Identifying a Relationship between the Number of CAG Trinucleotide Repeats and the Incidence of Alzheimer's Disease

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Introduction

Ataxin-1 (*ATXN1)* is a gene that in healthy humans contains 4-39 repeats of the CAG trinucleotide which codes for the amino acid glutamine. Genes with variable number of repeats in their coding sequence are called "advantageous mutators", as change in the number of repeats may lead to a change in their function. For example, over 40 or more CAG repeats in *ATXN1* will cause a neurodegenerative disorder called spinocerebellar ataxia type 1 (SCA1) (Banfi et al., 1994). SCA1 is characterized by motor deficits such as lack of motor control and speech misarticulation (Harding, 1982) as well as by cognitive deficits, such as impaired executive function. Intriguingly, the deletion of *ATXN1* is associated with lower IQs and autism spectrum disorders (Celestino-Soper et al., 2012) indicating that *ATXN1* may play a role in cognition. *SCA1* null mice showed significant impairments on the spatial Morris water maze test (Matilla et al., 1998), reiterating that the deletion of *ATXN1* may lead to learning deficits.

In addition to causing SCA1, ATXN1 was found to have a correlation with Alzheimer's disease (AD) in a genome wide association study (GWAS) (Zhang et al., 2010). AD is a fatal neurological disease associated with cognitive impairment and is the sixth leading cause of death in the United States ("US Death Rates from Alzheimer's Disease", 2017). A GWAS is an investigation into the association of genetic variants with particular traits. In this specific case, AD was the trait and *ATXN1* was the genetic variant. Although the correlation between *ATXN1* and AD has been established in multiple studies (Zhang et al., 2010; Bettens et al., 2010), there have been no examinations of the correlation between the number of CAG repeats and occurrence of AD using human DNA. In this project, we analyzed human DNA sequences for

the number of CAG repeats in order to determine whether a correlation between number of CAG repeats and AD exists. This was accomplished by analyzing DNA sequences to determine number of CAG repeats in *Atxn1* obtained from three different groups - AD patients, patients with mild cognitive impairment who are at a greater risk for acquiring AD, and control healthy individuals. We hypothesized that the number of CAG trinucleotide repeats in the DNA sequence is directly proportional to the incidence of AD because CAG repeats have already shown to be correlated with the onset of cognitive impairments in SCA1 (Banfi et al., 1994).

Materials and Methods

DNA Sequences

DNA from three different groups of individuals of ages 65 and above - individuals with AD, individuals with mild cognitive impairment, and healthy individuals that served as the control group - was obtained from the Dr. Ling Lee Laboratory. 5 samples from each group were obtained, with a total of 15 DNA sequences analyzed.

DNA Sequencing

The DNA corresponding to CAG repeat containing region of *ATXN1* was amplified using polymerase chain reaction (PCR). The primers used to amplify this repeat region were Rep-1/Rep-2 (Orr et al., 1993). Agarose gel electrophoresis was performed and the fluorescent bands were excised from the gel. DNA sequencing was performed by dideoxynucleotide chain termination method using a Zymoclean Gel DNA Recovery Kit (Zymo Research). Sequencing was completed by the University of Minnesota Genomic Center.

Results

15 DNA sequences from the three different groups were analyzed, with five samples in each group. The mean number of CAG repeats increased from the normal group to the group with

mild cognitive impairment and increased further in the AD group (Figure 1). There was no significant difference of number of CAG repeats between the three groups, with a p value of 0.215 obtained from a one-way ANOVA.

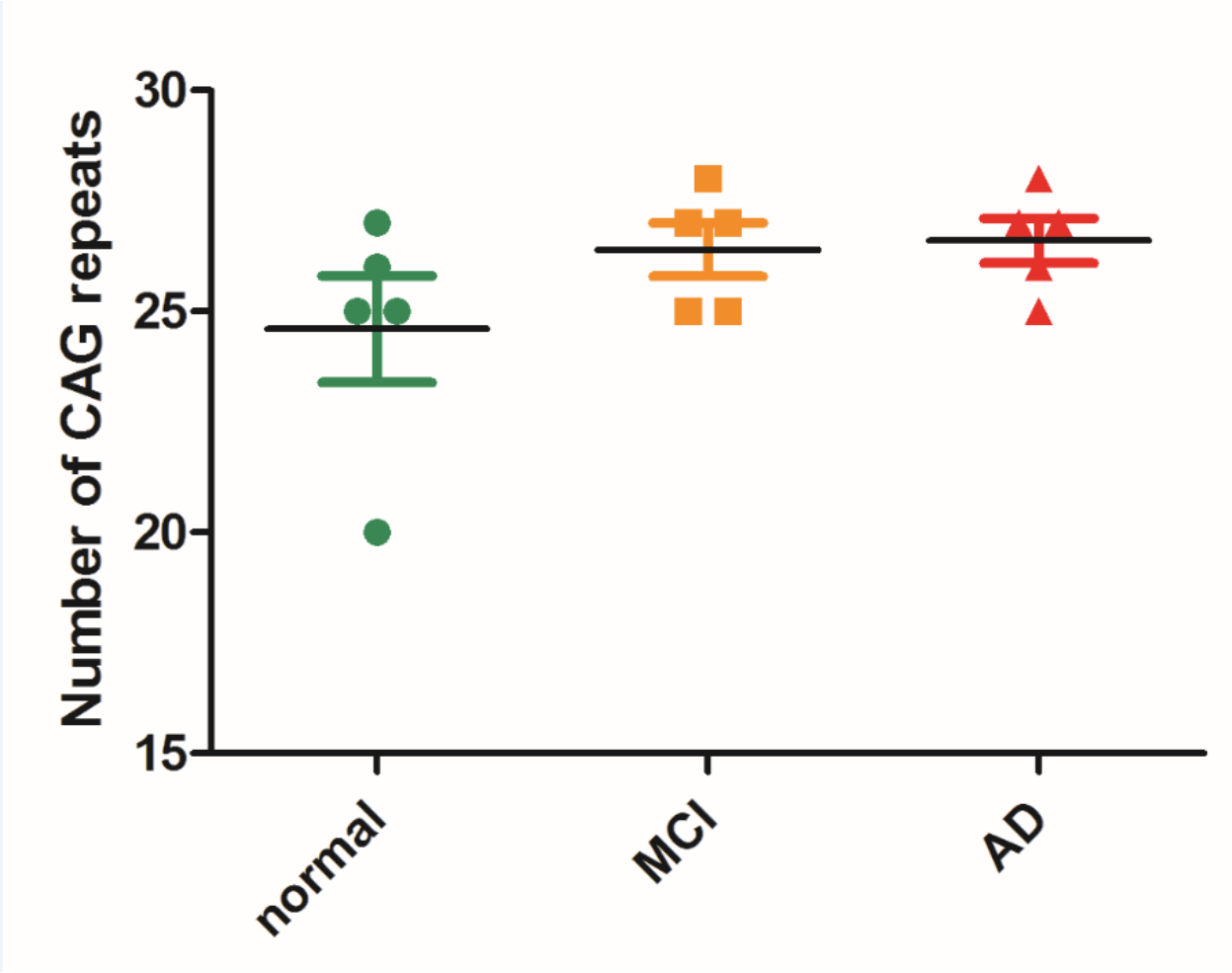

Figure 1. Number of CAG repeats for DNA sequences from normal individuals, individuals with MCI, and individuals with AD. One-way ANOVA (p = 0.215) showed that there was no significant difference between the three groups.

Discussion

A correlation between AD and *ATXN1* has been established in multiple studies (Zhang et al., 2010; Bettens et al., 2010), but there have been no studies exploring the correlation of the number of CAG repeats and occurrence of AD. Here we analyzed DNA sequences from three

different groups of individuals above the age of 65 - individuals with AD, individuals with mild cognitive impairment, and healthy individuals. Although the results of this experiment were not statistically significant, an increase in the number of CAG repeats has been proven to be associated with SCA1 and Huntington's disease (Banfi et al., 1994). These findings warrant a further examination of the correlation between CAG repeats and AD, in which the sample size should be increased in order to conduct a more conclusive analysis.

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