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Investigating Evidence of Symptomatic Osteoarthritis in Baboons

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Introduction

In the spring of 2013 I interned at the Texas Biomedical Research Institute under Dr. Havill, a research scientist whose laboratory investigates osteoarthritis using baboon skeletal remains.—The institute has two main departments: Virology and Immunology, and Genetics; Dr. Havill works in the genetics department at Texas Biomed. This internship opportunity allowed me to develop a hypothesis to further the team’s research goals relating to manifestation of OA symptoms and severity of OA. I became part of a team of researchers that reflects the extremely interdisciplinary nature of work at Texas Biomed. Dr. Havill’s staff included four individuals: A post-doctoral scientist with a Ph.D. in molecular biology; two Senior Research Assistants (one acting as lab manager), one with a B.S. in Biology, and the other with a B.S. in biology and a B.A. in biological anthropology; and a Research Associate with a B.S. in molecular biology and an M.P.H. and a C.P.H.

The Texas Biomedical Research Institute

The Texas Biomedical Research Institute has a long and prestigious history. The institute was founded in 1941 by Thomas B. Slick Jr., and is located on a 200-acre campus in San Antonio. The campus contains multiple lab and administrative buildings for each department, a library, veterinary facilities for the animals, and several different holding areas for the various nonhuman primates (for example, there is a “retirement village” for older chimpanzees no longer used in studies). There are 75 doctoral-level scientists and 28 principle investigators working at

Texas Biomed, with more than 200 active research projects. Some of the more impressive accomplishments of the researchers include the identification of genes that influence heart disease, obesity and diabetes; as well as the development of vaccines and antibodies for viruses and infectious diseases such as Ebola, botulinum neurotoxins, and anthrax (Vandeberg et al. “Texas Biomed Quick Facts”). Texas Biomed scientists in the Dept. of Genetics work to identify genetic influences on common, complex diseases to allow for a better understanding of disease risk, origin, and progression.

Lorena Havill’s Research Lab

A main project of Dr. Havill’s laboratory is with osteoarthritis (OA) in the baboon population at the Texas Biomed Research Institute. The onset of OA generally occurs around age 40, and 85% of the population has clinical or radiographic evidence of OA by the age of 75 (Sack 1995). This pathology, also known as degenerative joint disease (DJD), generally occurs in weight-bearing joints, as opposed to all over the body as seen in rheumatoid arthritis (RA). The changes associated with OA include osteophyte (bone spurs) formation, degradation of the cartilage, and hypertrophy (enlargement) of the joint. Originally, researchers assumed OA was entirely tied to the “wear and tear” of the joint over time. Recent studies have indicated that OA is multi-factorial, and researchers are uncertain what process of the degeneration begins first and why (Loeser et al. 2012). The risk factors include genetics, lifestyle (an umbrella term that can encompass diet and activity level), and previous injuries.

OA is a significant health problem, and the epidemiology of the disease is not well understood. While there is correlation between the bone cartilage breakdown and surrounding tissue degeneration, it is not known if these are contributing factors (Hill et al. 2007; Katz et al.

2009). The advanced stages are recognized, but early detection and preventing the advancement of the disease is still a hurdle in the biomedical community. Most people only see a doctor when their pain is severe, and so little is known about the early stages of OA and how each factor (calcification of cartilage, growth of osteophytes) contributes to the progression of OA.

A major advantage of utilizing the primate model in this kind of research is that the principle investigators (PIs) have the clinical history of the animal as well as the genetic information. The animals are also kept within a controlled environment. Therefore, variables that may not be completely controlled in a human study, such as the diet, do not confound the results. Dr. Havill and the researchers in her lab also have the advantage of examining the entire knee joint from the skeletal remains. This allows them to observe small changes in the cartilage and menisci of the knee joints that are missed with MRI and arthrogram scans. This helps with understanding the early stages of the disease.

Researching Osteoarthritis

The discrete project assigned to me by Dr. Havill focused on ibuprofen distribution in baboons with OA. A meta-analysis by Dijk et al. (2006) revealed that a correlation between pain severity and the progression of OA in humans was not consistently supported in the literature. The hypothesis we were testing was that there is a correlation between severity of OA in the knee joint and ibuprofen distribution to the baboons. The results might also reveal a relationship between the certain markers for OA and symptoms. For example, baboons with osteophytes in a certain area might display more symptoms of OA. It could be important to make this distinction since OA progresses differently at varying rates for each individual. If there is no correlation, it would support previous studies that show a lack of correlation. This would indicate that the

variation in the experience of symptoms is not solely tied to sociocultural differences in how pain is perceived and reported.

The veterinarians distributed nonsteroid anti-inflammatory drugs (NSAID; e.g. ibuprofen or Advil) for animals with diagnosed OA or displaying symptoms of OA. The documented clinical history, contained in a database called CAMP, provided information on drugs distributed, the duration, and the date for each animal. Also in the database were medical notes about the animal's surgeries, clinical trials, study-related tests (such as radiographs examining OA in aged animals), and general observations by the veterinarians.

To test the hypothesis I had to review the clinical history (held in the CAMP database) of all 600 animals included in Dr. Havill's OA study. For this task, I compiled an Excel spreadsheet detailing the clinical history of each animal. I recorded the date and duration of NSAID distribution and if it was related to a diagnosis of OA in the knees. I noted comments about limited range of motion, and other notes that might have indicate OA symptoms. For example, one clinical history detailed that the baboon had a "slow gait" and favored his right leg. In addition to this, I also noted the diagnosis of crepitation (audible popping or cracking) in the knee joints, range of motion, confirmed diagnosis of OA, and dates of radiographs. This would help Dr. Havill later correlate information about crepitation, range of motion, with severity of OA in the remains, as well as possible genetic signatures for those observations in OA individuals.

Although the main hypothesis Dr. Havill assigned to me was fairly straight forward, the other aspect of this task was to bring to her attention any other information that might affect her results. I found that several animals in the late 1990's received fibulae or tibiae defect implants as part of another study. I mentioned this to Dr. Havill, since those animals might not be

appropriate to include in the general OA studies. In other words, their gait was altered artificially so that environmental influence might distort genetic contributions to OA, or their development of OA could be due to different genes that OA resulting without an obvious bone/joint injury. Although frustrating to cut a few animals from the study, it demonstrated the value of the intern program in that careful review of records was possible so that the purity of the sample could be insured for the project.

I also had the opportunity to shadow the research staff in the laboratory when the data was collected from the animal tissues. Multiple observations and types of data were obtained from the skeletal remains. The meniscus was examined for tears, calcification, and overall damage by the project leader. The tibial plateau (area of tibia that articulates with the femur in the knee joint), meniscus, and femoral condyles were photographed. She also collected information of the degree of OA in the knee joint. The info sheets for OA included a diagram of the femoral condyles, where she noted the area of visible OA change in the cartilage. The severity of OA was also scored on a scale of 1-4, (1 = normal, no OA, 2 = observable loss of reflection, 3 = areas with erosion present, 4 = area of complete erosion to bone). Synovial fluid (the fluid that lubricates the knee joint) and cartilage were also collected and preserved for each animal. After all the data was collected, the knee joints were either preserved in a buffered 10% formalin solution or frozen for future study.

This was my first opportunity to examine a whole joint not fully skeletonized. I could actually see the calcification of menisci and cartilage degeneration in animals with OA. Although not always visible to the untrained eye, small “bumps” of calcified meniscal cartilage can be felt in the tissue. Healthy cartilage looks bright white and shiny; degeneration is present

when the area appears to have “dull” spots in the cartilage. As degeneration continues, the cartilage may wear away completely in some areas, and osteophytes may be present.

While compiling the spreadsheet of data of my internship, it became clear to Dr. Havill and me that the veterinary notes within the CAMP database were not always consistent. Therefore, we will have to take this into consideration if we do not see a relationship between severe knee OA and NSAID distribution in the baboons. However, a summer opportunity provided me with the funds to further explore this research question.

Other Duties and Opportunities in the Lab

In addition to observing data collection of baboon knees, I helped in a few organizational tasks in the lab. I arranged and documented the placement of samples collected from the baboons to place in the large sub-zero freezers. These samples are collected to use in other research projects currently in process at the lab, or for general data collection for future research. I also helped to chicletize serum and synovial fluid for future biomarker tests. Exactly as it sounds, this process entails making small packets of serum fluid the size of chiclets to keep in a freezer. This liquid is used to mix with other substances to test various biomarkers (such as detecting metabolite or hormone levels). But the substances to be measured in the fluid and serum can be quite sensitive to thawing and refreezing. Thus, these small packets are made so that a single chiclet may be thawed and tested without compromising the rest of the serum. This process allowed me to see how much time is spent simply to prepare a sample or study, and how important each step is in that process.

Aside from my main project and lab assisting tasks, I also attended the weekly lab meetings on Tuesdays. This allowed me to hear from all members of the lab about their various projects, as well as how the administrative aspect of a lab operates. The Research Associate in the lab is testing bone biomarkers in samples from a population in Nepal to measure variation in bone metabolism, and one of the Senior Research Associates is examining synovial fluid in the OA baboon population. Dr. Havill kept tabs on everyone's projects and this was also a time for people to suggest ideas about new research questions or bring up any road blocks in their work. One of the more exciting meetings included an overview of the recent Orthopaedic Research Society (ORS) meeting. Each member of the lab summarized their favorite talk to the group and provided insight as to how that might be included in the research in Dr. Havill's lab. It was exciting to see how the scientific network of researchers allows for the exchanging of ideas and the implementation of new methods or techniques in other labs.

Another weekly meeting involved the Southwest Research Institute (SwRI) mechanical engineers. Their work included a great deal of applied research in the field of biomechanics, such as examining force trauma and hip arthroscopy (hip replacement) improvements. These meetings provided a glimpse into up-and-coming biomechanical research and how these two groups negotiated paper and project designs.

Conclusion

When I began my internship, I worked with Dr. Havill on an application for a paid summer internship at Texas Biomed to conduct an osteoarthritis-related research project with her. Though the application process was competitive, I successfully earned a spot for the summer. The internship includes a small budget for Dr. Havill to cover project-related expenses

and she plans to use these funds to purchase activity monitors to place on osteoarthritic baboons to further explore how OA affects activity level and mobility. It was difficult to draw conclusions from my previous study using the CAMP database because of the time it took to bring order to the inconsistent notes on OA symptomology. Real-time data from activity monitors would provide more reliable information for evaluating the impact of an animal's OA on their functionality. Pain is notoriously difficult to measure in humans, and even self-reported pain is siphoned through multiple cultural and personal filters that make cross-study comparisons difficult. A baboon model allows researchers to examine mobility in situations less affected by complex sociocultural factors that can confound human studies.

In addition to furthering the activity level and OA project this summer, I will examine the relationship between meniscus damage and OA in the knee. This is also relevant to translational medicine since meniscal damage and OA symptoms are related but not epidemiologically understood (Pauli et al. 2011). I intend to use this opportunity to further my understanding of OA and work with Dr. Havill to present and publish the results of our research.

Works Cited

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