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A Pharmacogenomic and Protein Analysis of Human Lacrimal Fluid in Varying Age Groups

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STATEMENT OF THE PROBLEM

Background

- Proteins are large biological molecules located within all cells. They are considered the basic functional components of cells that allow them to operate appropriately. Genes consist of both DNA and RNA, and are the cellular components that code for the proteins. A biomarker is any cellular component that is an indication of a biological state. Therefore, genetic and protein biomarkers are specific genes and proteins, respectively, present in cells that indicate a specific biological state of a cell. Identification of proteins and genetic biomarkers in relative quantities has been found to reflect various disease states and age groups in humans.
- MicroRNAs were found not only intracellularly but also in many of the body's fluids (plasma, saliva, tears, urine). MicroRNAs can act as informative biomarkers due to the correlation in the amount and type of these microRNAs with disease.
- A reference list of 1543 proteins that they discovered in lacrimal fluids.
- The decreased tear levels of proteins in dry eye disease are related to impaired lacrimal function, and tear levels of nerve growth factor (NGF) are more closely related to corneal epithelial damage. These findings suggest that proteins could become useful markers of dry eye severity.
- Comparisons of possible techniques for collecting lacrimal fluids from human subjects which could potentially be utilized in the design of the study.

Significance of the Problem

- The exploratory nature of this project could follow many different avenues and has many potential uses, all of which depend on the findings. By looking at the variation of proteins and genetic biomarkers in the lacrimal fluids of patients in different age groups, we hope to identify any variations that would link a patient with a particular age group and possibly a specific disease. The differences in these lacrimal fluid components may also correlate with the individual's eye health. We hope to identify links between variations in biomarkers (protein and genetic) and specific age groups that could potentially contribute to future research in their correlation with disease states.

OBJECTIVES

- To gather and analyze proteins and genetic biomarkers in human lacrimal fluid in varying age groups.

HYPOTHESIS

- There will be a significant difference in the amount and type of protein and genetic biomarkers in the lacrimal fluid of individuals in different age groups.

PROJECT TIMELINE

- The IRB will be submitted in December 2012 and, pending IRB approval, subject recruitment will take place between January 2013 and April 2013. In August 2013, the researchers will receive training from Dr. Florkey on how to correctly utilize the capillary method in collecting lacrimal fluid from the subjects. Collection of subject information and lacrimal fluid samples and the analysis of the samples will occur between September 2013 and April 2014.

LIMITATIONS

- The limitations of this study include the unknown time that will be required to recruit the needed number of subjects, the potential for human error in the analysis of the samples, and the potential for a lack of generalizability with the results.

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PROPOSED METHODS

Study Design

- Subjects will be recruited and further divided into one of two age groups: 18 to 24 years old and over 50 years old.
- 30-50 microliters of human lacrimal fluid will be collected from the subjects' lacrimal lake using a microcapillary tube and a pre-developed method.
- The collection of the lacrimal fluid will occur within Cedarville University's simulation clinic in the Health Sciences Center.
- Once the lacrimal fluid samples are collected, they will be stored in a secure -80° Celsius freezer in the Pharmaceutical Sciences laboratory, also located in the Health Sciences Center.
- All of the sample collection and analysis will be completed by professional students of the Cedarville University School of Pharmacy, under the instruction and validation of a faculty advisor and a practicing optometrist.

Sample

- Subjects will be enrolled using a quota sampling strategy.
- The quota for the study has been set at 20-25 individuals for each group.
- Individuals will be conveniently selected through the Cedarville University School of Pharmacy and a local practicing optometrist's patient database. If the quota has not been met, snowball sampling may be utilized to recruit further subjects.
- The subjects will be divided into two different age groups: 18 to 24 years old and over 50 years old.
- Individuals who have worn contacts within the past year will be excluded from the study.

Sample Collection

- Each subject will be provided with information about the study upon their arrival and prior to sample collection. The subject will enter the simulation clinic with two researchers and be asked to sign a consent form. If the subject indicates his or her willingness to participate in the study, he or she will be assigned a subject number and data collection will begin.
- The first component of data collection consists of a short interview to record any aspects of the subject's personal history that could affect the protein and genetic components of his or her lacrimal fluid.
- Once the interview has concluded, one of the researchers will collect a sample of the subject's lacrimal fluid. While wearing gloves, the researcher will use a microcapillary tube to collect a 30-50 microliters sample of lacrimal fluid from the lacrimal lake of the lower eyelid without touching the surface of the eye. The researcher will then place the fluid-filled microcapillary tube into a microfuge tube labeled with the subject number. These microfuge tubes will be transported to the Pharmaceutical Sciences laboratory for either immediate analysis or storage in a freezer.
- The samples will be stored in a secure -80° Celsius freezer until the time of analysis.

Measurement

- In order to analyze the lacrimal fluid samples, preservation extraction buffer will be added to each sample in an amount equal to the lacrimal fluid samples (30-50 microliters). Each sample will then be run through a real-time PCR Machine to test for the presence of microRNA and a poly-acrylamide gel electrophoresis to analyze the protein content of each sample.

FUTURE DIRECTIONS

- The results of this study could potentially establish new biomarkers, have diagnostic applications, and prompt continued research into possible correlations between genetic and protein information with varying age-groups.

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