Increased cytokine levels in BA21 brain region of African Americans relative to Caucasians with Alzheimer’s Disease

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ABSTRACT

Tissue preparation: AD-confirmed tissue samples of BA21 (middle temporal gyrus) (see Figure 1) were obtained from U.S. brain banks which also contained the AP-2(2) genotype. Age at death, Braak Stage, and health status (where available) were similar across ethnicities. Tissue was pulverized under liquid nitrogen and maintained at -80°C.

A Bio-Plex Cytokine kit was used for protein assays according to the manufacturer’s protocol. The cytokine and buffer were homogenized using a UVP Fastprep, spun in a microcentrifuge for 20 min at 15,000 RPM and 4°C. The supernatant was aliquoted and stored at -80°C.

Methods: Protein assays were conducted using a Pierce BCA protein assay kit according to the manufacturer’s protocol. The 56 well plate reader was used to generate a standard curve and analyze the protein content of each sample with absorbance measured at 562 nm.

Multiplex assays: Cytokine assays were conducted using a Bio-Plex 200 multiplex assay system with an automated Bio-Plex Pro wash station. Standard curves for all assays were fitted using logistic SLP regression. Protein content for each sample was normalized to the recommended protein range.

Cytokines were assayed using the Bio-Plex Pro Human Cytokine panel. Lysate samples were normalized to a final protein concentration of 600 µg/ml. Only those analyses which were above the limit of detection for at least 90% of the samples included in statistical analyses as has been previously done (4).

Statistical Analyses: Levels of each analyte were analyzed via SigmaPlot using a two-way analysis of variance (ANOVA) with ethnicity, gender, and the interaction as factors. Where the data were not normally distributed, a base 10 log transformation was done.

METHODS AND RESULTS

Significant Ethnicity Effects: IL-10 levels were elevated in African Americans (p<0.01) (Figure 2); IL-8 levels were decreased in African Americans (p<0.05) (Figure 3); and CCL3 levels were elevated in African Americans (p<0.04) (Figure 4).

Figure 2: IL-10 levels are increased 106% in African Americans

Figure 3: IL-8 levels are decreased 36% in African Americans

Figure 4: CCL3 levels are increased 262% in African Americans

CONCLUSIONS

These results are the first describing ethnicity-related differences in cytokine levels in post-mortem brain tissue of AD cases.

Although levels of IL-8 in the CNS are reported to be increased in AD (1), IL-8 is also thought to be somewhat neuroprotective (7).

Increased CNS levels of IL-10 are commonly described in AD (5) and can result from activation of the NLRP1 inflammasome which is now believed to be critically involved in AD (6).

There is some evidence that CCL3 levels are increased in APoe4 or APoe2 background (3); however, APoe status was not available for our subjects.

The ethnicity-related differences in IL-8, IL-10, and CCL3 are consistent with increased AD severity in African Americans.

Whether such differences might result in ethnicity-related differences in language processing in those with AD is not clear.

It is not known if the significant gender differences are specific to AD cases or would be apparent in control cases as well.

REFERENCES


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BACKGROUND

- African Americans are disproportionately affected by Alzheimer’s Disease (AD), exhibiting a higher incidence of dementia and AD and increased symptom severity.
- There is evidence that increased incidence is related to higher risk even after adjustments for education, family dementia histories, and hypertension comorbidity (10).
- Increased knowledge of AD in African Americans and other minority populations is crucial for the goals of precision medicine.
- GWAS and other studies have identified AD-related genes and SNPs involved in inflammatory processes and chronic neuroinflammation is thought to exacerbate or even cause various neurodegenerative diseases.
- Potential ethnicity-related differences in neuroinflammation have not been thoroughly studied.
- The BA21 region (studied here) is critically involved in language processing and generation in humans and has been shown to be significantly affected by AD (2, 6, 11).

Figure 1: The middle temporal gyrus (in green) illustrating the BA21 region image from: https://www.ontbraininc.com/interactivemap/brainviewer.html