Modulation of Alzheimer's protein Amyloid Beta by Gut Microbes in *Drosophila melanogaster*

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Abstract

Drosophila melanogaster, or the fruit fly, is commonly used in labs because approximately 75% of human disease-causing genes are believed to have a functional homolog in the fly (Pandey 2011). This close genetic makeup as well as minimal expense makes Drosophila an ideal model to investigate potential effects of gut microbiota in human disease. Gut microbes have been shown to influence a host of diseases such as obesity, type 2 diabetes and kidney disease (Musso 2010). In this study, a protocol for generating GF (germ free) flies was developed and verified. We used a *Drosophila melanogaster* strain expressing human amyloid-β (Aß) protein, a key protein responsible for Alzheimer's disease, in a temperature dependent manner (Singh 2013). Surprisingly, GF Aß flies did not exhibit the Alzheimer's phenotype. This technique is crucial to investigating the role of commensal microbes in the genesis and progression of this disease and could be used to study the role of commensal microbes on any human disease homolog in the fly.

Hypothesis

Eliminating gut microbes will rescue the the amyloid-β 42 phenotype in the Alzheimer's model strain (GMR>Aß42) of *Drosophila melanogaster*.

Materials and Methods

Fly Strain

The Aβ-42 protein was misexpressed in the differentiating neurons of the fly's eye using a GMR-Gal4 driver, which results as well as the complete fusion of ommatidia (Tare 2011). This phenotype is also temperature dependent, and at 18 degrees, the phenotype is less dramatic than when reared at 29 degrees (Tare 2011). The GMR>Aβ-42 flies are a proven model for the study of the Alzheimer's protein in *D. melanogaster*, and therefore provide an ideal model for the study of gut microbiota on the Alzheimer's phenotype.

Fly Food

Yeast, corn meal, soy flour, agar, corn syrup, water were all added to an Erlenmeyer flask and cooked until mixture became thick. The media was sterilized by autoclaving and poured into petri dishes which were sterilized via UV radiation (Lakovaara 1969).

Materials and Methods Continued

Dechorionation

Embryos of the GMR>Aß42 flies were collected from stock flies and placed on double stick tape. Under a microscope, the embryos were gently rolled on the tape to remove the chorion (Kiehart 2007).

Sterilization

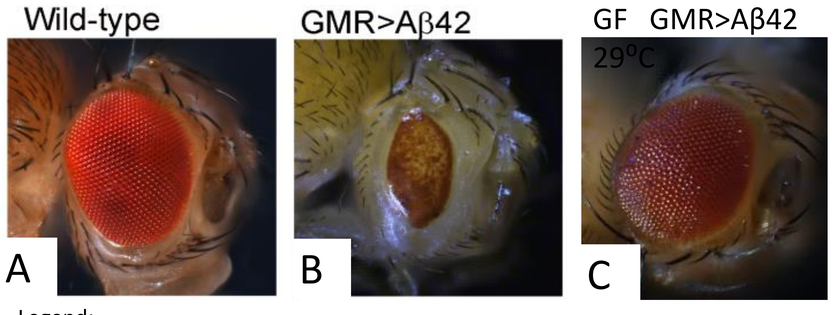
The dechorionated embryos were placed on sterilized filter paper and soaked with bleach for two minutes. The bleach was removed by vacuum filtration; embryos were washed twice with ethanol, followed by two washes with sterile water. The filter paper containing the embryos was aseptically transferred to a plate of sterile fly media. Plates were sealed with parafilm to prevent desiccation of the embryos and/or infestation by mites.

Sterilized embryos were incubated at 18 or 29^{o}C for one week. After one week flies were examined by bright field microscopy for phenotypic differences of the eye.

Confirming Sterility

Adult flies were homogenized, plated on standard growth media , and observed for the growth of microbes. PCR analysis (Polymerase Chain Reaction) was used to amplify bacterial DNA to identify all microbes present in the fly gut.

Results



Legend:
A Wild-type eye **B** conventionally reared (nonGF) GMR>Aβ42 **C** GF GMR>Aβ42

We obtained adult flies from the sterilized fly embryos incubated at either 18 or 29^{o}C incubator. Since the amyloid-β flies express their condition in the eye, the eyes of the flies were examined. GF GMR>Aß42 flies exhibited eyes similar to wild-type eyes (Figure 1).

Results Continued

The GF fly eyes did have some areas of necrosis like the conventionally reared GMR>Aß42 (nonGF) flies but did have formed ommatidia like the wild-type eyes. The same was true for GF flies grown at 18^{o}C which proved to be sterile based upon no growth on standard microbiological media.

Discussion

These preliminary results support our hypothesis. GF flies had almost wild-type eyes which indicates that one or more commensal gut microbes interact with the host signaling pathways in controlling Aß42 protein expression. Complete elimination of microbes was not necessary as some of the flies with almost wild-type eyes were not sterile, albeit the numbers of microbes was substantially reduced. The use of conventional antibiotics also does not result in completely germ free conditions so it is possible that elimination of one or several groups of microbes is all that is needed to reduce Aß42 protein expression to the point that function is restored. With these results we will be able to identify which microbe(s) in the gut of the fly contribute to the Aβ42 phenotypic eye by adding back microbes singly and in combination to GF flies.. We will also be able to compare sterilized flies as per the protocol in this study with those fed commercially available antibiotics to reduce microbial numbers but not achieve sterilization.

References

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