prior to loading the gel, ethidium bromide was loaded into the buffer on the right side of the gel box and to the gel itself before it had solidified. This allowed the DNA to move down the gel depending on size and to be

seen under UV light following the completion of the DNA migration. The visualization of bands under UV light allowed us to genotype patients and determine the frequency of the mutant alleles within our two populations.

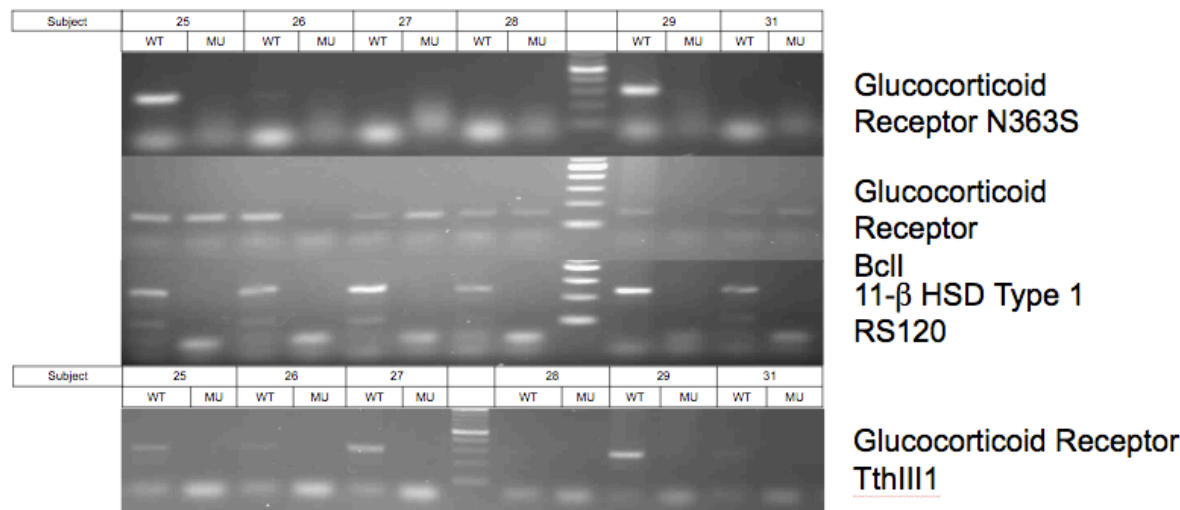


Figure 7 Example of gels ran for patients 25, 26, 27, 28, 29, and 31 for each of the optimized primer sets.

Data Analysis

Mutant allele frequencies were calculated by adding the percentage of the population with a mutant genotype and the half the percentage of the population with a heterozygote genotype. The percentages of the population were calculated by dividing the number of people with each genotype by the total population of successful tests.

Results

As described previously our data analysis consisted of calculating and comparing mutant allele frequencies. These results are listed in Table 2. For BclI, our obese sample population size was 79 and our random undergraduate population size was 43. The mutant allele frequency for the bariatric population was 39.2%, while the mutant allele frequency of the random population was 31.4%. The N363S bariatric population size was 54 and the random population size was 41. The mutant allele frequency for the

bariatric population was 4.6% and for the random population was 4.9%. The TthIII1 bariatric population size was 29 and the random population size was 36. The mutant allele frequency of the bariatric population was 1.7% and within the random population was 6.94%. The bariatric population size for rs12086634 was 23 and was 32 for the random population. The mutant allele frequency for the bariatric population was 23.9% and was 14.1% for the random population.

Table 2 Results of sample testing for BclI, N363S, TthIII1, and rs12086634.

Gene Polymorphism			Genotype Frequency								Allele Frequency			
			Random population				Bariatric population				Random population		Bariatric population	
Name	Name	Phenotype of mutant	WT	Hetero	MT	n	WT	Hetero	MT	n	WT	MT	WT	MT
GR	RS41423247 (BclI)	Hypersensitivity to cortisol	47	44.2	9.3	43	37	48.1	15	79	68.6	31.4	60.8	39.2
GR	RS6195 (N363S)	Hypersensitivity to cortisol	93	4.9	2.4	41	91	9.3	0	54	95.1	4.9	95.4	4.6
GR	RS10052957 (TthIII1)	Resistance to cortisol	92	2.8	5.6	36	97	3.4	0	29	93.1	6.94	98.3	1.7
11b-HSD														
Type 1	RS12086634	Type II Diabetes	75	21.9	3.1	32	61	30.4	8.7	23	85.9	14.1	76.1	23.9

Discussion

Findings

In our study, we hypothesized that MSX was a cushingoid-like state caused by hypersensitivity of the GR and or hyperactivity of the enzyme 11 β -HSD1. Because of this we expected to find unique mutant gene frequencies within our bariatric population when compared to a random undergraduate population.

Prior to genotyping, we expected our bariatric population to have a larger mutant allele frequency for BclI, N363S, and rs12086634 than our random population. Additionally, we hypothesized the TthIII1 mutant allele would appear less frequently in our bariatric population when compared to our random population. As seen in our results, these hypotheses were mostly supported by our data. BclI had a higher mutant allele frequency within the bariatric population than the random population. This was also true for rs12086634 as well. TthIII1 had a much lower mutant allele frequency in the bariatric population than in the random population.

However, one piece of data we did not expect to see was the high mutant allele frequency for N363S within the random population. While the bariatric population and the random undergraduate population had essentially the same mutant allele frequency, according to dpsnp, an online database that compiles information about known SNPs, the expected mutant allele frequency for a random population is around 1.15%¹³. This was not supported by our data, and our hypothesis for N363S was ultimately not supported.

Limitations

One of the biggest limitations of this study was our sample size. Our lab was only able to collect samples when we were physically present at Ellis Bariatric Center; there were no samples collected on our behalf by the nurses or the staff of the center. Additionally, some patients denied our request for a buccal sample. While this did not happen often, there were few occasions where the patient did not feel comfortable participating. Furthermore, the current procedure for DNA collection and isolation is effective, but not always successful. For some of our participants there was an extremely limited amount of DNA actually contained within the sample. This made performing the

PCR reactions difficult for these samples. Finally, there was occasional PCR error and the PCR conditions would just not give us results. This cut our sample population and reduced the sample size, especially for rs12086634 and Tthlll1, which did not have previous year's research to build off of.

Additionally, another limitation of this study is our reliance on BMI as an accurate measure of obesity. BMI is one of the quickest and easiest ways to classify someone as underweight, normal weight, overweight, obese, or morbidly obese. However, this formula does not take into account some extremely important factors such as muscle content. There are much better measures of obesity such as body fat content, waist circumference, and waist to hip ratio, but these methods are slower and often more expensive.

Directions for Future Research

Primarily we would like to continue our statistical analysis of our current data, and complete haplotype analyses. But, our first avenue of true future research is to continue to collect samples for this study. In order to establish clinical significance, we need a much larger patient population, and this is one of the major long-term goals of our laboratory. Also, by collecting more samples, we increase the likelihood that we have patients who are five to ten years post-surgery. Collecting samples from this population will allow us to continue to study the success of each type of surgery as defined by weight regain. With a large enough patient population we can begin to see trends in the types of surgeries that work best for those who has the single nucleotide polymorphisms.

In addition to collecting more samples, developing a new more reliable protocol for sample collection is a priority. Using cotton swabs versus a saline wash could aid in the collection of cheek cells, which is ultimately what we gather DNA from.

Adding additional SNPs, in combination with the existing SNPs we have been studying, will allow us to cover a broad range of mutations that exist within the GR and the enzyme 11 β -HSD1. Three such SNPs, which we have discovered in the literature, are rs7701443 and rs2963156 within the GR, and rs846910 within 11 β -HSD1.

Furthermore, upon studying the hyperactivity polymorphisms within 11 β -HSD1, this lead us to examine what else large amounts of cortisol can do within the body in addition to metabolic effects. One such effect is on the mineralocorticoid receptor (MR). When there is excess cortisol, due to the similarity between the structures of aldosterone and cortisol, the MR can be activated. Having both the GR and the MR activated at the same time may cause interesting and unforeseen affects within the body. Additionally, there are known SNPs within the MR itself that may effect this even more significantly. Finally, we wish to use similar methods in order to study HPA axis dysregulation and its affects on clinical depression. HPA axis dysregulation has been implicated as one of the possible causes of clinical depression, as we saw in the possible clinical manifestations of Cushing's Syndrome. By examining SNPs indicated in the literature, we will begin to collect samples from patients with depression in partnership with Albany Medical College. We will use the same PCR and gel electrophoresis techniques to examine the frequencies of indicated SNPs within this new population.

Clinical Significance

Personalized medicine is the future. Within every field, researchers and scientists are looking to give patients an individualized approach to treatment that diminishes side effects and increases success of treatment. This is the long-term goal of our study.

Because of the patient population we are studying, we have begun to notice as our patient population has grown, that when a patient is homozygous mutant or heterozygous for the polymorphisms we are investigating they are more susceptible to weight regain following certain types of surgery. This was observed by Sebastian Gingras, a former member of our lab, specifically with the adjustable gastric band. Ultimately, having the ability to screen our patients for a panel of SNPs, we will be able to formulate an exact treatment plan and recommend the best possible type of surgery for their genetic makeup. This would reduce the risk of weight regain and eliminate unnecessary risk for patients.

Conclusion

Obesity is an epidemic that has not only been plaguing the United States, but the world as a whole. In fact, the World Health Organization (WHO) estimates that since 1980 worldwide obesity has doubled¹⁴. In addition to the growing number of obese, we also see a growing number of those suffering from the comorbidities of obesity, specifically heart disease and diabetes¹⁵. This is a problem we need to be devoting time, resources, and research to, particularly the role of the HPA axis. As this study has shown, the HPA axis and dysregulation of it may have implications in the development of obesity, MSX especially. If there is indeed a genetic component to the development of obesity and obesity-related syndromes such as MSX, then physicians can be better prepared to recommend treatments following genetic screenings. This will ultimately help patients suffering from this disease lose the weight and keep the weight off, as well

as reduce the obesity epidemic as a whole. Fighting the epidemic will not only help the lives of millions, but will also ultimately reduce the costs our health care system has had to shoulder.

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Appendix A



Bellevue Woman's Center
Ellis Health Center
Ellis Hospital

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12308

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4011 www.ellismedicine.org

December 23, 2014

Anne Jones RN
Brian D Cohen PhD
Ellis Medicine Bariatric Care Center
2125 River Road Suite #302
Niskayuna, NY 12309

Re: Prevalence of Glucocorticoid Receptor Polymorphisms in Morbidly Obese Patients

Dear Ann and Dr. Cohen

Thank you for attending the December 18, 2014 meeting of the Institutional Review Board. Under full board review the protocol, informed consent (with changes) and other materials presented for the above mentioned study were approved. The study was approved for 2 years with an interim report due at the June 2015 IRB meeting. As a reminder, no additional changes may be made to this project without first submitting the changes to the IRB for review. Any inquiries or unanticipated problems must also be promptly reported.

Thank you for your continued interest in medical research.

Sincerely,

Michael V. Pasquarella

Michael V. Pasquarella Pharm.D., R.Ph.
Chairperson
Institutional Review Board

cc: Pat Biggica

Appendix B

Informed Consent Form

The purpose of this research project is to look for a correlation between polymorphisms of the glucocorticoid receptor (variations in the DNA sequence of the natural receptor for the hormone cortisol), the enzyme 11-Beta Hydroxysteroid Dehydrogenase (11- β HSD 1) (the enzyme responsible for converting inactive cortisone to its active form cortisol), and clinical manifestations of obesity and related comorbidities; elevated blood sugar, blood pressure, increased waist/hip circumference ratio, and altered serum lipid profiles. We have previously been screening for two hypersensitivity polymorphisms, but we will be adding two additional polymorphisms within the glucocorticoid receptor as well as two polymorphisms within the enzyme 11- β HSD 1.

Agreeing to participate in this study means that you will allow the testing of your DNA for the purposes of identifying receptor variations and you are agreeing for relevant medical data to be provided to the researchers to compare with the receptor variations. You will not receive information about your individual results from this study. Your DNA will not be used for any other purpose or analyzed in any other way. After 2 years, your DNA sample will be destroyed.

Your decision about whether or not to participate will not affect your treatment by Ellis Bariatric Medicine. You will not be compensated for your participation in the study.

If you decide to participate you will be asked to perform a mouthwash with sterile saline that will allow us to recover cells from inside your cheek. Some people find the salty taste of the saline wash a little unpleasant but should provide no significant discomfort or risk for you.

Identification of which genetic variant(s) of this gene you have will not affect your treatment in any way. As information is gathered about the relationship between variations of the cortisol receptor gene and obesity (and related diseases), it may eventually help identify opportunities for complimentary therapies for obesity, but this is beyond the scope of the present study. Currently we are strictly interested in determining if there is a connection between these genes and obesity (and related comorbidities) and any discovery will not affect your treatment plan.

Your DNA sample will be coded with your patient identification number instead of your name before it is given to researchers at Union College for DNA analysis. The researchers will also be given access anonymously to relevant medical information from your records required to complete the study. Records given to Union College researchers will only have your patient ID number and not your name. Relevant medical information will include but not strictly be limited to:

HPA Axis Dysregulation and MSX

1. Your weight before you began treatment
2. Your height
3. Your waist to hip ratio or waist circumference (where available) before you began treatment
4. Fasting blood sugar
5. Serum triglycerides
6. Serum LDL and HDL
7. Blood pressure
8. Related medications that you are taking that might affect these measures
9. Treatments you receive at Ellis Bariatric such as medications and surgical procedures

Your DNA sample will be kept in the laboratory of Dr. Brian Cohen at Union College and will only be accessible to him or to his student researchers working on this research project. Similarly, relevant medical information made available to Dr. Cohen will only be available to him or his student researchers and will not be accessible to anyone else at Union College.

Although we do not anticipate making any discoveries that would alter your care or cause you to wish to drop out of the study, if any such discoveries are made the medical staff of Ellis Bariatric will contact you and give you the option to withdraw from the study. Choosing to withdraw from the study will in no way affect your care as a patient of Ellis Bariatric. We anticipate that more than 100 patients will be a part of the study and the more patients that are included, the more significant any findings will be.

If you have any questions, please ask Anne Jones, RN, CBN, at Ellis Bariatric or Dr. Brian Cohen, Union College. Their contact information can be found below. You will be asked to sign one copy of this informed consent form and will be given a copy to keep for your records.

The choice to be in this study and to stay in this study is strictly voluntary. Refusal to participate will involve no penalty or loss of benefits which you are otherwise entitled. You may discontinue your participation at any time with no penalty or loss of benefits which you are otherwise entitled. If you wish to leave the study, please contact Ms. Anne Jones and inform her that you wish to withdraw from the study. You will be asked to sign a written form indicating your desire to withdraw. Ellis Bariatric will then inform Dr. Cohen of your ID number and your DNA sample will be destroyed and your relevant medical information will be removed from the data set.

Thank you for considering being a part of this research study. If you have any questions before participating or at any time during the study, please do not hesitate to contact us.

HPA Axis Dysregulation and MSX

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1. I have been informed about the purpose of this genetic test and experimental research project.
2. I understand that I will not receive the results from the testing.
3. I have been informed as to who may have access to my biological sample, and that the laboratory may retain any leftover sample until the end of the study, at which point it will be destroyed.
4. I have read the material provided and this consent form in full. My questions have been answered to my satisfaction.
5. I consent to provide a sample for genetic testing and to have relevant medical data provided anonymously to the researchers.
6. I understand that my participation is completely voluntary and I may withdraw at any time without affecting my care as a patient of Ellis Bariatric.

Signature

Date

Appendix C

INFORMED CONSENT FORM

Our names are Brianna Godlewski and Justin Salm and we are students at Union College in Schenectady, NY. We are inviting you to participate in a research study. Involvement in the study is voluntary, so you may choose to participate or not. **Participation in the study will not affect your grade in BIO225. Your instructor will not know if you have participated or not.** A description of the study is written below.

Our research is interested in determining if there is a relationship between variations in sequences of genes associated with stress responses and the prevalence of obesity and depression. You will be asked to provide access to the DNA sample that you generated for your BIO225 lab and ask you to answer a few questions including your height and weight and if you have ever been diagnosed with depression. If you choose to participate in the study, we will ask you to put your DNA from lab into a new tube labeled with an arbitrary number and also to fill out a separate form marked with the same number. This will take approximately 5 minutes. There are no foreseeable risks to taking part in this study. If you no longer wish to continue, you have the right to withdraw from the study, without penalty, at any time.

Your responses will be anonymous: the arbitrary number will not be connected to you in any way so we will not be able to identify who filled out each form or whose DNA is being analyzed.

All DNA samples collected will be destroyed after analysis, no later than 7/1/17.

By signing below, you indicate that you understand the information above, and that you wish to participate in this research study.

Participant Signature
Date

Printed Name

Appendix D

Isolation of Crude Human Genomic DNA samples suitable for PCR

The source of DNA that you will use for PCR reactions next week will be your cheek cells, which will be obtained by a sterile saline mouthwash. The cells are collected by centrifugation and resuspend in a solution containing the resin "Chelex", which binds metal ions that would otherwise inhibit the PCR reaction. The cells are then lysed by boiling, and centrifuged to remove cell debris and the Chelex resin. The result is a crude genomic DNA prep that is "good" enough for PCR!

You should have the following materials available **before** you start:

Materials:

Microfuge tubes and racks; micropipettors and tips
One 15 ml plastic "Blue-top" tube
One Non-sterile (but unused) plastic "Dixie" cup
One plastic transfer pipette
Labelled Waste container for "spit" waste
Microfuge tube containing 1.2 ml of 10% Chelex (labeled "Chelex")

Shared Materials:

Clinical Table-top Centrifuge for pelleting cells
Boiling water baths with "floatie" for boiling samples
Microfuge

- 1) Get a boiling water bath ready for use in step 8. Fill up an ice bucket halfway using crushed ice obtained from the Biology "autoclave room" on the third floor.
- 2) Label a 15 ml tube (blue-top) with your initials on the top and the side of the tube, and pipette (or carefully pour) 10 ml of sterile saline into the tube. Label a new microfuge tube with your initials, also on the top and side of the tube.
- 3) Pour all of the saline solution into your mouth (don't swallow it!), and vigorously swish for 15 seconds. Carefully expel the fluid into a plastic Dixie cup, and then pour the liquid back into the 15 ml tube and reclose the cap.
- 4) Place your tube in a clinical centrifuge balanced against another tube opposite it in the rotor. Try and fill up the rotor if you can with tubes from other members of the class. Centrifuge for 10 minutes at 1,000 X g for 10 minutes to pellet the cells.
- 5) After the spin is over, use a transfer pipette to pipette off as much of the supernatant as possible into the labeled waste container (we'll autoclave this later). Pipette off the liquid so that the cells remain with only a minimal amount of liquid (approx 200 μ L). You might have to briefly (1 min.) spin the tube again if the cells get dislodged from the side/bottom of the tube during this process. Resuspend the cells in the minimal volume of remaining supernatant by vortexing, and then transfer the whole volume using the same plastic transfer pipette to a new, labelled microfuge tube. Vortex this tube containing the cells for 5-10 seconds, making sure that there are no clumps of cells remaining.

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- 6) Resuspend the Chelex beads (in the microfuge tube labeled "10% Chelex") by vortexing for 10 seconds. Before the beads have had a chance to settle, pipette 500 μ L of Chelex solution into your microfuge tube containing the cells.
- 7) Resuspend the cells in the Chelex by vortexing for 10 seconds. Make sure that no cell clumps remain.
- 8) Incubate the microfuge tube containing the cells/Chelex mixture in the boiling water bath for 10 minutes, using a plastic "floatie". Other peoples' tubes will also be boiled at the same time in the water bath, so make sure that your tube is labelled well!
- 9) Remove the floatie carefully from the boiling water bath, take your tube and incubate it on ice for two minutes. Spin the tube in a microfuge (max. speed) for 1 minute to pellet the Chelex and cell debris to the bottom of the tube.
- 10) Using a 200 μ L pipette, carefully transfer 200 μ L of the supernatant (pipette from the top) to a new, labeled (with your initials) microfuge tube. Avoid transferring any of the pelleted cells or the Chelex. Discard the tube containing the pellet. YOUR PREP IS DONE AT THIS POINT! You should store your genomic DNA in the fridge in a labeled box or tube rack.