Association of Glucocorticoid Receptor Polymorphisms with Metabolic Characteristics and Bariatric Surgery in Bariatric Patients

By

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ABBREVIATION INDEX

GR: glucocorticoid receptor

CS/D: Cushing's Syndrome or Disease

SNP: single-nucleotide polymorphism

MS: Metabolic Syndrome

% EBWL: percent of excess body weight loss

LDL: low-density lipoprotein

HDL: high-density lipoprotein

BMI: body mass index

WHR: waist to hip ratio

BP: blood pressure

HPA: hypothalamic-pituitary-adrenal

DNA: deoxyribonucleic acid

RNA: ribonucleic acid

CRH: corticotropin-releasing factor

ACTH: adrenocorticotropic hormone

WT: wild-type

MUT: mutant

ABSTRACT

GINGRAS, SEBASTIEN Association of Glucocorticoid Receptor Polymorphisms with Metabolic Characteristics and Bariatric Surgery in Bariatric Patients. Department of Biological Sciences, June 2016.

ADVISOR: Dr. Brian D. Cohen

Cortisol acts on target tissues through the glucocorticoid receptor (GR). When in high concentration in the blood, it causes an obesity phenotype with hyperglycemia, hyperglyceridemia, hypertension and weight gain, known as Cushings' Syndrome or Disease (CS/D). Hypersensitivity-causing single-nucleotide polymorphisms (SNPs) of the GR have been shown to lead to a similar phenotype. Because of the symptomatic resemblance between CS/D and Metabolic Syndrome (MS), we and others hypothesized that the MS may be a Cushingoid-like state with absence of hypercortisolemia, but with GR hypersensitivity. Additionally, the link between GR SNPs and the success of bariatric surgery as measured by the percent of excess body weight loss (%EBWL) following bariatric surgery has been largely left untested.

We tested 62 obese bariatric patients and 51 normal weight college students for the presence of the BclI and N363S SNPs using a polymerase chain reaction technique with allele-specific primers. We first compared the prevalence of the SNPs in our populations and found them to be higher in the obese population for both SNPs. We also obtained a variety of metabolic parameters on the bariatric population and looked for significant differences across genotypes using one-way ANOVAs. Homozygous mutants for the BclI SNP showed a significant difference in triglyceride levels from heterozygotes. While all other parameters were non-significant, BclI mutants tended to have higher LDL cholesterol levels and lower HDL cholesterol levels with only slightly higher BMI, and N363S heterozygotes seemed to have higher LDL levels, systolic BP and blood glucose than homozygous wild-types. Our only BclI-positive/N363S-positive patient also tended to have higher blood glucose, LDL cholesterol, and systolic blood pressure, when compared to BclI-positive/N363S-negative patients. Finally, BclI-positive patients who underwent a gastric band tended to lose less weight following bariatric surgery than patients on whom other weight-loss surgeries were performed.

Taken together, these results support the hypothesis that GR SNPs have a significant impact on metabolic profiles, suggesting that GR SNPs could contribute to the development of disease states such as the MS, and affect the success of gastric band surgery.

INTRODUCTION

Cortisol and the HPA Axis

Cortisol is one of the most versatile hormones in the human body. It belongs to a group of steroid hormones called glucocorticoids that have a wide variety of effects on human peripheral tissues, mainly in response to physiological and psychological stress[1]. Glucocorticoids mediate inflammation by causing immune system suppression, and are involved in mood maintenance and cognitive processes[1]. They also play a part in regulating circadian rhythms[1]. Some evidence even suggests that they modulate the process of programmed cell death - or apoptosis[2-4]. More importantly, they are essential to the maintenance of energy homeostasis by regulating glucose, fat and protein metabolism[1]. By acting through its receptor, cortisol is an essential hormone involved in the catabolism of lipids and carbohydrates from peripheral tissues such as adipose tissues[1]. It acts on the liver to promote gluconeogenesis, the synthesis of glucose from smaller derivatives, and glycogenolysis, the breakdown of glycogen into glucose[5]. It also increases the synthesis of LDL and free fatty acids, and enhances the activity of lipase, the enzyme that catalyzes the breakdown of lipids into fatty acids, resulting in elevated triglycerides and LDL cholesterol levels[5]. Finally, it causes insulin resistance, which also contributes to increased lipid levels[5]. For these reasons, glucocorticoids and their effects on the body represent an intriguing area of study in the field of obesity.

In humans, cortisol is the primary glucocorticoid. It is produced by the adrenal glands following activation of the hypothalamic-pituitary-adrenal (HPA) axis by stressors (Figure 1)[6]. Stress causes the release of corticotropin-releasing factor (CRH) from the hypothalamus, and this hormone acts on the corticotrophs of anterior pituitary to produce

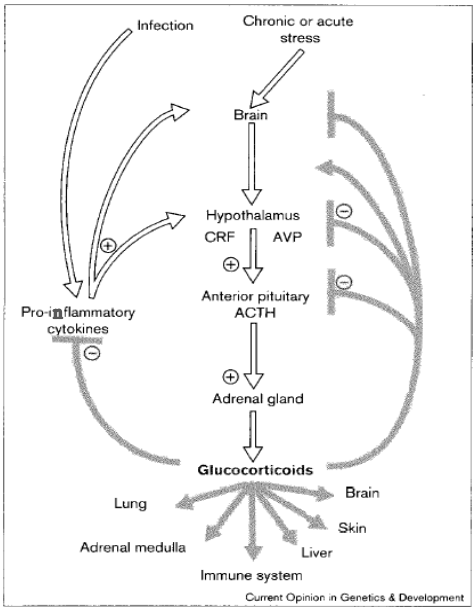


Figure 1[3]. The components of the HPA axis. The HPA axis contains negative feedback loops typical of the endocrine system. It culminates in the production of glucocorticoids, which are essential for many organs and systems.

adrenocorticotropic hormone (ACTH) [6]. ACTH binds to receptors on the cortex of the adrenal glands, and initiates the production of glucocorticoids and sex hormones in the zona fasciculata and zona reticularis, respectively[6].

Cortisol is derived primarily from the metabolism of cholesterol, which can be synthesized *de novo*, but is obtained mostly through an individual's diet[7]. Cholesterol, normally stored in lipid vesicles as an ester, is hydrolyzed into free cholesterol and transported to the mitochondria where it is converted to pregnenolone in the rate-determining step of steroidogenesis[7]. Then, enzymes in the mitochondria and endoplasmic reticulum catalyze the conversion of pregnenolone into a variety of steroid intermediates, and finally into steroid hormones such as cortisol (Figure 2)[7]. The enzymes present in a particular tissue, thus, determine which steroid hormone is likely produced and secreted by that tissue.

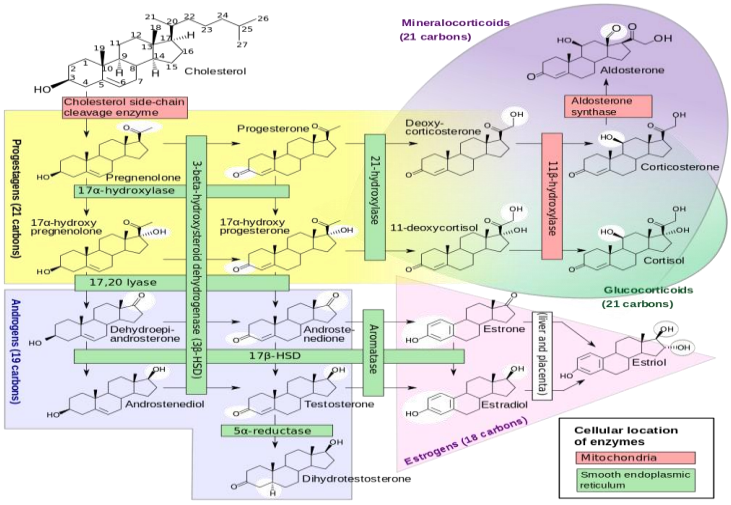


Figure 2[34]. Steroidogenesis. Important for our purposes are the intermediates and enzymes required for the conversion of cholesterol to cortisol.

In response to ACTH, cells in the zona fasciculata of the adrenal cortex increase cholesterol mobilization and transport into the mitochondria, which leads to the production of cortisol[7]. Once synthesized, cortisol is released into the circulation mostly bound to corticosteroid binding globulins, and is delivered to a variety of target tissues[8]. Because of their properties, steroid hormones must constantly be produced and released to maintain adequate levels in the blood, and usually produce slower and more sustained responses[7]. To respond to cortisol, a tissue must have a receptor that responds to the hormone (Figure 3)[9]. In most cases, cortisol exerts its effects by binding the glucocorticoid receptor (GR), which is present in most cells of the body[1]. Interestingly, when high levels of cortisol are in the blood, cortisol can function as a mineralocorticoid and bind to the mineralocorticoid receptor, causing the effects normally associated with mineralocorticoids[3].

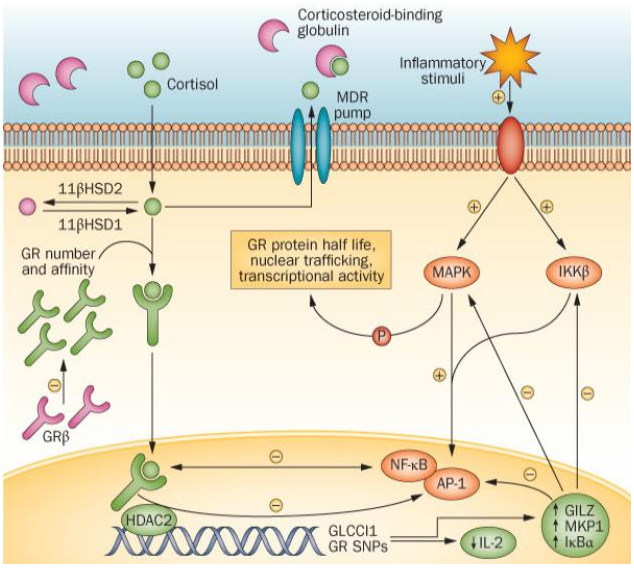


Figure 3[1]. The interactions of the cortisol-bound GR with other cellular components in target tissues. This very simplified schematic attempts to depict the complexity of GR function in human peripheral cells.

Cortisol's impact on tissues is also controlled peripherally by 11β-HSD I, which converts the inactive cortisone to cortisol, its active form, and 11β-HSD 2, which catalyzes the reverse reaction[10]. Research suggests that the tissue-specific functions of these two enzymes may differ between obese and normal weight individuals[10].

Finally, as is commonly seen in the endocrine system, cortisol suppresses CRH production in the hypothalamus and ACTH production in the anterior pituitary in a negative feedback fashion (Figure 1)[11].

Glucocorticoid Receptor Structure and Function

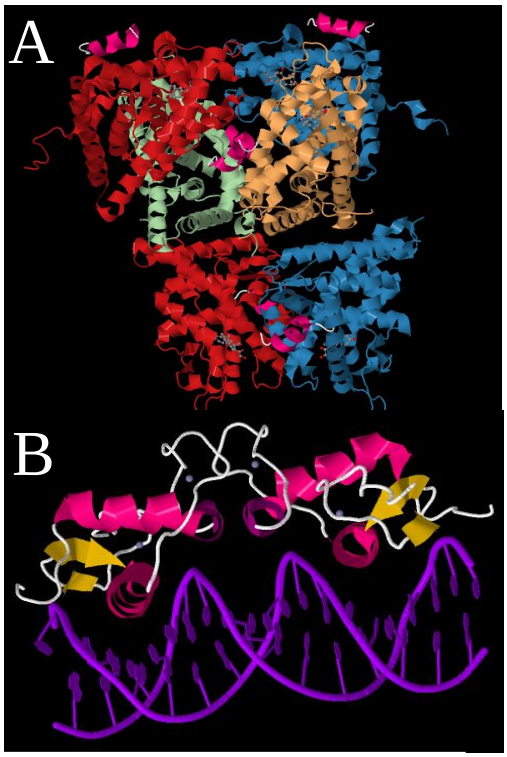


Figure 4[35,36]. Ribbon structures of the GR.
A: Cortisol-bound ligand-binding domain,
B: DNA-binding domain

The GR is part of the nuclear hormone superfamily, whose members are characterized by their similar structure and their ability to bind DNA to alter gene expression[12]. The GR receptor is highly polymorphic and the unique gene encoding it gives rise to drastically different variants of the receptor[13]. Structurally, the receptor consists of three major domains: a 250 amino acid carboxyl terminal ligand-binding domain, a central 60 to 70 amino acid DNA-binding domain, and a variable amino terminal transactivation domain (Figure 4)[1,13].

In the classical model, cortisol binds to the ligand-binding region of the GR in the cytosol, causing a conformational change in the protein that allows it to enter the nucleus, bind DNA through its DNA-binding domain, and act as a transcriptional switch that turns genes on and off (Figure 3)[13]. However, more recent studies highlight the complexity of the intracellular and intranuclear interactions of the GR. They suggest that, once bound by a ligand, the many different isoforms of the GR interact with a variety of cytosolic

molecules such as chaperones, kinases, phosphatases, nuclear shuttling proteins and the proteasome, in addition to numerous transcription factors and coactivators (Figure 3)[1,13].

The GR is encoded by only one gene, the NR3C1 gene, located on chromosome 5q11-q13, a puzzling fact given its highly diverse functions[1,13]. Figure 5 describes how such a wide variety of functions are made possible. First, as depicted in 5A, gene expression is controlled by 3 promoter regions that have the ability to recruit unique combinations of transcription factors, and that lead to the transcription of different parts of the GR gene[13]. This process gives rise to alternative transcripts that are believed to vary depending on the cell type and the environmental conditions, and account for some

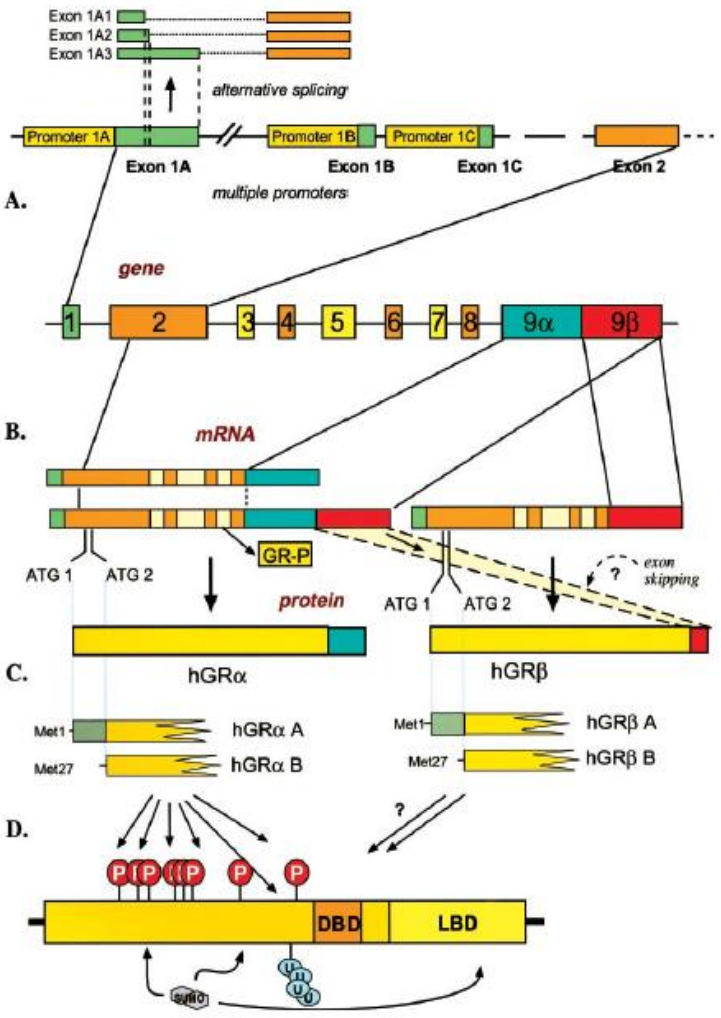


Figure 5[11]. Generation of the different isoforms of the GR that give rise to its huge functional diversity. Contributing to this diversity are isoforms due to A: the 3 promoter sequences modulating the 9 exons of the unique GR gene, B: alternative splicing of the transcripts, C: variable translation start locations and D: post-translational modifications.

of the huge diversity in GR expression[13]. In addition, as shown in 5B, these alternative transcripts can undergo alternative splicing, which increases the diversity of messenger RNAs even further. Evidence also suggests that translation initiation may begin at different locations on the messenger RNA, forming translational isoforms that have different biochemical properties, as is portrayed in 5C[13]. Finally, segment 5D shows that the GR can be modified by other intracellular proteins such as kinases and phosphatases following translation[13]. All of these mechanisms permit the creation of a large database of GRs that differ in function and interact intricately with the environment to produce a wide variety of effects on the body.

The importance of the GR for life was highlighted in mice with GR-knockouts; they have been shown to die only a few minutes following birth due to lung collapse caused by uncontrolled inflammation of the lungs[3].

Glucocorticoid Receptor Single-Nucleotide Polymorphisms

The many isoforms of the GR, which serve integral functions in the human body, originate from a single gene, causing the GR to be strongly affected by mutations in the gene that codes for it. A wide range of single-nucleotide polymorphisms (SNPs) of the

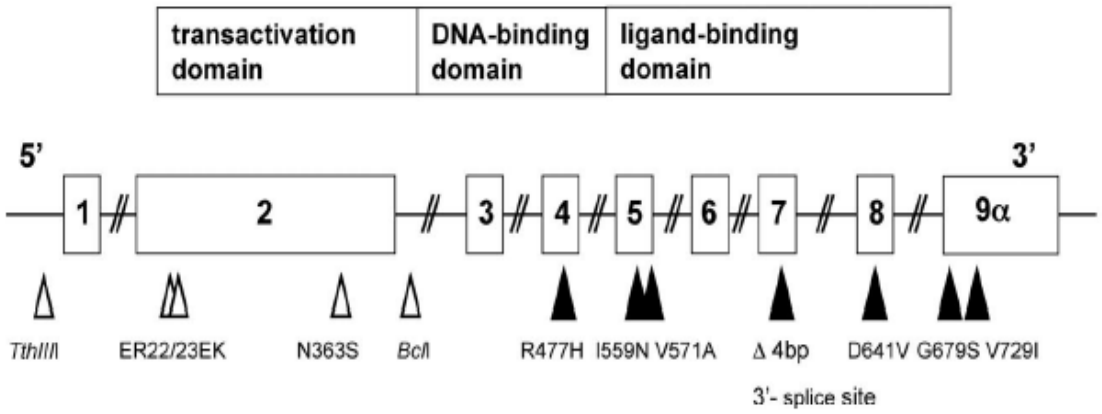


Figure 6[8]. The main SNPs of the GR. The N363S SNP is located in exon 2, and the BclI is intronic. While some polymorphisms cause GR hypersensitivity, others cause resistance of the GR.

GR gene have been reported, some of which are thought to cause altered lipid and carbohydrate profiles.

Yet, because of the many different functions of the GR, the phenotypic impact of SNPs at the organismal level has been difficult to describe accurately. In fact, while the research literature on SNPs is extensive, conclusive results remain to be obtained, in some part due to inconsistencies in the size, age, gender and race/ethnicities of sample populations[10]. While some SNPs have been associated with GR resistance, others have been shown to cause hypersensitivity of the GR to glucocorticoids[11]. Two SNPs that fall into the latter category are the BclI and the N363S[11].

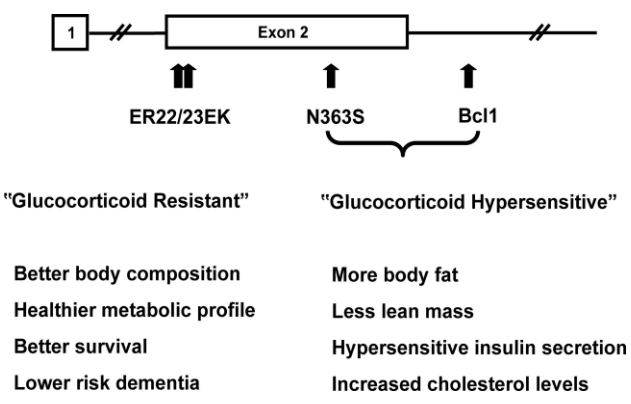


Figure 7[8]. Clinical effects of hypersensitivity-causing and resistance-causing SNPs of the GR.

The BclI polymorphism consists of a C → G nucleotide change in the intron downstream of exon number two, 646 nucleotides away from the exon-intron junction[10]. It is estimated that 38.0% of the population are positive for the G allele[1]. The first analysis of the impact of the BclI SNP on metabolic parameters was performed by Weaver et al. (1992), and found no difference in the prevalence of the mutant allele between obese and non-obese populations of premenopausal women, although it did find that the obese individuals had higher insulin levels and were more insulin resistant than the non-obese individuals[10]. Some subsequent studies have found increased body mass index (BMI), waist-to-hip ratio (WHR) and abdominal obesity in the G-allele carriers, but others have shown no such associations[11]. The BclI SNP has also been linked to higher

risk of hypertension, but once again, no correlation has been found between the presence of the mutant allele and BP, triglycerides or blood glucose in replicate studies using different populations[11]. The only parameter on which the evidence seems to agree is total cholesterol, which is believed to be higher in BclI carriers[11].

On the other hand, the N363S polymorphism is an AAT → AGT change in codon 363 of exon 2 of the GR gene.[10] Its prevalence is significantly lower than the BclI SNP at about 3.5% of the population[1]. Just like for the BclI SNP, the findings regarding the N363S SNP are mixed. Some research suggests an association with BMI and WHR, but these results haven't been successfully replicated by other groups[11]. However, there is strong evidence for a correlation between the presence of the N363S allele and higher serum triglycerides and LDL cholesterol levels[11].

The mechanisms by which the BclI and N363S SNPs cause glucocorticoid hypersensitivity are still unknown, though some theories are presently being investigated[11].

Cushing's Syndrome/Disease and the Metabolic Syndrome

Because of cortisol's importance in lipid and carbohydrate homeostasis, it is not surprising that alterations in the HPA pathway can cause serious metabolic dysfunctions, such as are seen in Cushing's Syndrome or Disease (CS/D)[14]. CS/D is characterized by hypercortisolemia, which can be caused by deficiencies in many components along the HPA axis[14]. Its clinical presentation is extremely varied, but has many commonalities with symptoms associated with obesity such as dyslipidemia, hypertension, glucose intolerance, and increased visceral adiposity[14,15].

The rapidly increasing prevalence of obesity in our society has caused a related increase in obesity-related symptoms such as abdominal adiposity, insulin resistance, dyslipidemia, elevated BP, and prothrombotic and proinflammatory states, a cluster of symptoms that is diagnosed as the Metabolic Syndrome (MS)[16]. Yet, a biologically-based, mechanistic explanation for the MS has yet to be formulated[17].

Meanwhile, with this debate still ongoing, clinical cases of patients with low or normal cortisol presenting with symptoms of CS/D have begun to appear. In 1990, Iida et al. presented the first case of a patient with Cushing-like symptoms and hypocortisolemia[18]. They found no abnormality in the HPA axis or cause for his hypocortisolemia, and the patient's skin fibroblasts were shown to be hyperreactive to glucocorticoids *in vitro*[18]. The researchers thus suggested that the hypocortisolemia may have been caused by a hypersensitivity of peripheral tissues to cortisol[18]. Al-Shoumer et al. (2008) reported the case of a 32 years old woman with similar characteristics[19]. This has led some groups to suggest that the MS may be a Cushingoid-like state with normal cortisol levels, but with hypersensitivity of the GR.

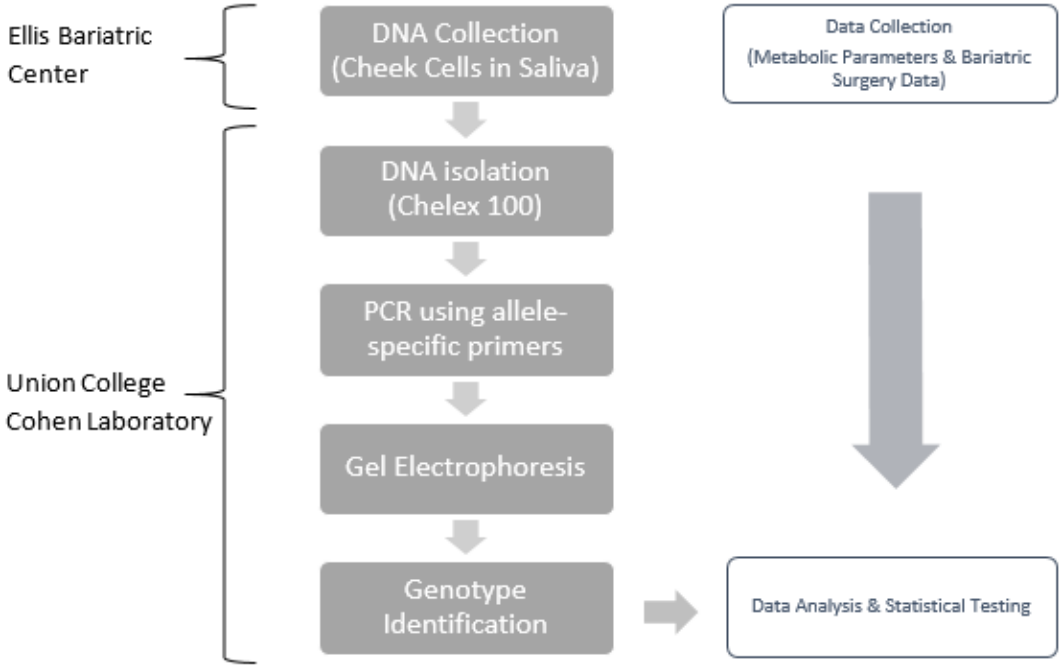
Bariatric Surgery

The obesity epidemic has led to a rise in prominence of bariatric surgery in the treatment of morbid obesity[20]. There are three main types of bariatric surgery, most of which are now performed laparoscopically: the Roux-en-Y gastric bypass, the sleeve gastrectomy and the adjustable gastric band. A large body of evidence suggest that bariatric surgery causes large changes in endocrine pathways, such as the HPA axis, that mediate weight loss and target comorbidities such as type 2 diabetes, hypertension, hyperlipidemia, heart disease and depression[20,21]. For example, the Roux-en-Y has been

shown to greatly reduce the development of type 2 diabetes in a majority of patients[21]. More specifically, massive weight loss, such as is associated with bariatric surgery, has been shown to decrease insulin resistance in adipose tissue, lower cortisol secretion rates, and reduce glucocorticoid production through inhibition of key steroidogenic enzymes[22]. Yet, perhaps surprisingly, only one study to date has looked at how genetic markers correlate with bariatric surgery success as measured by weight loss or regain, and none have targeted GR SNPs specifically[23]. Nevertheless, GR SNPs' effects on metabolic parameters make them likely candidates for the genetic susceptibility of some individuals to reduced weight loss or increased weight regain following bariatric surgery.

The purpose of our studies is thus threefold: first, we wish to evaluate the prevalence of the BclI and N363S hypersensitivity-causing SNPs in an obese, post-bariatric population as compared to a normal weight population of college students. Second, we are interested in how the presence of these SNPs correlates with metabolic parameters, specifically BMI, blood glucose levels, serum triglycerides, HDL and LDL cholesterol levels, and systolic and diastolic BP. This could support a mechanism for the development of the MS in which hypersensitivity of the GR plays an important role. Thirdly, given the importance of the HPA axis in weight management, we want to look at how the presence of SNPs correlates with success of bariatric surgery as measured by percent of excess body weight loss (%EBWL) following surgery. These findings could guide decisions to undergo invasive weight loss surgery and improve the care of morbidly obese individuals.

METHODS



DNA Collection

The buccal cheek cells of 62 patients at Ellis Bariatric Care Center in Niskayuna, NY, and of 51 undergraduate students at Union College in Schenectady, NY, were collected. Approval to collect bariatric patient samples was obtained from Ellis Hospital's Institutional Review Board (Appendix, Section A), and the Union College Human Subjects Review Committee granted us approval to collect student samples (Appendix, Section B). The purpose of the study and the rights of the participants were explained, and informed consent was obtained prior to saliva collection for both groups (Appendix, Section C). The participant poured 10 mL of 0.1% saline solution contained in a 15 mL centrifuge tube in his or her mouth, swished it around for 30 seconds, and spat the saline in a plastic cup. The content of the cup was then manually transferred back to the 15 mL

centrifuge tube to limit the amount of saliva lost in the procedure. The tubes were then labelled with the participant's medical record number (MRN), and stored at 4^0C before DNA extraction.

DNA Isolation

The DNA was isolated using Chelex 100 beads by Bio-Rad©. See Appendix, Section C for a detailed protocol.

PCR Using Allele-Specific Primers

Because of inconsistent early trials, purified DNA concentration was quantified using a Nanodrop© prior to the PCR procedure. Our inquiries being solely qualitative, the goal was simply for the template concentration in the PCR tube to be high enough for the PCR reaction to be successful. Thus, the concentration value obtained using the Nanodrop© guided our evaluation of the amount of template used for the PCR reaction (Table 1).

Table 1. Amount of Template Used for PCR reactions Based on Original Concentration of BclI and N363S samples

	BclI	N363S
Below 20 ng/µL	30 µL	25 µL
Between 20-30 ng/µL	25 µL	22.5 µL
Above 30 µL	20 µL	20 µL

Every PCR tube used in the PCR reactions contained a total volume of 50 µL.

Table 2. Reagent Quantity in a Typical PCR Tube	
Reagent	Quantity
1) Deionized Water	50 µL – (2+3+4+5+6+7)
2) Buffer	5 µL
3) dNTPs	1 µL
4) Common Primer	1 µL
5) DNA polymerase	0.5 µL
6) WT or MUT Primer	1 µL
7) Patient Template	Amount based on Table 1

Based on known DNA sequences, wild-type (WT) primers were specifically designed to bind only the common GR gene sequence at the BclI and the N363S sites, respectively. In contrast, mutant (MUT) primers were designed to bind the GR sequence of interest for both the BclI and the N363S polymorphisms with specificity (Figure 9).

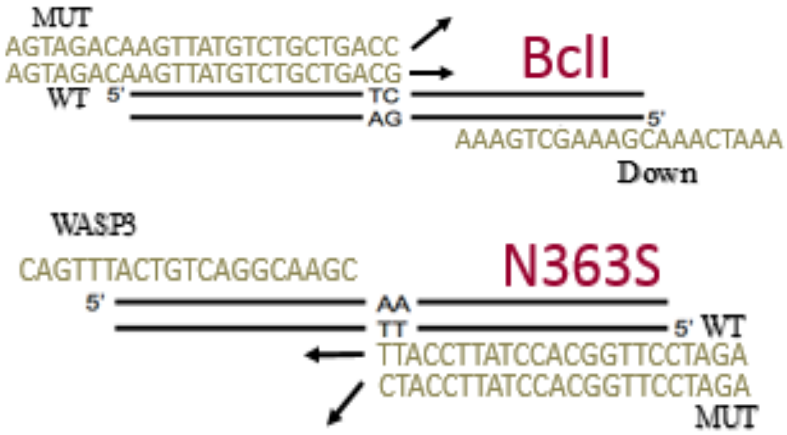


Figure 9. Upstream and downstream primer sequences used for the BclI and the N363S SNPs.

The PCR reactions were run in a Bio Rad C1000 Thermal Cycler©. The conditions are shown in Table 3.

Table 3. PCR Conditions for both SNPs.

	BclI	N363S
Denaturation Temperature	95°C	95°C
Primer Annealing Temperature	56°C	55°C
Primer Extension Temperature	72°C	72°C
Number of Cycles	36	36

Gel Electrophoresis

PCR reactions were electrophoresed on a 2% agarose gel at 100 V for approximatively 10 minutes. The use of ethidium bromide permitted the visualization of the bands under UV light, and provided a method for genotyping patients.

Data Collection

Metabolic data was obtained from each patient's medical records kept in Ellis Hospital's electronic database. The data collected consisted of height, fasting blood glucose, serum triglycerides, LDL cholesterol, HDL cholesterol, systolic and diastolic BP, surgery type and date, original weight with date, pre-surgery weight with date, lowest weight with date, and current weight with date.

Data Analysis and Statistical Testing

One-way ANOVAs using IBM SPSS Statistics© were used to evaluate the relationship between genotype and BMI, fasting blood glucose, serum triglycerides, LDL cholesterol, HDL cholesterol, and systolic and diastolic BP. A significance criterion $p < 0.05$ was used. We also observed the relationship between genotype and success of bariatric surgery as measured by %EBWL.

RESULTS

Genotyping was performed as described in materials and methods. Section E of the Appendix shows the agarose gel electrophoresis of the PCR reactions. A complete accounting of individual genotype assignments and other clinical information - where available - can be found in Section F of the Appendix for the bariatric patients and Section G for the undergraduate students.

Prevalence of the SNPs

From the genotyping results, we were able to compare the prevalence of the BclI and N363S SNPs in both the study population and the control population (Appendix, Section H). The prevalence of both SNPs was higher in the obese population than in the normal weight population.

Association between SNPs and Metabolic Parameters

One-way two-tailed ANOVAs using a $p<0.05$ significance criterion were performed using IBM SPSS© to look for differences in the bariatric patients' metabolic profiles based on their genotype for the BclI SNP (Appendix, Section I). Homozygous MUTs for the BclI SNP were only significantly different from heterozygotes and homozygous WTs in serum triglyceride levels; they had higher levels of triglycerides than the other groups ($p<0.05$). All other associations were non-significant. However, homozygous MTs tended to have higher LDL cholesterol levels, slightly higher BMIs, and lower HDL cholesterol levels (Appendix, Section J for BclI and Section K for N363S).

Only 1 participant tested positive for the N363S SNP, making statistical analyses impossible. This patient was heterozygous for the N363S SNP and heterozygous for the BclI SNP. When compared to BclI-positive/N363S-negative participants, this patient tended to have higher blood glucose levels, LDL cholesterol levels, and systolic BP (Appendix, Section L).

Association between SNPs and Bariatric Surgery

The participants' %EBWL following bariatric surgery was organized based on the surgery they underwent and their BclI genotype (Appendix, Section M). For both the Roux-en-Y gastric bypass and the vertical sleeve gastrectomy, there was no difference in the average %EBWL between genotypes. However, for patients who underwent the gastric band, both heterozygotes and homozygous mutants tended to have a lower %EBWL than homozygous wild types. In addition, the BclI/N363S double heterozygote, who underwent a Roux-en-Y gastric bypass, tended to have a lower %EBWL than BclI-positive/N363S-negative patients.

DISCUSSION

In the present study, we hypothesized that 1) the prevalence of the BclI and N363S SNPs would be higher in an obese population than in a normal weight population, 2) the presence of the SNPs would be significantly associated with poorer metabolic profiles, and 3) the presence of the SNPs would be associated with less success of bariatric surgery.

Prevalence of the SNPs

The results obtained in this study suggest that there is a difference in the prevalence of both the BclI and the N363S SNPs in obese populations compared to normal weight populations. This supports the theory that SNPs may be involved in the development of obesity. Many studies have looked at the prevalence of the SNPs in either obese or normal weight populations. A study by Gergics et al. (2006) found that the prevalence of the BclI SNP was 34.4% in a normal weight population, supporting our findings[24]. However, interestingly, Di Blasio et al. (2003) obtained a lower value, 27%, in their obese population[25]. This may be a direct consequence of the different populations that both studies used, highlighting the importance of characterizing the prevalence of the SNPs for different populations based on age, sex and ethnicity, among others. While we found no participant with the N363S SNP in our normal weight population, 0.9% of the normal weight population recruited by Di Blasio et al. (2003) were positive for the SNP[25]. Finally, Dobson et al. (2001) found that the prevalence of the N363S SNP was at 3% in their obese population, and Di Blasio et al. (2003) reported it to be at 2.3%, both measurements being slightly higher than the value we found in our population[25,26]. Overall, our results agree to some extent with previous studies on the topic, and suggest

that the prevalence of the SNPs in obese populations is higher than in normal populations and thus, may in fact contribute to weight gain.

Association between SNPs and Metabolic Parameters

The main assumption of our study was that the MS may be a Cushingoid-like state characterized by hypersensitivity of the GR. In other words, the presence of glucocorticoid hypersensitivity may contribute to more morbid obesity phenotypes. By looking for associations between the presence of the SNPs and metabolic parameters characteristic of the MS, we hoped to provide support for the claim that glucocorticoid hypersensitivity may be the biological mechanism driving the MS. The results of previous studies have been mixed on the topic, but none to my knowledge have found a significant difference in serum triglycerides between BclI-positive and BclI-negative individuals. Some, however, have found associations with BMI, waist circumference and visceral adiposity[11].

The one major conclusion that can be taken from our findings and from multiple previous studies on the topic is that there is an enormous amount of variability in the phenotypic representation of SNP-positive individuals. Whether this is due to misguided attempts to make holistic generalizations from results obtained in drastically different populations, whether it can be ascribed to an overly narrow focus on SNPs of the GR, or even whether it can be attributed to the large amount of functional diversity of the GR, understanding why such inter-study or inter-population variability occurs remains an important goal to understand the role of SNPs in disease and obesity. To achieve that goal, it is essential that the structural effect of the BclI and N363S SNPs on the GR be

investigated further, so that the functional impact of the mutated GR protein be elucidated.

One strategy is to look at the effect of multiple SNPs on phenotype. Comparing our only Bcl-positive/N363S-positive participant, who was heterozygous for both SNPs, to homozygous MUT for the BclI, we found some evidence that looking at the effect of multiple SNPs may be a valuable strategy. Our double heterozygote seemed to have higher blood glucose, LDL cholesterol and systolic BP, while being similar for all other parameters. This is in accord with Di Blasio et al (2003)'s findings that BclI-positive/N363S-positive individuals have significantly higher LDL and total cholesterol, and tend to have higher systolic and diastolic BP[25]. It remains to be confirmed whether blood glucose is also elevated in those individuals.

A further strategy, which has been termed "haplotype analysis", is to look at contributions from all hypersensitivity-causing and resistance-causing SNPs in the GR gene on a patient's phenotype. A recent study by Yan et al. (2014) has looked at the combined effect of 4 different SNPs on metabolic parameters characteristic of the MS[5]. Overall, as expected, the researchers found that presence of hypersensitivity-causing SNPs with an absence of resistance-causing SNPs correlated with MS-like phenotypes, while the presence of resistance-causing SNPs with an absence of hypersensitivity-causing SNPs were associated with favorable metabolic profiles. However, depending on the combination of the SNPs present in an individual's genome, there were instances where resistance-causing SNPs appeared to mediate the effects of hypersensitivity-causing polymorphisms, suggesting that it may be important to look at the effect of all SNPs, hypersensitivity-causing and resistance-causing, on phenotype.

Finally, some studies suggest that other abnormalities in an individual's HPA pathway may be responsible for metabolic deficiencies. One likely candidate based on other studies, 11β-HSD 1, which has been shown to have different expression and activation patterns in the liver and adipose tissues, has been linked to the development of central obesity[27,28]. In addition, SNPs in the gene encoding the enzyme have been found to cause metabolic abnormalities[29,30]. All in all, a full understanding of the role of the HPA axis in the development of obesity and the MS may necessitate a combined investigation of multiple genes involved in important regulatory steps of this pathway.

Association between SNPs and Bariatric Surgery

In recent years, bariatric surgery has emerged as the best treatment option for obesity. However, for reasons still unknown, its success varies across individuals. Because of the invasiveness of the surgery, an important goal of researchers in the field has been to find ways to predict the success of surgery. Still et al (2014) identified a set a preoperative variables such baseline BMI, initial weight loss and iron deficiency that have been shown to be helpful in predicting the success of bariatric surgery[31]. In 2011, the same group found four SNPs in different obesity-related genes that were associated with poorer weight loss outcomes following gastric bypass surgery[32]. There is thus some evidence that an individual's genetic makeup may play a role in his ability to lose weight after bariatric surgery. This has prompted a recent large study by Seip et al. (2016) that grouped successful and unsuccessful patients based on BMI drop pre and post gastric band and gastric bypass surgeries, respectively, and compared the prevalence of a large amount of SNPs in these groups[23]. They found no differences in the allele frequency of GR SNPs between successful and unsuccessful patients who underwent a gastric bypass.

However, interestingly, they found a SNP in intron 2 of the GR gene, a location near the BclI SNP, which was present in significantly higher proportion in the unsuccessful gastric band group. This polymorphism has yet to be identified as hypersensitivity-causing or resistance-causing, but these results suggest that it may be hypersensitivity-causing. As a whole, this research supports our findings for an association between the presence of the BclI and poorer weight loss outcomes following gastric band surgery. More research is required to establish whether these loci may represent important data for predicting favorable outcomes of bariatric surgery.

In any case, the relationship between GR SNPs and gastric band surgery requires further investigation. It remains unclear as to why SNPs of the GR affect weight loss in patients who undergo a gastric band, but not in patients who undergo a gastric bypass, or based on our findings, a gastric sleeve. One possibility is that the hormonal changes associated with the gastric bypass and gastric sleeve surgeries may mitigate the negative effects of the GR SNPs on weight homeostasis. A study by Korner et al. (2009) found that individuals who had undergone a gastric bypass had a higher GLP-1 response and lower ghrelin, leptin, fasting blood glucose and insulin levels than individuals who got the gastric band surgery[33]. There is thus a clear difference in the hormonal impact of more invasive surgeries such as the gastric bypass or sleeve, compared to less invasive surgeries such as the gastric band. Whether and how these endocrine changes mediate GR SNPs' effects on metabolism is yet unknown, but this could help understand one of the many ways bariatric surgery has such drastic impact on weight homeostasis.

Clinical Significance

The present findings also have important clinical implications. It remains possible that the MS is caused by hypersensitivity of the GR. However, our findings suggest that inclusion of many SNPs or even better, an individual's haplotype, may be better predictors of obesity phenotypes and thus, may be responsible for the MS and for its variability in symptomatic presentation.

As for the GR SNPs' impact on the success of bariatric surgery, it seems that particular attention must be paid to individual's undergoing the gastric band. The gastric band may not be the best options for patients who have GR hypersensitivity-causing SNPs. Alternatively, patients who test positive for these SNPs and who prefer the gastric band for alternative reasons, may have to be kept on a GR-blocker such as RU-486 - clinically known as Korlym - following their surgery. Taken together, these results highlight the potential of full-genome sequencing in the treatment of obesity, and in improving the success of bariatric surgery.

Limitations

In order to understand the implications of this study, it is important to note some limitations. Firstly, we were not able to obtain the patients' waist circumference, which is the criterion used to diagnose the MS because it is more representative of an individual's central adiposity. Secondly, a larger sample size would have been necessary to better establish the significance of our results. Thirdly, our sample consisted mostly of Caucasians, and to some extent, of individuals with a history of post-surgical

complications, and thus, one should be careful when generalizing to the whole obese population.

Conclusion

All in all, obesity is an extremely complex multi-causal phenomenon, and undoubtedly, more research will be necessary to truly understand the role of the HPA axis and of the GR in fat accumulation. However, the present study provides evidence for the impact of genetics in the development of the obesity, more precisely the MS, and in the recovery of patients following bariatric surgery. Our hope is that these findings may guide clinicians' interventions to slow the progress of obesity, and help obese patients improve their quality of life.

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APPENDIX

Section A – Ellis Hospital IRB Approval

1101 Nott Street Schenectady, New York 12308

Phone: (518) 243-4011
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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

Section B – Union College Human Subjects Review Committee Approval

Greetings,

The project/s listed below is/are granted conditional approval by the acting chair of the Union College Human Subjects Review Committee. Please make the following changes:

1. Please change third paragraph of informed consent to read "... your name with your DNA material, your height, or your weight"

2. Please state the DNA material will be destroyed after use (and ensure that this is indeed done)

3. Ensure that the instructor is not aware (and will not be made aware) of which students opted to participate and which did not. Ensure that students understand that the instructor will not know of students' participation or lack thereof.

If these changes are amenable, please send a note to me stating that you have made them (and, in the case of #3, how).

Please keep in mind:

1. Any changes to the proposal must be reviewed before implementation.

2. The approval for this study expires one year from the approval date presented below.

George Bizer

Acting Chair, Union College HSRC

15070 29-Sep-15 Gingras, Sebastien Cohen, B BIO Prevalence of Glucocorticoid Polymorphisms in an Undergraduate Population Conditional per 9/29 email

- -

George Y Bizer, PhD

Professor and Chair of Psychology

Union College, Schenectady, NY

- -

georgebizer.com 518-388-6228

- -

Section C - Participant Informed Consent Form

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) and clinical manifestations of obesity and related comorbidities such as elevated blood sugar, blood pressure, increased waist/hip circumference ratio, and altered serum lipid profiles.

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 this gene and obesity (and related diseases) 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:

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 518-243-

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Michael Pasquarella, PharmD, RPh

Chair, Institutional Review Board

Ellis Hospital

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1. I understand that I will not receive the results from the testing.
2. 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.
3. I have read the material provided and this consent form in full. My questions have been answered to my satisfaction.
4. I consent to provide a sample for genetic testing and to have relevant medical data provided anonymously to the researchers.
5. 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*

D – DNA Extraction Protocol

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 µL). 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.

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 µL 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.

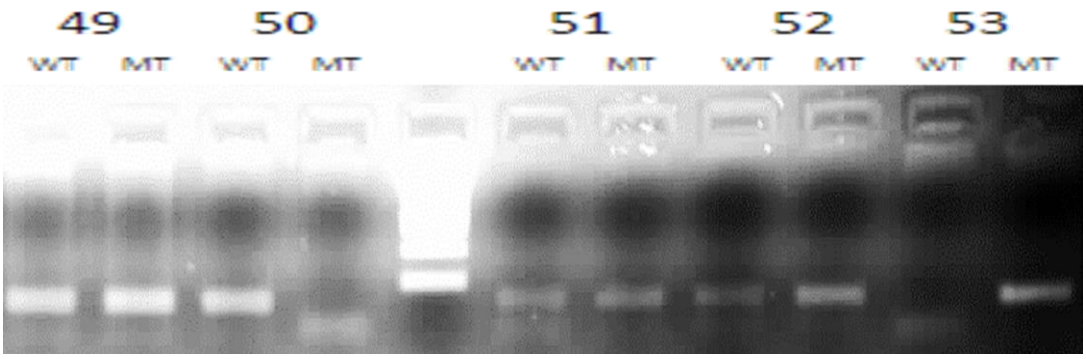
8) Incubate the microfuge tube containing the cells/Chelex mixture in the boiling water bath for 10 minutes, using a plastic "floatie". Other peoples' tubes will also be boiled at the same time in the water bath, so make sure that your tube is labelled well!

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.

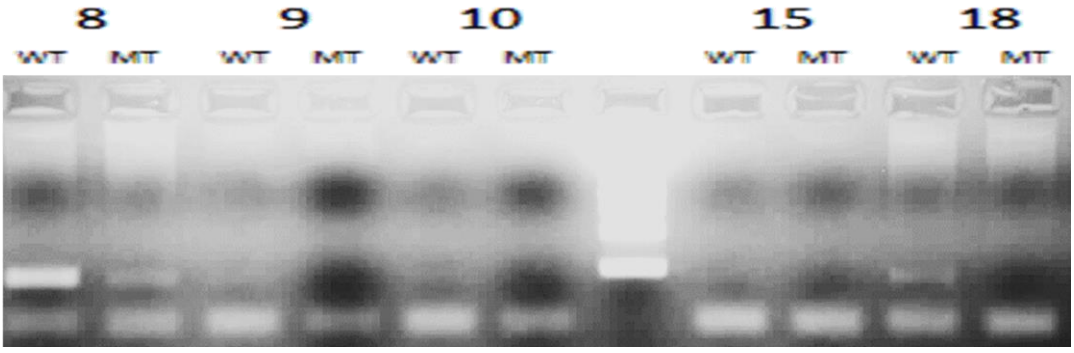
10) Using a 200 µL pipette, carefully transfer 200 µL of the supernatant (pipette from the top) to a new, labeled (with your initials) microfuge tube. Avoid transferring any of the pelleted cells or the Chelex. Discard the tube containing the pellet. YOUR PREP IS DONE AT THIS POINT! You should store your genomic DNA in the fridge in a labeled box or tube rack.

Section E – Examples of Agarose Gels for both the BclI and the N363S SNPs

BclI



N363S



Section F – Bariatric Patients' Metabolic Data and Genotypes

MRN	Original weight (lb)	Height (in)	BMI	Fasting Blood Glucose (mg/dl)	Serum Triglycerides (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)	Systolic BP	Diastolic BP	Surgery Date	Type of Surgery	BclI Genotype	N363S Genotype
31944	229	63	40.56	79	159	93	72	159	88	4/10/2008	band	hetero	
40803	385	73	50.79	127	179	115	32	126	60	8/20/2014	sleeve		
85377	227	63	40.21	88				130	70	5/13/2013	bypass		
89349	372	72	50.45	128	83	81	58	116	78	4/29/2015	bypass	wt	wt
128115	222	62	40.60	83	97	94	53	130	82	11/26/2013	sleeve	hetero	
262835	258	63	45.70					123	82	11/21/2011	band	wt	wt
318801	232	68.5	34.76	101	267	104	35	120	75	2/26/2008	band	wt	wt
324961	238	62	43.53	89	168	84	69	140	82	10/27/2010	band		
348386	281	65	46.76	83	72	106	52	112	80	Surgery Not Done		hetero	
356986	286	64	49.09	127	135	159	53	140	80	4/13/2015	gastric bypass	hetero	hetero
376477	268	64	46.00	87	69	106	73	116	72	7/23/2009	gastric bypass	hetero	wt
517936	287.2	68	43.66	123	152	74	29	110	80	4/23/2015	sleeve	hetero	wt
530840	229	61	43.26	89	164	152	43	140	80	6/11/2012	band		
585627	249	63	44.10	85	126	111	45	120	80	9/24/2014	bypass		
618643	241	65	40.10	90	67	102	75	132	94	8/13/2014	sleeve	hetero	
622361	297	61	56.11	243	371	79	41	134	82	5/24/2006	bypass	hetero	

634472	222	61.25		103	169	87	45	140	90	6/16/2015	sleeve	hetero	wt
660817	247.8	63.5	43.20	71	130	130	55	122	80	6/10/2015	sleeve		
685673	310	69	45.77	88	54	139	49	134	85	1/26/2009	band	hetero	wt
703105	236	63.5	41.15	132	309	120	40	100	70	4/15/2015	sleeve	mut	
704904	218	59	44.03	103	124	40	94	126	72	10/26/2009	bypass		
724485	306.7	63.5	53.47	78	126	120	57	124	74	11/16/2009	band	wt	wt
733809	237	62	43.34					122	78	1/8/2010	band	hetero	
1507325	221	65						106	74	4/14/2011	band	wt	
1508270	294	70	42.18	78	81	72	56	134	88	7/8/2011	bypass	hetero	wt
1513991	302.8	66	48.87	84	178	117	35	118	82	7/7/2011	band	hetero	wt
1516423	275	67	43.07	81	112	96	55	122	96	7/19/2011	gastric bypass		
1519044	256	67	40.09	99	96	77	54	112	80	5/4/2015	sleeve	wt	wt
1543716	202	60	39.45	93	158	93	61	110	72	4/23/2015	sleeve	mut	wt
1553936	341	63	60.40	142	52	82	95	162	80	11/16/2012	sleeve	wt	
1594114	306	69	45.18	88	215	125	40	126	80	4/20/2015	bypass	wt	wt
1594834	300	67	46.98	117	186	85	46	132	70	1/2/2014	bypass	wt	wt
1600340	456	74	58.54	80	169	100	44	150	88	3/10/2014	bypass	mut	
1623010	220	60.25		92	109	96	47	140	70	7/6/2015	bypass	hetero	wt

1624154	243	62	44.44	96	174	141	66	110	80	9/22/2014	sleeve	wt	
1624179	274	62	50.11	91	444	159	39	120	80	10/22/2014	sleeve	mut	wt
1630618	298	64	51.15	97	170	140	63	120	80	3/5/2015	bypass		
1631902	247	67	38.68	84	76	112	48	114	70	7/7/2015	bypass	hetero	
1635864	241	61	45.53	113	174	57	58	152	74	3/10/2015	bypass		
1640732	246	62.75	43.92	88	60	76	54	126	78	4/6/2015	gastric bypass	hetero	wt
1643405	310	64	53.21		124	91	67	136	76	4/20/2015	sleeve	wt	wt
1643886	282	62	51.57	82	119	116	36	112	80	5/29/2015	sleeve	hetero	
1644633	343	70	49.21	102	225	142	45	134	74	5/13/2015	sleeve	wt	wt
1644661	262	64	44.97	76	72	87	58	112	64	5/14/2015	gastric bypass	hetero	wt
1645170	287	63.75	49.65	84	74	96	42	130	80	5/4/2015	sleeve		
1648043	294	63	52.07	90	168	111	55	108	76	6/29/2015	bypass	mut	wt
1576626	369	67	57.79	85	95	129	35	126	74	7/1/2013	bypass		
1647433	370	64.5	62.52	106	94	114	54	120	80	6/17/2015	bypass	hetero	
658592	298	62	54.50	97	90	166	46	132	80	9/8/2014	sleeve		
300457	234	69	34.55	187	122	103	34	172	84	7/28/2014	bypass	wt	wt
1658769	388	71	54.11	120	188	119	36	110	70	10/14/2015	sleeve	hetero	wt
1556623	329	66	53.10	123	335	104	46	142	78	1/24/2013	bypass	wt	

1657589	184	58	38.45	97	207	90	44	100	80	12/9/2015	sleeve	mt	wt
1644767	292	70	41.89	108				136	92	10/6/2015	band	mt	
1531310	380	65.5	62.27	86	91	98	55	126	72	5/21/2013	bypass	mt	wt
1624819	257	60.5	49.36	86	533	232	38	116	58				
1543612	290	68	44.09	91	189	115	34	114	80	1/5/2015	bypass	hetero	
610595	318	72	43.12	73	174	96	39	150	90	11/19/2014	bypass	wt	
335315	218	61.5	40.52	64	188	114	60	148	82	3/18/2010	bypass	mt	
1651869	238	62.5	42.83	94	82	99	63	173	106	7/8/2015	sleeve	hetero	wt
1561130	248	60	48.43	102	97	113	56	130	90	6/1/2015	bypass	wt	wt
155283	267	64	45.83	182	105	95	38	132	90	7/12/2011	bypass	hetero	wt

Section G – Undergraduate Students' Metabolic Data and Genotypes

Student Number	Weight	Height	BclI Genotype	N363S Genotype
M1	170	65	wt	
M2	110	64	hetero	
M3	140	70	hetero	wt
M4	125	62	hetero	wt
M5	152	64	wt	
M6	145	66	hetero	wt
M7	106	60	hetero	
M8	100	64		
M9	135	71	hetero	wt
M10	189	70	wt	wt
M11	135	72	hetero	wt
M12	130	71	wt	wt
M13	190	68		
T1	182	70	wt	
T2	115	59	wt	
T3	150	63		
T5	160	70	wt	wt
T6	115	62	wt	
T7	141	66	hetero	wt
T8	145	71	hetero	
T9	162	73	wt	
T10	130	65	wt	wt
T11	110	66		
T15	210	73	wt	wt
W1	195	73	hetero	wt
W2	180	72	hetero	wt
W3	178	71	wt	wt
W4	104	62	wt	wt
W5	112	63		
W6	140	71	mut	wt
W7	128	64	mut	wt
W8	150	68	hetero	wt
W9			wt	wt
W10	180	72	hetero	wt
W11			hetero	wt
W13	190	72	wt	wt
W14	265	70.5		

Th1	152	55	hetero	wt
Th2	160	62	hetero	wt
Th3	160	67		wt
Th4	135	63	mut	wt
Th5	114	61	mut	
Th6	130	60		wt
Th7	185	72	hetero	
Th8	139	64	wt	wt
Th9	185	70		
Th10	117	59		wt
Th12a	130	66		wt
Th12b	140	65		wt
Th13	155	64		
Th14	165	73		wt

Section H – Prevalence of BclI and N363S SNPs

Prevalence of BclI and N363S Single-Nucleotide Polymorphisms (SNPs)

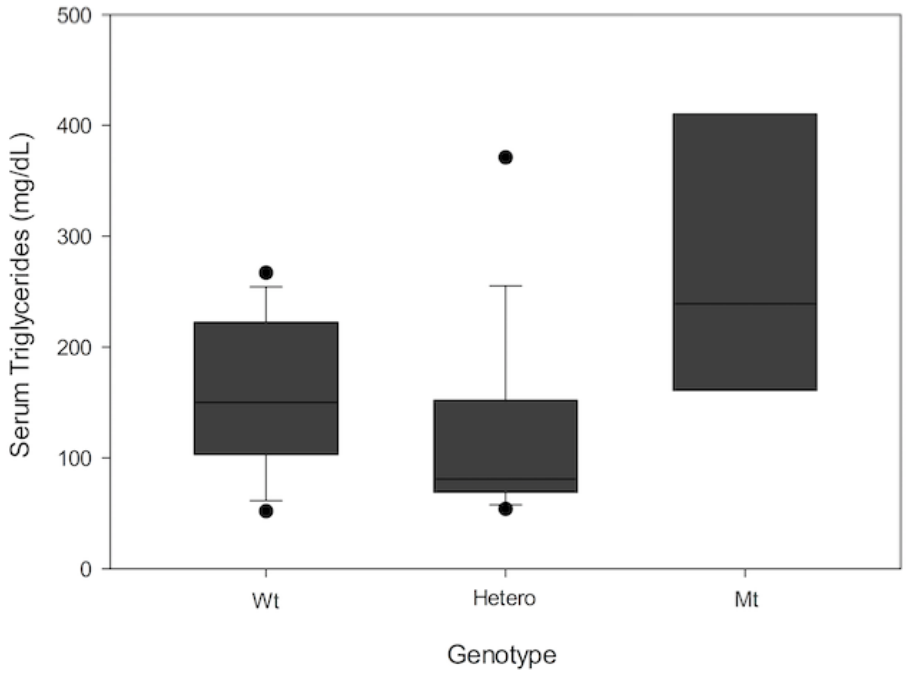
	BclI		**N363S**	
	Percentage	N	Percentage	N
Prevalence in normal weight population	33.78%	37	0%	32
Prevalence in obese population	42.71%	48	1.72%	29

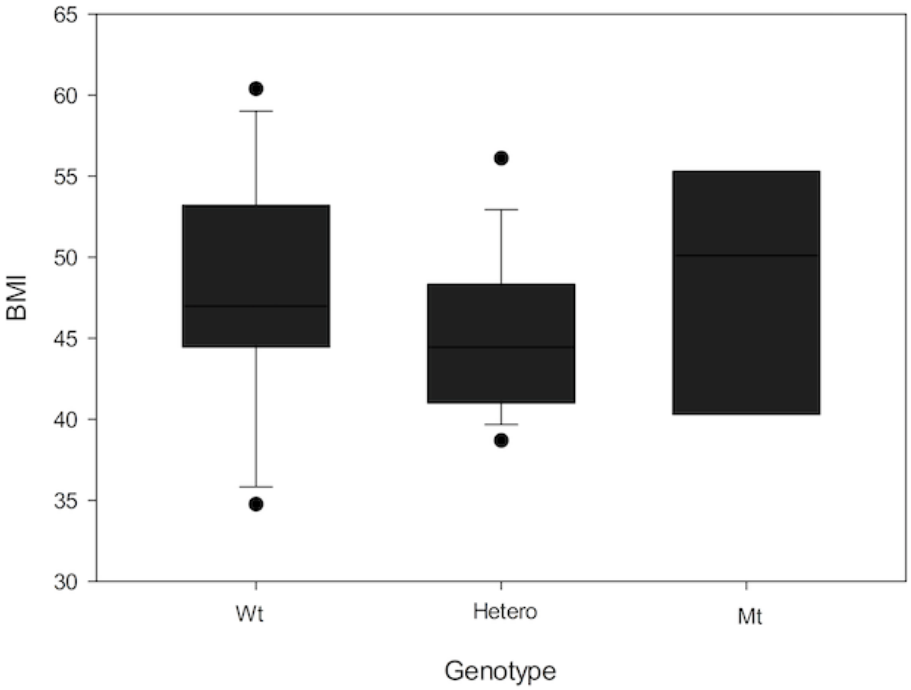
Section I – Results of one-way ANOVA

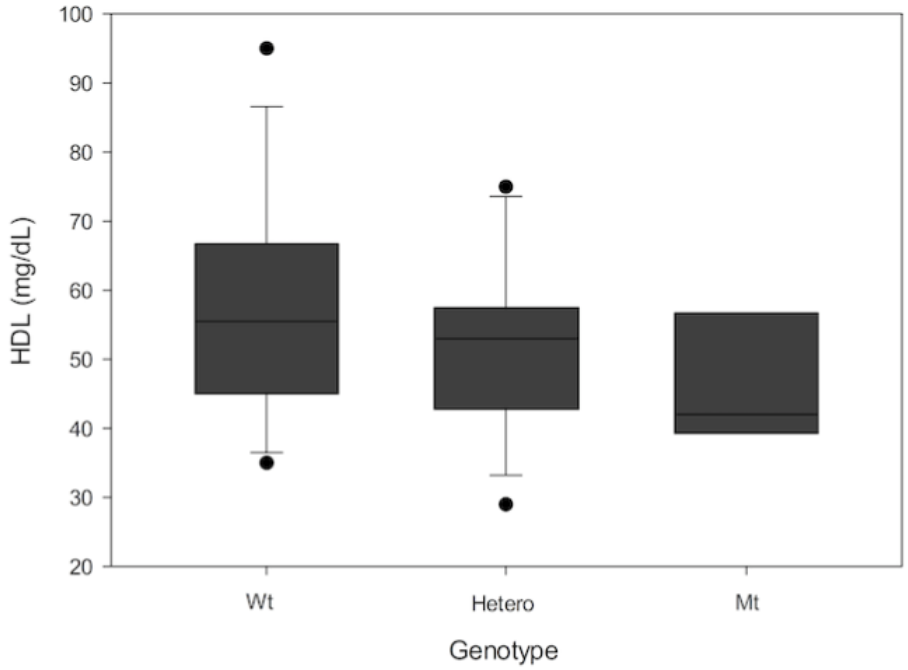
Results of one-way ANOVAs comparing homozygous mutants and heterozygotes

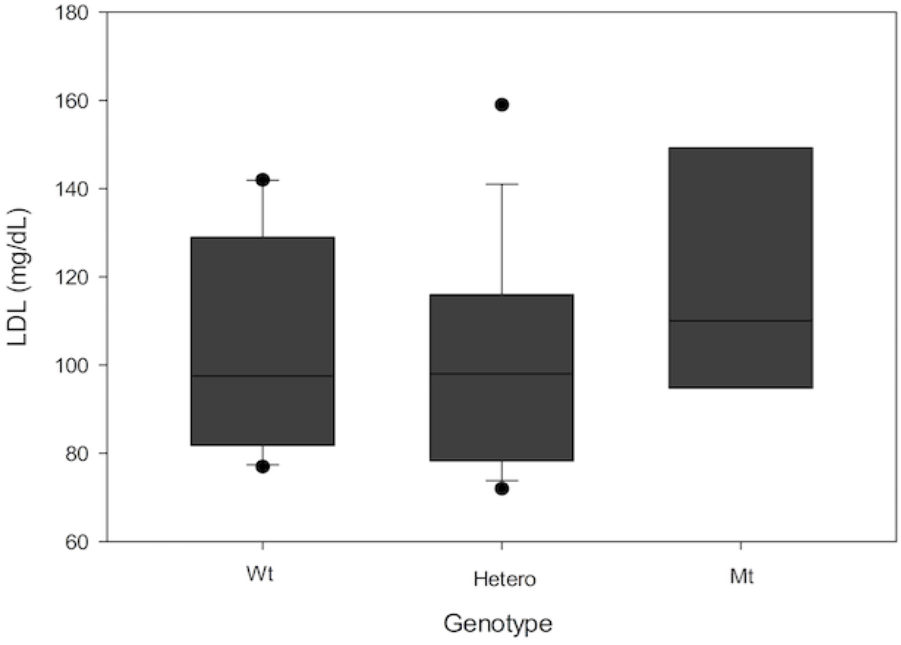
Metabolic Parameters	Significance
BMI	p = 0.575
Glucose	p = 0.989
Triglycerides	p = 0.016*
LDL	p = 0.510
HDL	p = 0.824
Systolic BP	p = 0.569
Diastolic BP	p = 0.623

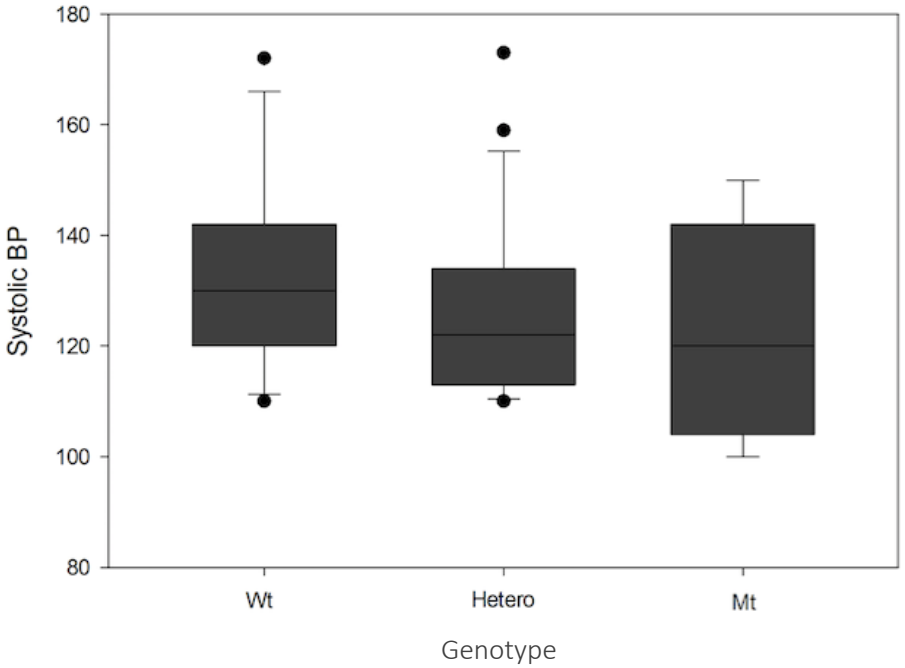
Section J – BclI Graphs

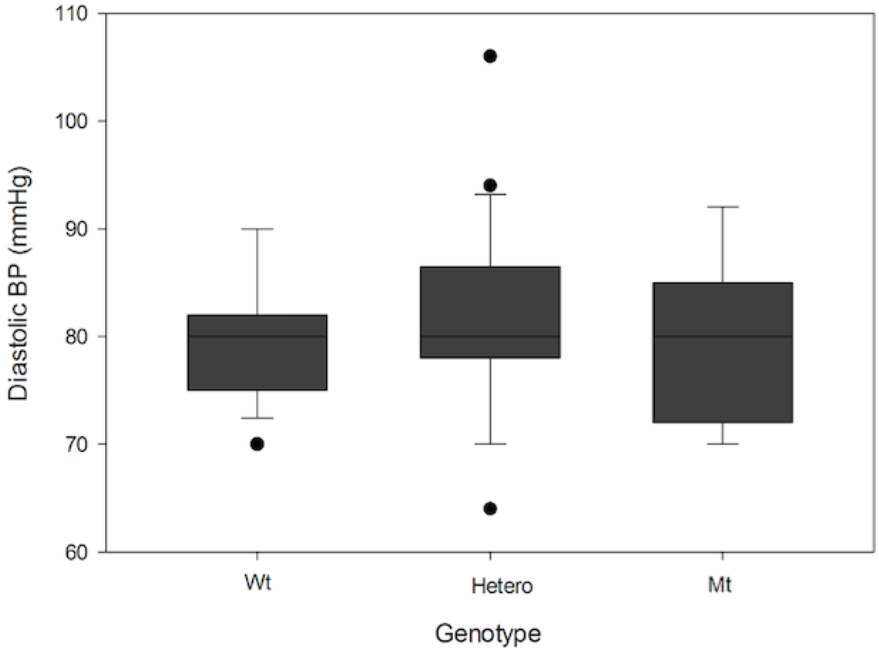


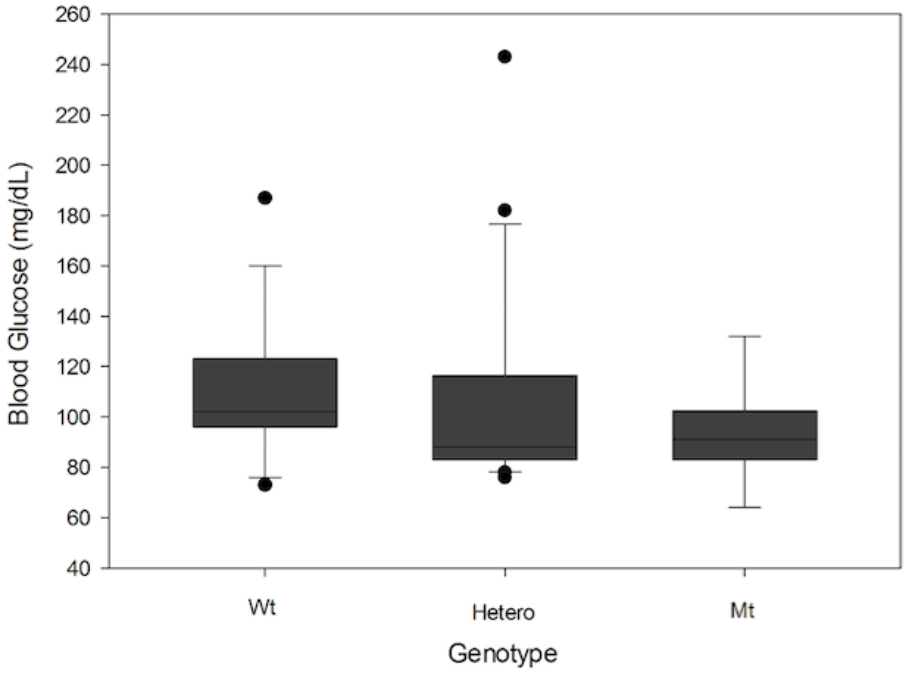



100
90
80
70
60
50
40
30
20
HDL (mg/dL)
Wt
Hetero
Mt
Genotype

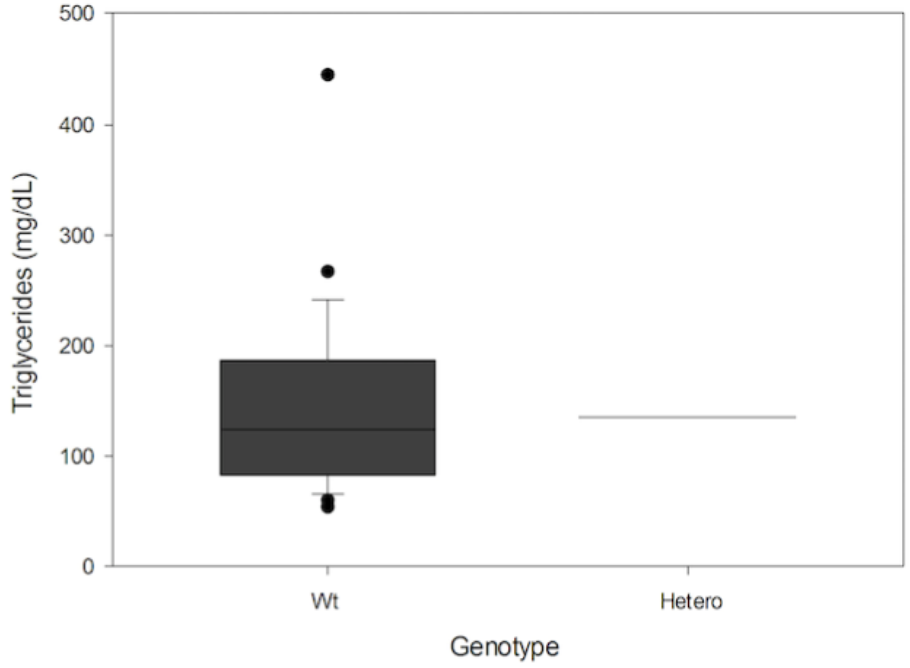

180
160
140
120
100
80
60
LDL (mg/dL)
Wt
Hetero
Mt
Genotype

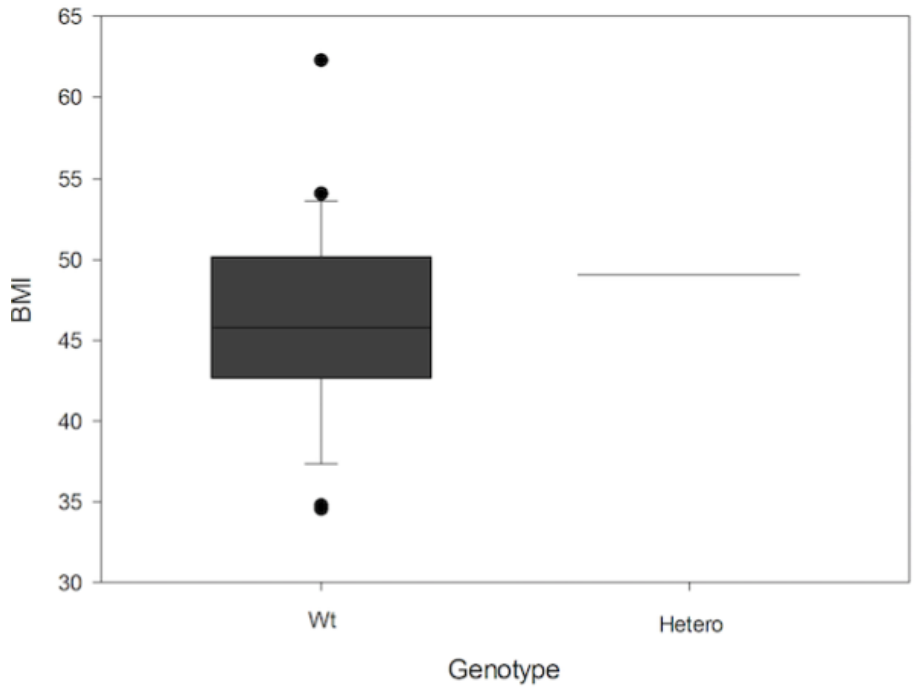

180
160
140
120
100
80
Systolic BP
Wt
Hetero
Mt
Genotype

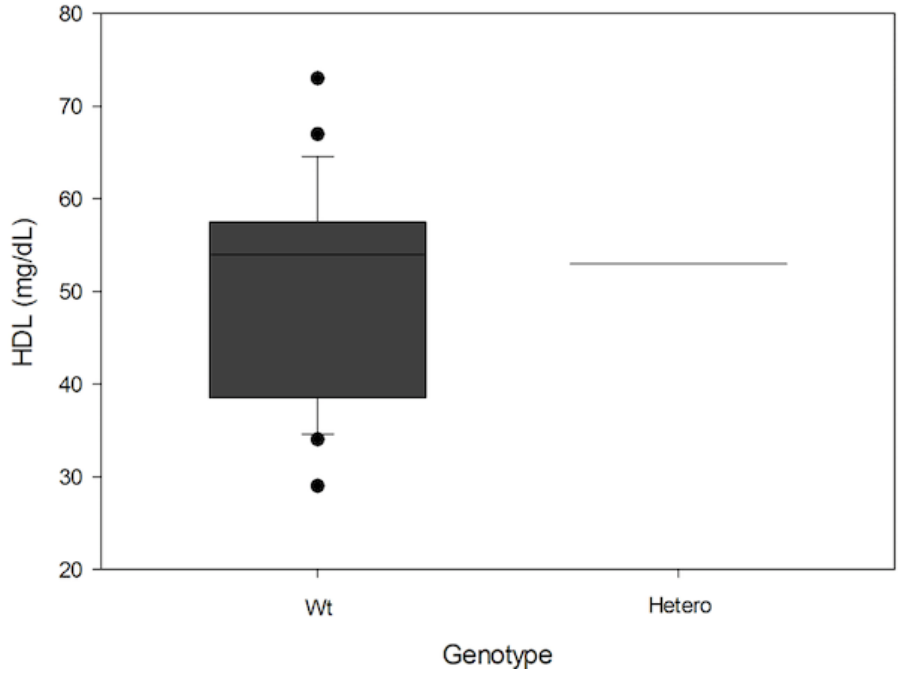

110
100
90
80
70
60
Diastolic BP (mmHg)
Wt
Hetero
Mt
Genotype

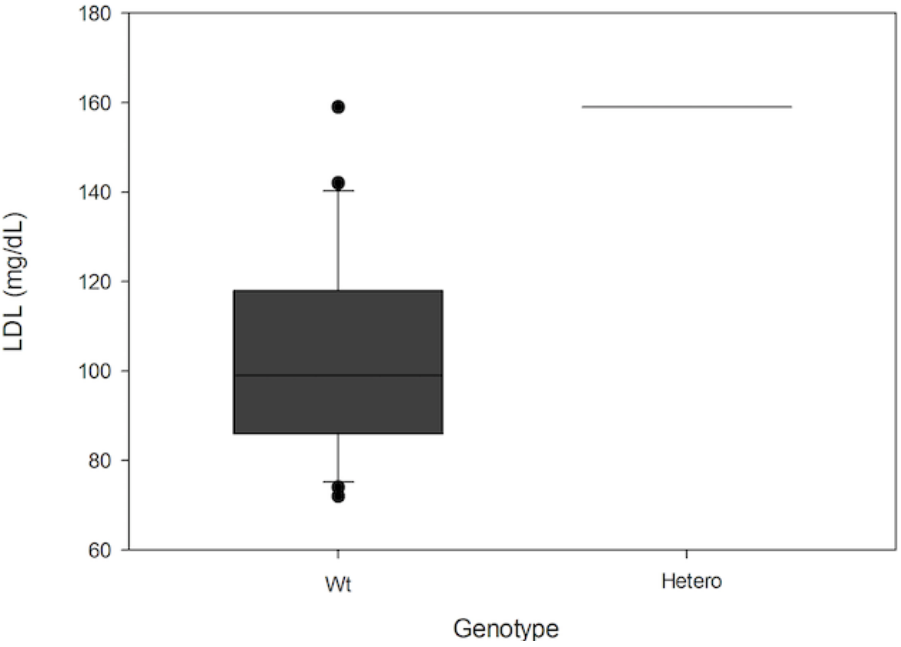

260
240
220
200
180
160
140
120
100
80
60
40
Blood Glucose (mg/dL)
Wt
Hetero
Mt
Genotype

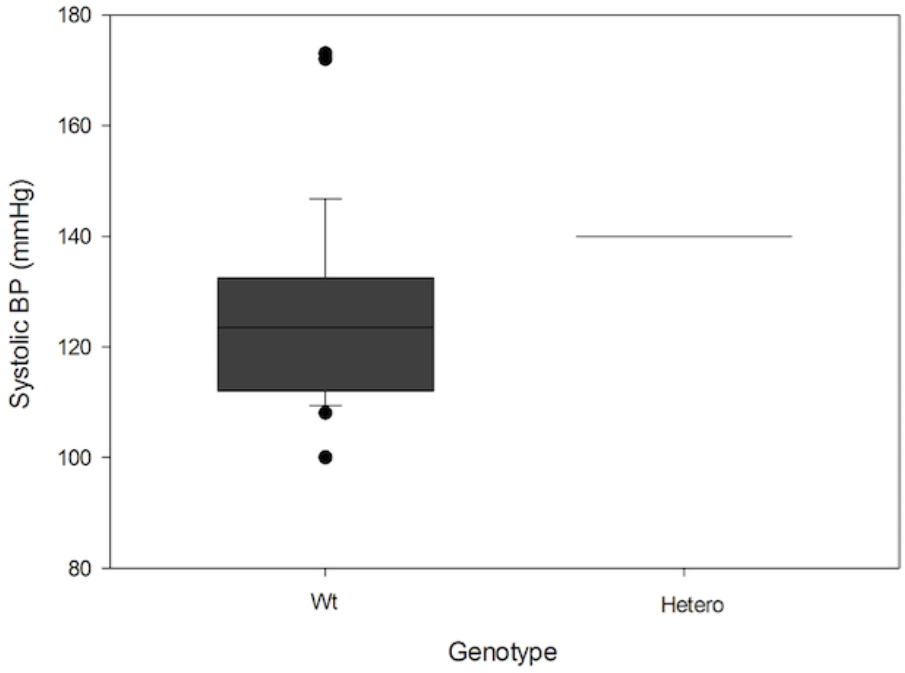
Section K– N363S Graphs

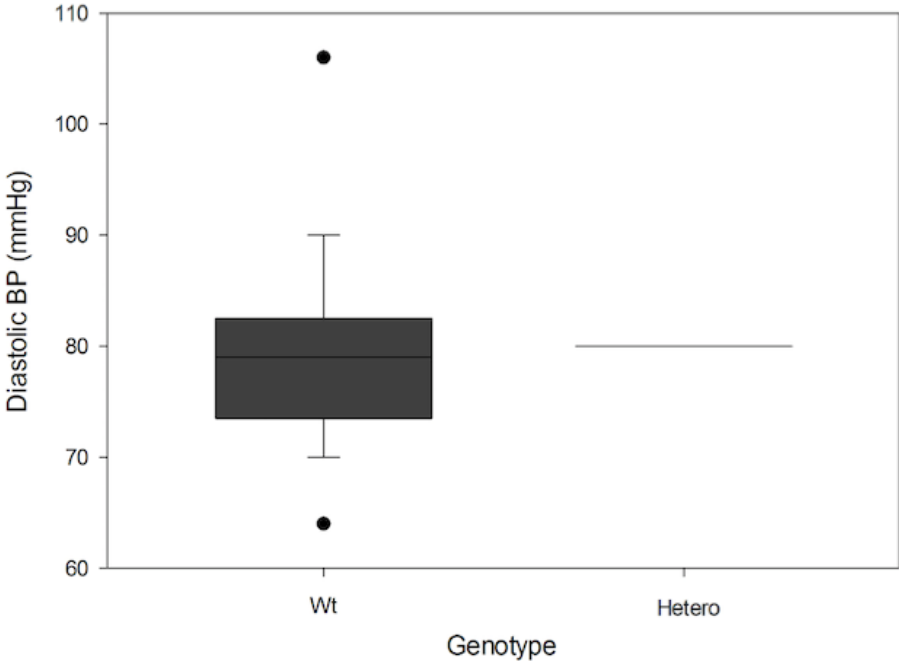


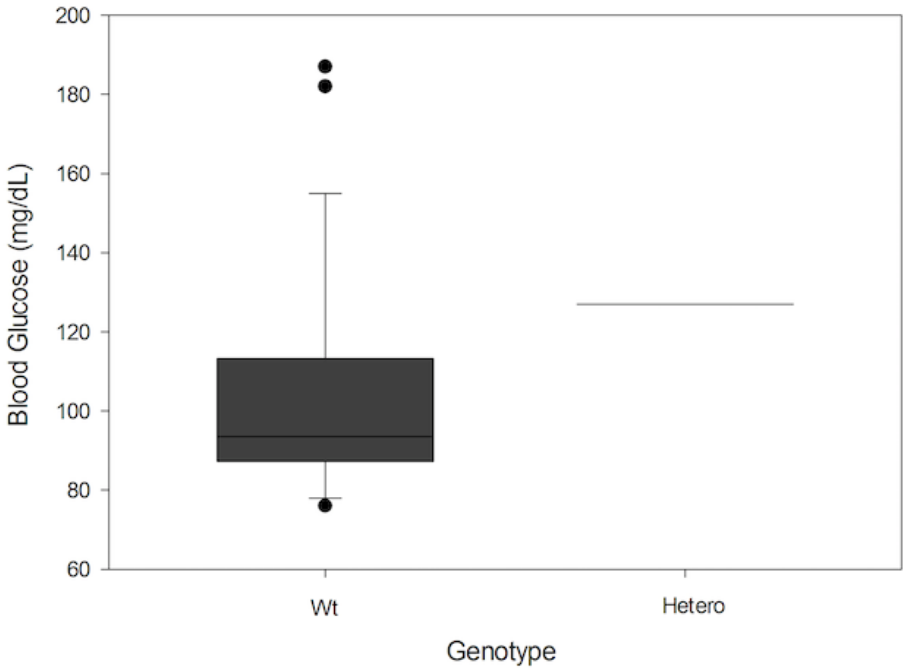



80
70
60
50
40
30
20
HDL (mg/dL)
Wt
Hetero
Genotype


180
160
140
120
100
80
60
LDL (mg/dL)
Wt
Hetero
Genotype


180
160
140
120
100
80
Systolic BP (mmHg)
Wt
Hetero
Genotype


110
100
90
80
70
60
Diastolic BP (mmHg)
Wt
Hetero
Genotype


200
180
160
140
120
100
80
60
Blood Glucose (mg/dL)
Wt
Hetero
Genotype

Section L – Association of SNPs and Double Heterozygosity with Metabolic Parameters

Association of SNPs and Double Heterozygosity with Metabolic Parameters (* p<0.05 compared to WT)

Alleles	**BclI**			**BclI/N363S**
Genotype	**WT**	**Hetero**	**MT**	**Hetero/Hetero**
Metabolic Parameters	Average (+/-se)	Average (+/-se)	Average (+/-se)	
BMI	47.63 (2.09)	45.14 (1.15)	48.26 (3.55)	49.08
Glucose	105.67 (6.66)	99.67 (10.95)	97.20 (9.00)	127
Triglycerides	154.80 (21.97)	117.47 (20.82)	249.60 (56.06)*	135
LDL	104.80 (8.01)	102.13 (6.34)	116.60 (11.56)	159
HDL	56.30 (5.44)	52.27 (3.55)	47.80 (4.35)	53
Systolic BP	126.82 (4.36)	125.31 (3.35)	117.60 (8.70)	140
Diastolic BP	77.18 (1.10)	80.19 (1.81)	77.20 (3.20)	80

Section M – Percent excess weight loss following bariatric surgery by surgery type and genotype

Percent excess weight loss following bariatric surgery by surgery type and genotype

Alleles	Genotype	Gastric band	Roux-en-Y Gastric Bypass	Vertical Sleeve Gastrectomy
BclI	WT	61% (16%)	73% (12%)	56% (8%)
	Hetero	34% (13%)	61% (8%)	65% (9%)
	MT	27%	61% (14%)	50% (10%)
BclI/ N363S	Hetero/ Hetero		31%	