Internship at the Texas Biomedical Research Institute or: How I Learned to Stop Worrying and Love Science

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Introduction

My internship with the Texas Biomedical Institute took place during summer 2014. I spent my internship in the genetics department of one of the most recognized biomedical research institutions in the world, working with one of the most knowledgeable scientists in the field of genetics. The following report is an account of my experiences with the Texas Biomedical Research Institute, entailing basic laboratory procedures I learned and a brief background regarding a few of the projects with which I was involved.

The Texas Biomedical Research Institute

Texas Biomed has contributed to major advancements in cancer research. It has developed animal models that help study many public health problems we face, and has helped identify genes that affect complex diseases like diabetes, obesity, and osteoporosis. Texas Biomed employs approximately 85 doctoral level biomedical scientists. Perhaps even more interesting is that scientists with a biological anthropology background founded the genetics department in Texas Biomed.

Texas Biomed sits on 200 acres in northwest San Antonio, surrounded by a gate that restricts access due to the sensitive materials found within. People familiar with its location recognize the campus by the monkey cages seen from the highway. The cages in

question are the residence to a baboon colony, and they are not the only nonhuman primates that call Texas Biomed home.

One of the most amazing resources Texas Biomed has is its colonies of almost 3,000 nonhuman primates. The majority of these primates are baboons, accounting for two-thirds of the primate population. The remaining colonies are made up of chimpanzees and macaque monkeys, spread throughout the campus. These primates are important to the research done at Texas Biomed, allowing scientists to study and better understand the diseases that affect both primates and humans. When I park near the chimpanzee colony, I am reminded of my safety briefing detailing the many methods chimps use to get your attention (such spitting or flinging feces) and hope none of these methods are used on me.

Texas Biomed is also home to the world's largest computer cluster for human genetic and genomic research. The human genome is made up of approximately 30,000 genes containing three billion base pairs of DNA (http://Txbiomed.org/about/extraordinary-resources/genomics-computing-resources). Analyzing genes significant to particular research used to be a difficult task that took months. But with the AT&T Genomics Computing Center, which houses 8000 computer processors, computer clusters partition complex analyses and increases the speed with which those analyses can be computed. Thanks to funding from the National Institutes of Health (NIH), Texas Biomed was capable of creating a software package called Sequential Oligogenic Linkage Analysis Routines (SOLAR), which takes care of the overwhelming math behind the science.

Texas Biomed is also home to the only operational biosafety level-4 (BSL-4) laboratory managed by a private institution. To fully understand the significance of this, biosafety is categorized into four levels. The amount of care in practices and techniques increases with the biosafety level of the lab (Chosewood, 2009). The BSL-4 lab, located in the virology department, is designed to ensure containment of incurable diseases and protection for everyone inside and out; however, I spent my time in a more agreeable BSL-2 lab in the genetics department.

I interned with Dr. Anthony Comuzzie in a lab conducting research on different biological processes to look for genetic factors in obesity and obesity-related illnesses such as diabetes and atherosclerosis. I prepared for my interview with Dr. Comuzzie the same as I would for any job interview. I studied up on his biography found on Texas Biomed's website and arrived to the campus early and suited up, complete with the red conservative tie that conveyed, "I am a professional. I will be a great contribution to your team." Dr. Comuzzie was not wearing a tie when I shook his hand. His attitude was very relaxed and accompanied with a great big smile and genuine laughs. As we walked towards his office, I knew immediately that the atmosphere at Texas Biomed was very casual. I also met with Dr. Comuzzie's laboratory technician, Vicki Mattern, who helped me recognize the easy-going scene. Dr. Comuzzie's laid-back personality doesn't diminish the respect he has earned, however. Having earned a Doctorate in Biological Anthropology, Dr. Comuzzie works with diseases like coronary artery disease and atherosclerosis. He also studies the effects of diets on these diseases. He is one of the leading experts on the genetics of obesity and the public health concerns associated with

obesity. However, stating that he investigates the genes that influence obesity-related phenotypes is oversimplifying his work.

The Complexities of Obesity

Before my experiences with Texas Biomed, I always understood body fat as this inert part of me that needed to be burned away in the gym. In actuality, adipose tissue, something we are more familiar with as *body fat*, is part of the endocrine system (Nogueriras, 2010). Leptin is one of the hormones secreted by fat tissue, the first hormone identified to be secreted by fat. Leptin plays a role in the regulation of body fat (Comuzzie, 1997), and leptin levels are associated positively with an individual's fat mass, which in turn are connected with obesity. It was unsettling to realize that my fat tissue was playing an active role trying to "stick around."

Obesity is defined by the excess accumulation of adipose tissue. Obesity is also the first step in predicting more serious chronic diseases like diabetes mellitus type 2 and coronary artery disease (Comuzzie, 1997). Obesity is measured by a body mass index (BMI) of 30 or more, but the size of a person is subjective and not sufficient enough to explain the significance of obesity. The term is relevant in a clinical environment (Comuzzie, 1998), but the "fatness" of a person doesn't explain the biological processes working underneath. An athlete could be considered obese but still be healthier than someone not considered obese. There has been extensive work to identify the genes that influence variation of excess adipose tissue.

Obesity is known as a complex phenotype. Complex phenotypes are affected by both multiple genetic and non-genetic factors. Complex phenotypes are not discrete Mendelian traits described by either the presence or absence of something, but instead

show a continual variation in the expression of a specific, observable characteristic (Comuzzie, 2001). Obesity, however, is not necessarily clinal because this variation exists within the population.

Obesity influences many phenotypes intertwined together. Besides body mass index, other characteristics like skinfold thickness, fat mass, and body weight are heritable traits associated with obesity. Inside the digestive system, there are many gut hormones that affect obesity. Some people are more predisposed to eat more. Some people secrete hormones that trigger hunger or don't secrete enough hormones that regulate hunger. Some people just retain fat more efficiently than others, or their metabolism is slower. Perhaps a person's genes make them more resistant to a specific hormone related to hunger and metabolism.

Dr. Comuzzie investigates obesity along with many traits related to obesity. Because obesity is a characteristic that follows a normal continuous variation and distribution pattern, we know obesity is not limited to one single gene hidden in our chromosomes. Dr. Comuzzie therefore studies both polygenic genes, in which many non-allelic genes work together by each contributing is small amounts, and oligogenic genes, which involve a few genes that contribute disproportionate, measureable effects.

Part of what makes obesity a complex phenotype besides all the genotypes working together is the inclusion of the environment. There are environments and behaviors that lead to obesity. People in the US display variation from "healthy" to "morbidly obese." Obesity is more prevalent in richer economies like the US due the convenience of highly palatable and high caloric foods. Emerging economies like Mexico have also seen a rise in obesity rates for these similar reasons. Populations that do not

have access to fast food establishments will probably exhibit similar BMI levels. I would add that, from a cultural standpoint, that statement "I'm starving" is entirely different between when I say it and when someone from a developing nation says it. But by gaining a better understanding of the underlying genetic contributions of the complex phenotype and being able to separate the genetic element, environmental factors will become more manageable (Comuzzie, 2001).

Identity by Descent

It has been established since the 1980's that there exists a genetic component to obesity. There are studies that suggest alleles (one particular form of gene) of some populations predispose them to obesity-related phenotypes (Comuzzie, 1998). The goal has shifted from asking if these genes exist to finding where these genes are. Dr. Comuzzie's lab investigates genes and looks for similarities inside a population, similarities in genome that could be linked to obesity. The genome is mapped utilizing the analytical power of the AT&T Genomics Computing Center. With these computer clusters, the genetics department can collect genetic information from large extended families, take into account kinship relationships, and analyze for the presence of inherited diseases.

In trying to locate the genes in question, one of the sampling strategies employed is to follow the family structure of a special population that shares greater homogeneity and linkage disequilibrium (Comuzzie, 1998). This sample is known as a pedigree study and scientists can use linkage analysis to locate and identify specific genes that express phenotypes in related individuals. DNA is considered "identical by descent" if you can find alleles shared among relatives (Comuzzie, 2001). Identical alleles found in random

sampling, however, are considered “identical by state,” and random sampling requires data from large populations to achieve any statistical significance. Using an established pedigree strengthens the statistical data when mapping genes because there is already a known relationship between individuals with the same alleles.

The San Antonio Family Heart Study (SAFHS) is one of the pedigrees studied at Texas Biomed. Before, genetic research on heart disease had been done on populations of Northern European ancestry. The SAFHS is the first large, population-based genetic study in Mexican Americans (Mitchell, 1996). The study began in 1991, as part of a collaborative project between Texas Biomed and the University of Texas Health Science Center (UTHSC). The population consists of 40 large Mexican American families and totaling more than 1,400 individuals. Information regarding family relationships, demographic characteristics, medical histories, and environmental risk factors has been collected from men and women and their various family members. The data was collected from all willing participants without concern for specific medical conditions (Hixon, 1999).

Mexican Americans have an extensive admixture involving European, African and Indigenous American ancestry (León-Mimila, 2013). The study initially began in search of genetic risk factors for atherosclerosis and type-2 diabetes, which is considerably important to find in Mexican Americans due to the high prevalence of diabetes and obesity.

The SAFHS has provided Texas Biomed with evidence of genes influencing many complex diseases, including obesity. Serum leptin levels, insulin levels, and fat mass are some of the few phenotypes that are heavily influenced by these newly

discovered genes. As studies continue, Texas Biomed hopes to narrow down specific genes responsible for these obesity-related characteristics with the promise of providing beneficial therapies and lifestyle changes.

In addition to the SAFHS, the genetics department also uses a large population in their baboon colony. Because baboons and humans share a similar physiology that is susceptible to complex diseases like atherosclerosis and obesity, scientists can create models studying the interaction of diet on obesity-related phenotypes (http://txbiomed.org/about/extraordinary-resources/nonhuman-primates). There are approximately 1,200 baboons residing at Texas Biomed that are members of another established pedigree. Scientists have maintained family histories throughout successive baboon generations. The primates have been genotyped to create a genetic linkage map, positioning gene and gene markers for study. The controlled environment of the baboon colony coupled with manipulated diets allows scientists to see the effects on adiposity-related phenotypes.

Dr. Comuzzie is currently gathering data on a pilot study he is hoping to get funded for the next five years. The aim of the project involves two different diets and their interplay with baboons. One diet is labeled the “prudent diet,” a diet that subsists of complex carbohydrates and heart-healthy fats. A healthy diet is postulated to lower cholesterol and blood pressure (Szostak, 2013). The second diet involves high trans-saturated fats and simple carbohydrates, commonly referred to as the “Western diet,” although it had been the first I heard of this. Strong evidence links the intake of high-fat foods to complex diseases like coronary artery disease but the project hopes to observe

the effects of both trans-fats and simple carbs. The expectation is that simple carbs like sugary sodas and candy are actually more detrimental to our health.

Working in a "Wet Lab" and an Informal Lesson in Biology

The majority of my internship occurred in the laboratory. Samples for all of Dr. Comuzzie's projects were brought to the lab, and different protocols were created for each piece of sample being tested. The particular conditions for most of these tests required water and pipets –a lab tool used to deliver small fixed volumes of liquid— giving this particular lab the moniker of a "wet lab." Dr. Comuzzie was the Wizard of Oz, but his lab was the magic behind the curtain. Before I could participate in any capacity with research, I had to get familiar with the tools and chemicals of the lab.

Some of the various instruments I became acquainted with were easy to learn while others required more attention. The plate washer flushed a buffer solution to maintain the pH of tests. Some tests needed to incubate for a period of time to allow chemical reactions to take place and thus were placed in a microplate shaker. The ACE Clinical Chemistry System was a fickle machine used in running diagnostics of serums, capable of testing for glucose, electrolytes or liver functions.

During scientific procedures, I was in contact with many hazardous chemicals, ranging from boiling water (something usually present in kitchens) to corrosive acids (something not typical in homes). I was responsible with handling these chemicals and also in proper disposal procedures. When handling hazardous chemicals, it was always imperative to use either nitrile or latex gloves. When handling corrosive agents, wearing a lab coat provided a sense of security. Beyond the lab coat were showerheads

strategically present, in the event I happened to spill something on my person. The disposal of liquids involved bleaching the solution.

Before tests could be run I needed to understand how samples were stored, inventoried, and how to collect them. Samples were collected, either by veterinarians or technicians drawing blood somewhere offsite, and sent to the genetics department. These samples were usually aliquoted, which meant that the samples were divided from a larger unit and partitioned into smaller volumes. The samples needed to stay frozen to maintain the integrity of the proteins found within, and the freezers required special handling to prevent frostbite. When collecting the samples for a determined test, dry ice was used to keep them cold while I separated the aliquots to only what I required.

The specific assay –a scientific procedure used to measure a specific substance in a collected sample –I became knowledgeable with was an *enzyme-linked immunosorbent assay (ELISA)*, although I was able to participate in other assays. The ELISA involves the capture of a specific substance from the sample to the wells of a micro-titer plate that has been coated with a pre-titered amount of antibody (Ab). An antibody is part of the immune system, a protein that binds to antigens at its antigen-binding site. The antigen is anything that triggers an antibody response, which could be a pathogen, or in the case of an ELISA, a hormone. The sample is run through a series of reagents that collect in a micro-titer plate. Reagents are substances added to the sample to produce a chemical reaction. The test culminates with the quantification of immobilized attached antibody-enzyme pair by monitoring the chemical reaction of the peroxidase with a substrate. In the ELISA, the peroxidase is the enzyme reagent added to the test. The substrate is the compound that will react with the enzyme. The chemical reaction when the substrate is

added changes the color of the well proportionate to the concentration of specific substance being tested.

In many of these tests, the protocol entailed incubating the samples for an extended period of time. The ELISA was time consuming, and it was common for me to deal with only one test at a time. The test was sensitive to environmental influences, like drying out or at times being exposed to specific lights, so I needed to ensure I was ready to perform the next step in the test. The assay kits were also relatively expensive and a constant fear of wasting Biomed's money loomed over me. The results of the tests were not demanded at a certain time, which allowed me to gain confidence in performing these assays.

While running assays, my primary focus concerned the non-human primate study noted in the background on the baboon pedigree. Samples (blood) were drawn from the proband (member of study) at a baseline, or start of diet, again at 3 weeks into the diet and one final time at 7 weeks. The subjects were then taken off the manipulated diet and given time to return to a normal status with a normal diet. The assumption was that after enough time on their normal diet, the animals would be able to eliminate the effects of the previous diet. After a set length of time, these baboons were placed into a diet distinctly different from the first diet, and samples were collected again at 0 weeks, 3 weeks, and 7 weeks.

I was tasked with running an ELISA on the Glucagon-like-peptide-1 (GLP-1) of the baboon study 1399PC. The GLP-1 is a hormone found in the gut and is involved with blood glucose levels. The focus of the test was to analyze GLP-1 levels of baboons on specific diets. Upon completing the procedure, the plate containing GLP-1 levels of the

animals needed to be read by a plate reader that measures optical density of the samples at a specified UV wavelength. The first wells on the plate of an assay kit are used to create a standard curve through serial dilation from a known concentration. This curve helps in determining the concentration of the unknown samples. The data was collected and placed in an Excel spreadsheet.

Afterwards, I needed to conduct statistical analysis on the data to find a pattern that could be used for inference into the study. Because the sample size was so small, removing any outliers would have weakened the statistical strength of the data. However, if a value seemed distinctive, I would theorize why this value was peculiar. I used a paired t-test to compare the means of the two sets of data to find the p-value in hopes of finding statistical significance. I calculated the test using a significance level of 0.05 to compare GLP-1 means at different weeks between the same diets and between the different diets. The t-test of the effects of specific diets on the GLP-1 hormone showed no significant difference.

I have conducted and have been a part of other procedures on the samples for 1399PC. I ran an ELISA for the C-reactive protein, which is found in response to inflammation (Parham, 2009). I was also able to run a TBARS assay as well, which measures thiobarbituric acid reactive substances (TBARS) to screen and monitor lipid peroxidation, an indicator of oxidative stress in cells. Unfortunately, the amount of detail in other assays is beyond the capacity this report will allow. The other assays I became familiar with are conducted similarly, sometimes involving different reagents or a different method in creating a standard curve. Some assays involved magnetic beads to

lock the analyte in place, and sometimes an assay was capable of measuring several analytes.

I have also been helping with a project called *Genética de las Enfermedades Metabólicas en México* (Genetics of Metabolic Diseases in Mexico) or simply *GEMM*, a new collaborative study between Texas Biomed and Mexico. My specific responsibilities have involved performing ELISAs on the protein Human Ghrelin in plasma samples. Ghrelin, a hormone that affects hunger, circulates through the blood and is primarily made in the gut. While the stomach is empty, ghrelin increases hunger as it communicates through the central nervous system. The gastrointestinal tract stops secreting the hormone after eating. The GEMM study is in its infancy stages, but I imagine this study will have a larger role at Texas Biomed. I participated in only a small part of this study, but Dr. Comuzzie is investigating many analytes, or biological components, found in serum. The involved parties on the opposite side of the border are excited about this study, and the genetics department can only benefit with the data collected.

Conclusion

Having an anthropologist background allowed me to ask questions not immediately important to the genetics aspect. Dr. Comuzzie recalled a story in one particular study with Alaskan Natives. The genes of a parent did not match up at all with their child. In the background information collected, that child belonged to the parents, but the genes did not collaborate with this information. The discussion about the culture was then brought up. In this tribe, if an adult died, the next of kin would adopt the children of the deceased. However, in their culture, there is no distinction between an

adopted child and a child from birth. To the adult, that child was his or hers as if from birth.

I did not know what to expect to learn during my time at the Texas Biomedical Research Institute. Genetics remains mysterious to me, but I have found a greater appreciation and newfound desire to learn more about the components that affect variation and heritability in humans. When I began my internship with Dr. Comuzzie, he spoke rapidly about the science with which he was involved. He used large words I barely recalled from my genetics lectures.

I was already aware that public health is a serious concern but only now made aware of how much effort goes into researching serious diseases, diseases that people I personally know are dealing with. Texas Biomed is hoping to find the genetic components that make a Mexican American more susceptible to overeating, more likely to retain fat tissue, or more likely to become diabetic. Microbiology and genetics don't provide the entire picture when considering complex phenotypes. The SAFHS was chosen based on the low income and socioeconomic status of the families. It is important to understand the cultural and social background, especially when realizing that genes do not operate in a vacuum (a quote picked up by Dr. Comuzzie). Many of the environmental factors, diet being one very important component, should be discussed when researching something as involved as obesity. My own family, for example, ate meals consisting of three or four serving of carbohydrates. Flour tortillas, as delicious as they are, have considerable negative effects when wantonly consumed.

I am also grateful for the experiences in the laboratory. I could never have expected to learn so many techniques and skills in a lab environment and more still

become confident enough to apply for a position in a laboratory in the hopes I can learn more about the tests used in human diagnostics and pathology.

It may seem disparaging regarding school, but I learned so much more while interning at Texas Biomed than I have in many lectures. I approached Dr. Comuzzie, unfamiliar with almost everything concerning genetics and human diseases. But as I learned to measure certain analytes, I wanted to learn what it was these biological elements did. What processes were these analytes involved in? Why did some individuals have a larger concentration of these analytes? How did their diets affect it? And then I wanted to learn more about the background information why I was testing these analytes. What were we trying to learn about these people? What genes are we looking for? How close are we to finding them?

I can't say my experiences have convinced me to follow a certain path, but I still hope to learn more about the way our genes work to express certain characteristics. I am compelled to follow the research on the SAFHS because they are in the same circle on the census form, which with I identify. I am consistently amazed by the biological and chemical processes happening inside us, by the way our bodies can bounce back from the punishment we put them through, and by how something so small can end the ride.

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