# Assessing Glucocorticoid Receptor Polymorphisms in Obese Populations

By

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ABSTRACT

DOWDYE, AYANAH Glucocorticoid polymorphisms assessed in overweight populations as compared to normal populations.
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The glucocorticoid receptor (GR) is part of a family of nuclear receptors that control gene expression. In the presence of the steroid hormone cortisol, certain genes are expressed; the products of which control certain features of the body, including but not limited to, blood pressure, serum triglycerides, and blood sugar. There is evidence that these features are major contributors to obesity. Specific polymorphisms of the GR and other regulators of either GR or the closely related mineralocorticoid receptor such as heat shock protein 90 and 11ß-hydroxysteroid dehydrogenase type 1 have been found in our labs and others to be present at a higher frequency in obese populations. Our current research is investigating polymorphisms in these genes as well as the gene encoding the FK506 binding protein (FKBP) which works in conjunction with HSP90 to negatively regulate GR activity. This finding in combination with previous results will lead to a better understanding of the genetic underpinnings of obesity and may reveal novel therapeutic approaches.

In this research, participants from Ellis Bariatrics Hospital allowed us to collect DNA from their cheek cells, that were then isolated t run through PCRs to analyze whether or not mutations were present in these subjects. With the polymorphisms tested, there was an increase in mutant allele frequency in our bariatric sample for FKBP51, decreased frequency for BclI, and no mutations present for TthIII.

***Keywords:*** *bariatrics, cortisol, Cushing's Syndrome, DNA isolation, gel electrophoresis, glucocorticoid receptor (GR), hypothalamic pituitary adrenal (HPA) axis, Metabolic Syndrome (MetS), obesity, polymerase chain reaction (PCR), single nucleotide polymorphism (SNP)*

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**Introduction:**

The obesity epidemic has been around for many years, and continues to be an issue even in today's society. Obesity is defined as a given point where a specific weight for a defined height has been exceeded. You present with having excessive fat around that body that is not what you would see among the average population. In 2015, a worldwide estimate of 107.7 million children and 603.7 million adults have been reported to be obese (1). Since 1980, obesity rates have nearly doubled in approximately 70 countries, and continues to increase in many countries as well. It has been reported that a high body mass index (BMI) was reported as a contributing factor in 4 million deaths around the globe in 2015, and 60% of those individuals were reported to be obese (1).

According to more recent studies, the US has the highest average BMI among all high-income countries (2). Despite this, and the ongoing efforts to educate the general public, as of 2011, 34% of the adult US population, and nearly 20% of child/adolescent populations are obese (3). This is an important issue because obesity increases the risk of many diseases in obese individuals, such as cardiovascular diseases, diabetes, and cancers among others. This is an urgent public health issue, and due to the complexity of obesity, this has the potential to be one of the most complicated health issues we have had to face.

Obesity also impacts the US economy due to its extreme impact on the healthcare system. The national estimated medical cost in 2008 for obesity related treatment efforts were approximately $147 billion, but can range from $147 billion to $210 billion annually (4). Direct medical costs include, but are not limited to, preventative, diagnostic and treatment services related to obesity, while indirect economic costs revolve around lack of

productivity in the workforce due to being absent from work for obesity related health issues, decreased productivity of obese employees while at work, or premature mortality (4).

Some individuals suffering from obesity decide to undergo bariatric surgery. In essence, this treatment physically decreases the size of their stomach, thus decreasing the amount of food that makes the individual feel full, decreasing their caloric intake and ultimately allowing for weight loss. There are three different surgical methods that we will be analyzing in this research project. The LAP Band is the least invasive of the three surgical methods. It requires the surgeon to place an inflatable ring around the patient's stomach to reduce the size of the stomach, to make you feel fuller with less amount of food (11). The surgeon can adjust the size of the band as necessary, and can be reversed if wanted (11). Next is the vertical sleeve gastrectomy, where the surgeon removes a large portion of the patient's stomach, which will leave a banana shaped portion enclosed with staples (11). This method allows one to feel fuller sooner, but unlike the LAP band, cannot be reversed (11). The third method is the roux-en-y gastric bypass. It allows one to feel full sooner while eating less food. The surgeon staples the stomach in order to create a small pouch in the upper part of your stomach. Then, the small intestine gets cut and attaches to the small pouch directly, in order to bypass most of the stomach (11). This will also change the gut bacteria to influence appetite and metabolism

As previously mentioned, obese individuals are more susceptible to certain diseases and illnesses. Metabolic syndrome (MetS) is a specific type of medical problem that obese people typically face. MetS is not defined as a disease, but rather a cluster of symptoms or risk factors. The 5 factors that are part of the MetS constellation are high blood pressure, high blood sugar, low HDL cholesterol levels, high triglyceride levels, and increased

centripetal (or abdominal) fat (5). Having one of these risk factors does not mean you have MetS, but rather having at least 3 of the 5 risk factors may imply that you do indeed have MetS.

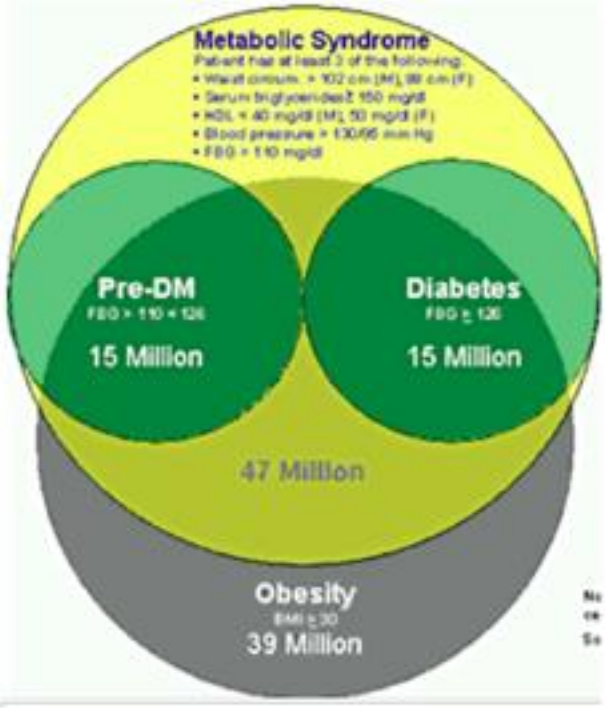


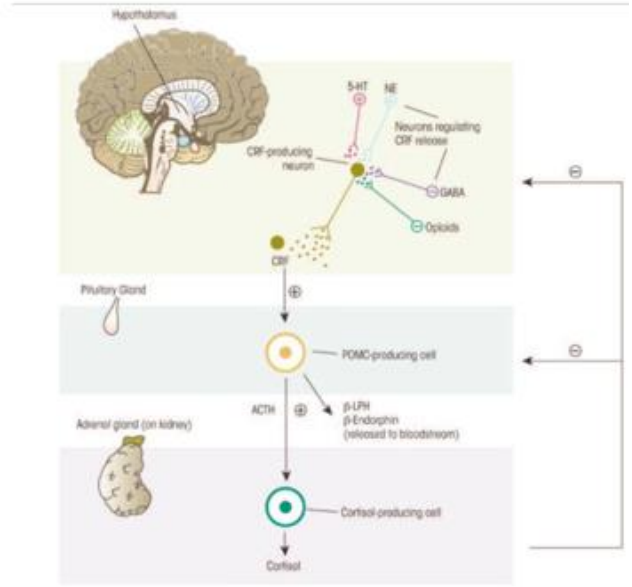

**Figure 1.** Distribution of cases of obesity related diseases. This demonstrates the overlap between MetS and obesity.

However, these risk factors are largely defined by lifestyle. Surplus food intake, sedentary life habits, and overall unhealthy lifestyles greatly contribute to MetS (5). While it most commonly occurs in obese individuals, one does not have to be obese to suffer from MetS.

MetS can be found in some Cushing's syndrome patients. Symptoms of cushing's syndrome include, but are not limited to, weight gain around the stomach (but not much around the arms and legs), high blood pressure, "buffalo hump", muscle weakness, purple striae, irritability, red face, poor concentration and more (6). Cushing's syndrome is a state

of having too much cortisol in the bloodstream, for long periods of time, otherwise known as hypercortisolemia. This most commonly is due to an over activation of the hypothalamic-pituitary-adrenal axis, or HPA axis (7).

The HPA axis is an important hormonal response system that allows your body to respond during periods of stress (9). The function of this hormonal response system is to




allow the body to respond rather quickly to a situation that is interpreted as stressful (9). The HPA axis is outlined in figure 2.

**Figure 2.** The Hypothalamic Pituitary Adrenal axis. This axis allows for the regulation of cortisol within the body. Stress is perceived, CRF is released, POMC is produced, gets cleaved into the hormone ACTH, and cortisol is eventually released

A stress stimulus is perceived by the brain, which then activates the hypothalamus. The hypothalamus then releases a hormone called corticotropin-releasing factor (CRF). CRF then finds its way to the anterior pituitary gland, where it stimulates the production of a protein called proopiomelanocortin (POMC), which is then cleaved to produce a hormone called adrenocorticotropic hormone (ACTH). ACTH then stimulates the adrenal gland, and

from the adrenal gland, you get the release of cortisol, a key contributor as a response hormone to stressful situations (9).

Cortisol is a steroid hormone produced by the adrenal glands (8). The purpose of cortisol is not only to help the body respond to stress, but also maintain blood pressure, maintain the immune system, and convert fat, carbs, and proteins into energy (8). Cortisol is a specific hormone under the umbrella term of glucocorticoids. As previously mentioned, hypercortisolemia is the underlying cause of Cushing's syndrome. MetS can be commonly found in most patients that suffer from Cushing's syndrome. However, we have found most peculiar at this time is that with Cushing's syndrome, you have hypercortisolemia, whereas with MetS, cortisol levels are normal. But sometimes, even though cortisol is normal, patients may still present as if they did indeed have hypercortisolemia. This requires one to know how hormones work.

Hormones are analogous to a key and a lock. Every hormone has its receptor. For example, cortisol's receptor is called the glucocorticoid receptor (GR). Once a hormone binds to its receptor, it triggers a series of events that causes the body to respond to that hormone. With that being said, we mentioned that with MetS patients, there isn't a presence of excess cortisol present, but sometimes, the body will respond as if there was a lot of that hormone. Which leads us to ask, how exactly is that phenomena happening?

Researchers have found that there may be a link between stress and obesity. More specifically, stress has been linked to bodily biological changes, that lead individuals to have cravings that can potentially lead to obesity.

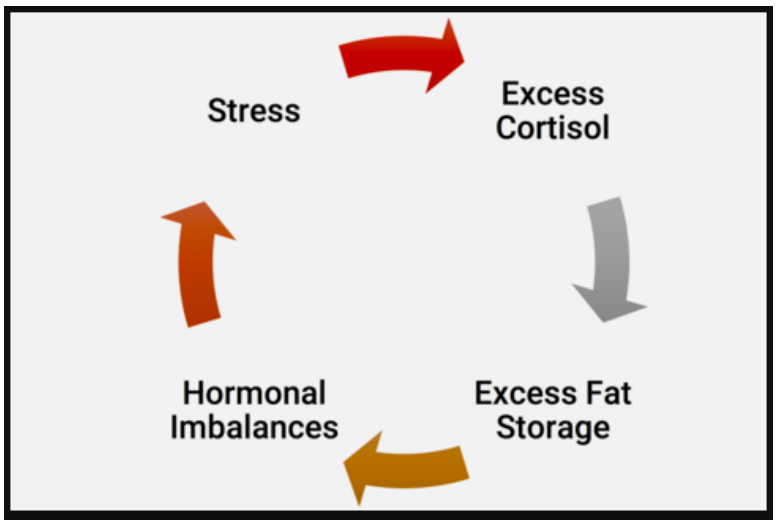



**Figure 3.** The cyclical nature of stress as it relates to cortisol. High stress levels cause the release of extra cortisol, which leads to excess fat storage, which can contribute to obesity.

This phenomenon has been noticed on numerous occasions in daily life. Refer to figure 3 above. People tend to have cravings for particular "comfort foods" that are typically either high in fat or high in sugar content. (10). This is in part due to cortisol, with its ability to not only manage fat storage and energy, but also known to increase appetite, and possibly increase cravings for sugar or fat (10).

We know that people that have an excess level of cortisol in the body can lead to a certain kind of obesity that we have already mentioned before, Cushing's Syndrome, which is accompanied by centripetal obesity. However, it is also noted that centripetal obesity can also be found in patients suffering with MetS. This lead the researchers in our lab to propose that MetS could possibly just be a subclinical Cushing's Syndrome. While individuals with

MetS may not have the hypercortisolemia, they somehow still have many of the same features as a Cushing's patient. Thus, these individuals are suffering with symptoms of hypercortisolemia without the excess cortisol. Rather, they have very normal cortisol levels. How can someone experience symptoms of hypercortisolemia without excess cortisol? Since we know that hormones work directly with a receptor, we proposed that something mechanistically can be malfunctioning with the receptor. It is possible that the receptor is hypersensitive to cortisol, and over expresses its function with even normal levels of cortisol. Because of this, we wanted to investigate whether or not people who have a tendency toward MetS have polymorphisms in their glucocorticoid receptors (GRs) that could make them more susceptible to obesity with their hypersensitive GRs to cortisol. This would allow the expression of the hypercortisol phenotype, or conversely, if these individuals have a lack of resistance polymorphisms, that can allow for the hypercortisol phenotype. In addition to this, our researchers are also interested in the idea that individuals that undergo bariatric surgery to cure their obesity sometimes end up regaining the weight right back. This lead us to hypothesize whether or not there could be a risk of weight regain associated with different GR polymorphisms.

With the direction medicine is going in, the future of medicine is personalized genomic medicine. To be able to help treat a patient based off of their own genetic makeup is the best and most effective way to treat someone. Thus, if we know that a patient has a genetic makeup that makes them susceptible to weight regain, we can try different methods of surgery depending on what we know would be best for that particular genetic makeup. This allows physicians and other healthcare professionals to make more informed choices about medical care if provided with some additional information. Within the context of this

research, we mentioned four different methods of bariatric surgery: the roux-en-Y gastric bypass, the laparoscopic adjustable gastric band (lac band), the sleeve gastrectomy, and the duodenal switch. If someone was successful with a lac band, and then removed the band, only to regain the weight, this can lead the person to feel depressed, and keep gaining weight, until a new method of surgery is required. If we knew that a person's genetic sequence makes them more susceptible to regaining weight if they were using lac band, then we would be able to immediately move on to a more invasive surgical method from the beginning, in order to skip the weight loss/ weight regain phases that the individual may come across. To be able to successfully do this would save individuals a lot of time, money, and mental exhaustion. Overall, this research has real medicinal implications that can be quite beneficial to the future of medicine.

**Experimental Approach:**

There is a total of **7** different SNPs that we wanted to look into in this research project. If these types of mutations are prevalent in more obese populations, this could help support the idea that this could play a role in the development of obesity. We hypothesized that polymorphisms at the cortisol receptors can lead to this oversensitivity of these receptors, or the resistance at these receptors of cortisol, which may contribute to obesity. The 7 different SNPs that we have identified, by colloquial polymorphism names, are BclI, N363S, TthIII1, RS120, RS84, RS207s, and MR55s. The chart below provides information regarding the mutated gene, the phenotypic expression of the mutated receptor, the location of the mutation, among other information.

| Colloquial name | Gene | Official name | Colloquial Polymorphism | Phenotype | Location | Predicted Annealing temp (˚C) | Tested Annealing Temperature(˚C) | Optimal | Q sol'n? |
|---|---|---|---|---|---|---|---|---|---|
| Gluccorticoid Receptor | NR3C1 | rs41423247 | Bcl I | Hypersensitivity | 41503 | 55.29 | 57 | 57 | N |
| Gluccorticoid Receptor | NR3C1 | rs56149945 | N363S | Hypersensitivity | 40761 | 54.9 | 57 | 57 | N |
| Gluccorticoid Receptor | NR3C1 | rs10052957 | TthIII1 | Resistance | 33377 | 58.85 | 61 | 61 | N |
| 11B-HSD | HSD11-B1 | RS12086634 | RS120 | Hypersensitivity | 25710 | 56.96 | 57 | 57 | N |
| 11B-HSD | HSD11-B2 | RS846910 | RS84 | Resistance | 20705 | 58.03 | 61-52 | 61 | Y |
| Mineralcorticoid Receptor | NRC2 | RS2070951 | RS207s | Hypersensitivity | 10659 | 56.26 | 61-52 | 61 | N |
| Mineralcorticoid Receptor | NRC3 | RS5522 | MR55s | Resistance | 11198 | 52.63 | 54-46 | 52 | Y |

We ran the samples to see how many of these patients have the mutation for each of the 7 polymorphisms we are testing. As previously mentioned, the DNA samples came from either patients that have already undergone bariatric surgery or are thinking of undergoing bariatric surgery. From there, we conducted analyses to see if there were any correlations between these obese patients and the propensity toward mutated glucocorticoid receptors. Due to time constraints however, we only had the time to test out 3 of our polymorphisms, which is discussed further in our results section.

## Materials and Methods:

*DNA Extraction*

The patient population we have pulled our DNA samples from come from Ellis Bariatric Hospital in Niskayuna, NY. We introduced ourselves as research students at Union College, followed by an overview of our research, and details on how to participate in our study if they so choose. These individuals were people that have either have already undergone or are seriously considering undergoing bariatric surgery.

We started the process of obtaining our data by extracting DNA from our patient sample population. We started by swabbing the inside of the patient's mouths for 15 – 30 seconds. Then we cut the swab handle, and put the swab side inside of a 1.5mL microcentrifuge tube. Since we typically did not perform the DNA extraction the same day, swabs were stored at 4 °C.

When we were ready to perform the DNA extraction, we added 600uL of 50mM NaOH. Then we closed the microcentrifuge tubes containing the brush and vortexed the mix for 10 minutes. After that, the tubes were then heated at 95°C for an additional 10 minutes. Next, we added 120uL of 1M Tris at a pH of 8.0 to the tube, and we removed and discarded the swab at this point. This was the extracted DNA samples that were used to continue with our research. We used approximately 2-3uL of DNA to run our reactions.

***Polymerase Chain Reaction***

Now that we had our extracted DNA samples, we could perform our Polymerase Chain Reactions (PCRs). For each DNA sample, we needed to have two PCR tubes, one for our wildtype (WT) primers and one for our mutant (MU) primers. So if we were running 8 DNA samples, we needed 16 PCR tubes. We added 2uL of DNA to each tube. We then created a

large mix in a microcentrifuge tube containing water, dNTPs, our common primer, 10X buffer, 5X Q solution (if needed, otherwise replaced with just water), and Taq polymerase depending on how many reactions we wanted to run. Then the mix was split into two. We then added our WT primer to one mix and the MU primer to the other mix. Then, we had put 13uL of each mix into their respective tubes (the WT mix would go into the PCR tube labeled WT, and likewise for the MU), so that the total amount in the tube was 15uL. The amount of each element in each tube was as follows:

**10X Buffer:** 1.5uL
**5X Qsol:** 3uL
**dNTPs:** 0.3uL
**Taq Polymerase:** 0.075uL
**Water:** 5.125uL
**Common Primer:** 1.5uL
**WT or MU Primer:** 1.5uL
**DNA:** 2uL

**Total:** 15uL

The purpose of PCRs is to make billions of copies of a segment of DNA in a matter of hours. It works in a series of three step processes: denaturation, annealing and extension. For denaturation, the temperature is high enough to unwind the DNA. For the annealing process, the temperature is lowered so that the primers (complementary to a short part of DNA that we wanted to amplify) can attach to the sequence that we were targeting. Then extension step, otherwise known as the elongation step, which is run at a higher temperature than that of the annealing step but lower than denaturation, takes the Taq polymerase (which moves along the strand of the DNA) and adds nucleotides to the annealed primer. This process repeats numerous times over a period of 3 hours to make billions of copies of the segment we targeted.

***Running Our Agarose Gels***

Once our PCRs were done, we added 3uL of DNA loading dye to each sample, so that we could visually see the bands. At this point, they were ready to be loaded into our agarose gels. We ran our products on 2% agarose gels, which were made in the lab. From there, we loaded the DNA ladder into the first lane, and the middle lane. Then the DNA samples were loaded side by side, by putting the WT products in the left lane, and the MU products in the adjacent right lane. We did this across the entire gel, or until all of our samples were all loaded. Then, by a process called gel electrophoresis, we ran our samples until they had separated far enough down the lane to be able to be differentiated. Gel electrophoresis takes advantage of the fact that DNA is negative, due to the backbone of the DNA, and thus the DNA 'runs' toward the positive electrode to separate. After the bands had been separated, they could be visualized in the gel visualizer for our DNA analysis.

**Results:**

While we had planned to run 7 different polymorphisms, we only had the opportunity to run through three: FKBP51, BclI, and TthIII1. In each of the gels starting at the left, patient 1 is represented in the first 2 lanes, patient 2 in the next 2 lanes, etc. The ladders are indicated with the arrows in each of the figures. For each patient, and for each pair of lanes, WT was loaded in the leftmost lane, while MU was loaded in the rightmost lane. If there is only a band in the left lane and not the right lane, then the genotype is homozygous WT. If there is only a band in the right lane, then the genotype is homozygous MU. If the band is present in both lanes, then the genotype is heterozygous. These gels provide the raw data

that needed to be analyzed. While I do not include all of our gels, representative gels are seen below.

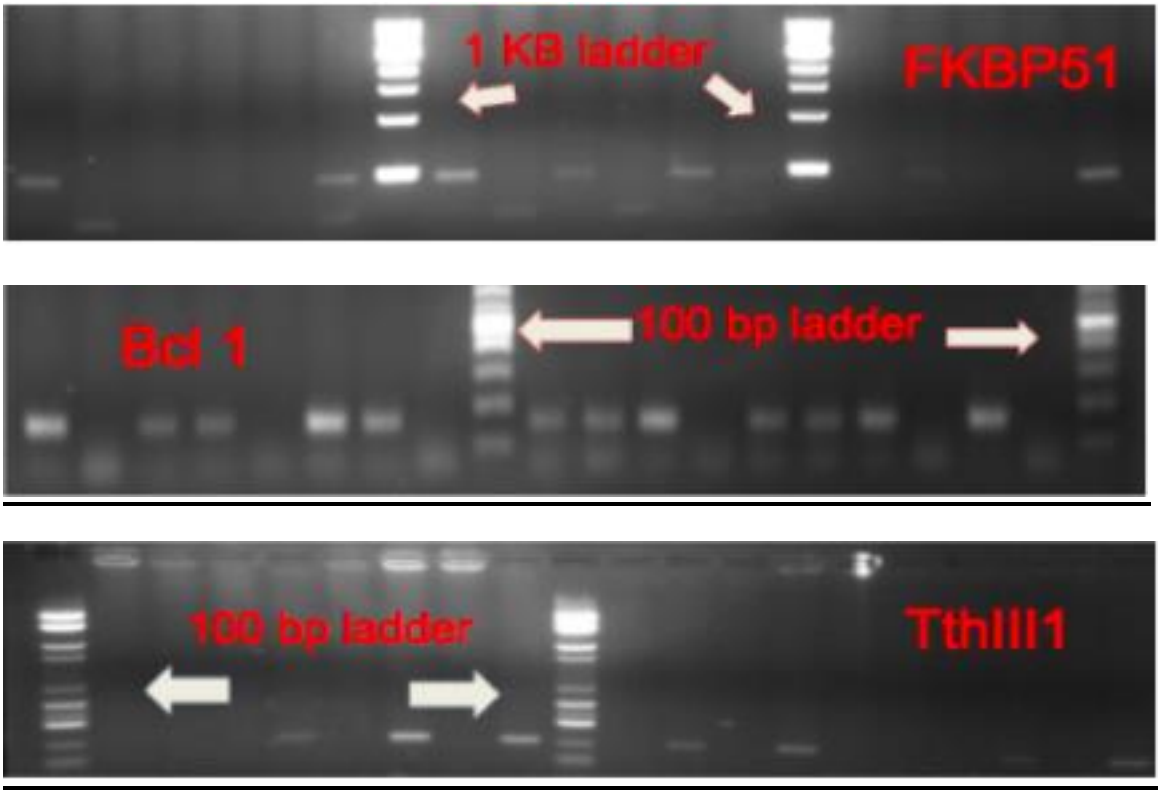



**Figure 4.** The patient DNA samples were run on 2% agarose gels. The top figure is to see detect any FKBP51 polymorphisms, the middle gel is to detect any BclI polymorphisms, the bottom gel is to detect any TthIII1 polymorphisms.

From the agarose gels, we had extracted the data and recorded the allele frequencies for each of the 3 polymorphisms. The blue section represents the frequency of the normal WT allele being expressed in the dataset. The orange section represents the frequency of the abnormal MU allele being expressed in the dataset. The normal population data is found in

the left column, which were obtained from statistics from the National Institute of Health. The overweight population data is found in the right column, which were obtained from willing volunteers at Ellis Bariatric Hospital. To get this data, we summed up how many times the WT allele or the MU allele appeared in each polymorphism, and calculated proportions from there.

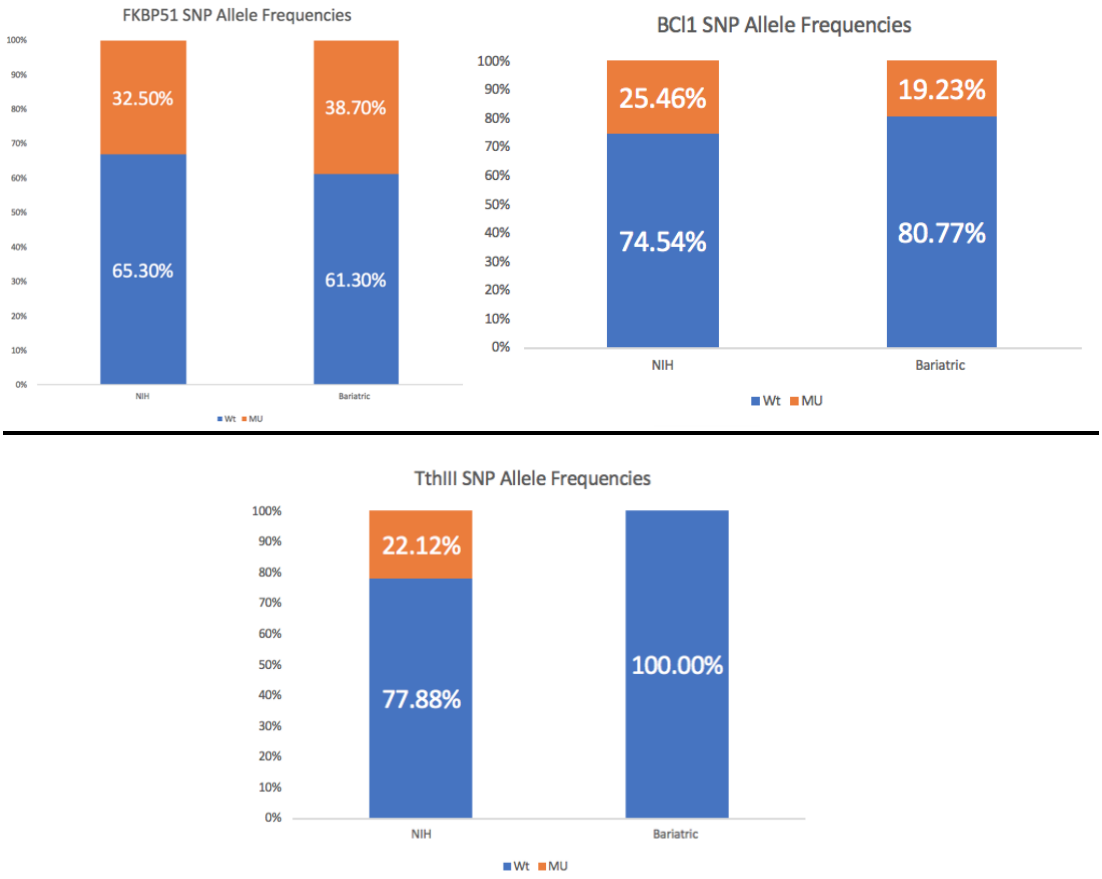



**Figure 5.** Allele SNP frequencies as compared between normal populations and our bariatric patient sample. The normal populations were extracted from the National Institute of Health data, with an N = 1000. Our bariatric patient samples were taken from Ellis Bariatric patient participants, with an N = 18.

**Discussion:**

Recall that our initial hypothesis was that SNPs associated with increased cortisol activity will be detected more frequently in overweight populations than that of normal weighted counterparts. As the data suggests, this is partially true, but there is not enough data to completely support this claim.

An important concept to know is that every hormone is only as good as it's receptor. The slightest of changes in its composition can change the overall efficacy of the receptor. The GR has a structured binding pocket of the glucocorticoid hormones, as illustrated in the image below.

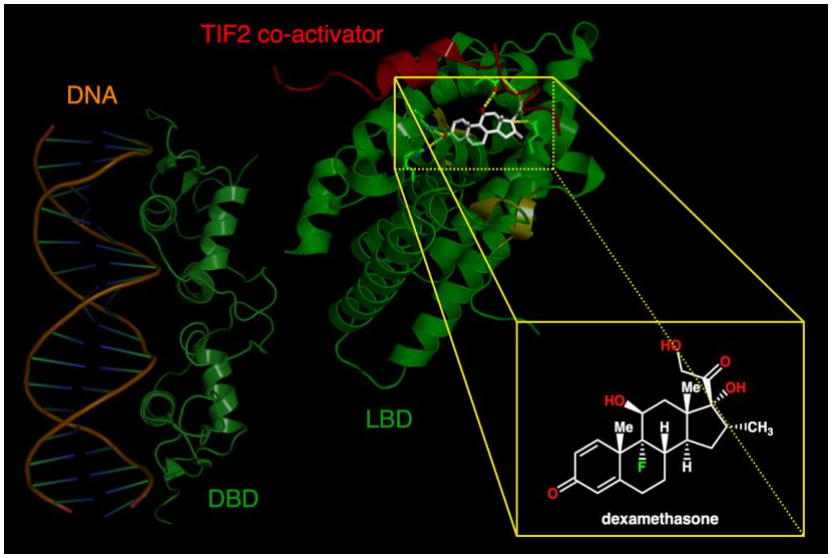



**Figure 6.** The glucocorticoid receptor has a binding pocket for different hormones. Changing the composition of the receptor can change how well the hormone will bind to the receptor.

Mutations in the structure of the GR can result in a phenotypic different in either overexpressing or under expressing glucocorticoids. From the data provided above, we have noticed that there was a higher occurrence of the FKBP51 MU allele in the bariatric patient

population sample that we collected, as opposed to the normal NIH data. Conversely, we found a lower frequency for the BCl1 MU allele in our bariatric patient population as opposed to the normal sample. And the most interesting data that we noticed was that the TthIII MU allele did not show up in our bariatric patient population at all.

When we thought about the data in greater detail, we deduced that it would support our hypothesis that the bariatric patient sample had a higher rate of occurrence of the MU allele for FKBP51 due to the fact that this protein interacts with other regulator complexes, such as HSP90, as a chaperone protein. Together, with the HSP90 complex, the affinity of cortisol to the GR changes. The 52 is a positive regulator that increases the affinity of cortisol to the GR, whereas the 51 is a negative regulator, reducing the affinity of the cortisol to the GR. Thus, if we hypothetically had a polymorphism that displayed the effects of hypercortisolemia, it could lead to obesity. If we hypothesized correctly, it would make sense for the bariatric population to have a higher occurrence of the FKBP51 mutation.

As for the BCl1 polymorphism, we would have expected that the bariatric patients had a higher frequency of this polymorphism, because a mutation in the BCl1 gene would have led to a hypersensitivity phenotype, indicating that with normal levels of cortisol, they were expressing hypercortisolemia. However, the data do not support this claim.

Finally, we were surprised that there were no TthIII mutations in our bariatric patient sample. After closer reflection, however, we realized that TthIII is a resistance polymorphism, indicating that while we are not seeing more hypersensitivity, we are seeing less resistance in our bariatric patient sample. This could also lead to a propensity toward hypercortisolemia, which is on par with our hypothesis. Let it be noted however, that our data may not be statistically significant at this point, due to our small patient sample

population, however it does give us hope for further research in the future to see if there are any clearer trends that come to the surface.

Unfortunately, although Claude and I wanted to analyze different aspects of the research, due to the time constraints, I did run out of time to look for any significant trends between the presence of these polymorphisms, and the propensity toward weight regain after surgery amongst the different bariatric surgery techniques that had been performed.

Each one of our polymorphisms had to be run on their own gels. Our TthIII on one gel, FKBP51 on another, BCl1 on another, etc. This takes up a lot more time because each PCR reaction takes about 3 hours to run, each gel about 30 minutes to make, and has to run for about no less than 30 minutes before it can be analyzed. However, if there was somehow a way to test two different polymorphisms that run through the PCR at the same temperature, with different expected product sizes, that could save a lot more time for data analysis. Thus, a multiplex assay was designed in order to do just this. We ended up getting one successful multiplex assay with FKBP51 and BCl1.

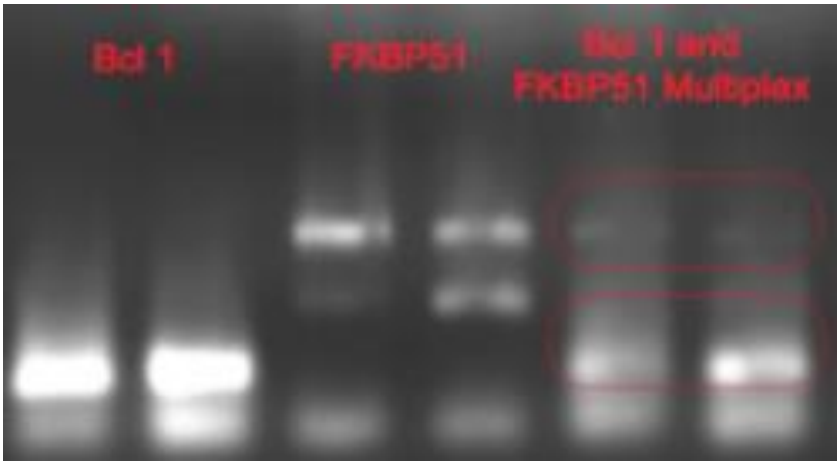



**Fig 3.** Gels show the multiplex assay for subject number 10. BCl1 and FKBP51 were first run separately to reveal the genotype for subject 10. Then they were combined to reveal the same genotype in the multiplex assay.

Two SNPs were put into a single reaction that created two separate and distinct sets of bands on the gel. The important considerations here are that these two primers were for different genes, that they both contain the same optimal annealing temperature, and they have distinguishable molecular weights to be able to tell the bands apart.

As for future directions of this research, we hope to optimize the multiplex procedure. This would help to save on materials as well as the amount of time it takes to get the raw data collected for data analysis. We also hope to be able to design primers that can run both the WT and MU alleles in the same lane, which again would save on both time and materials for this research. Additionally, we would hope to obtain more patient samples in the future. We got 40 DNA samples this year, however not all of the DNA was working with the primers, which could have been a mistake when isolating the DNA. Only 18 of our DNA samples worked consistently. Finally, we would hope to obtain more significant correlations toward the weight propensity and weight regain angle of this research.

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