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

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ABSTRACT

Introduction: Although some studies have indicated few or no differences in AD neuropathology between African Americans and Caucasians, minorities are disproportionately affected by AD, exhibiting an earlier age of onset and more severe symptoms. However, many research studies do not explicitly state the race or ethnicity of their subjects. Because neuroinflammation is thought to play a significant role in AD, we measured the levels of inflammatory cytokines in the BA21 brain region of African Americans and Caucasians with AD. This temporal lobe brain region has been implicated in cognitive task performance of those with AD (2).

Methods: Post-mortem BA21 brain tissue (n=6/sex/race) was obtained from five U.S. brain banks which also confirmed the AD pathology. Mean age at death was 81.1 years for African Americans and 81.6 years for Caucasians. Tissue was pulverized under liquid nitrogen, homogenized in lysis buffer, and centrifuged to obtain tissue lysates. Protein concentration was determined prior to analysis of cytokines using a multiplex assay system (Bio-Rad 200) and a 40-plex human cytokine panel. Levels of cytokines were analyzed via 2-way ANOVAs with sex, race, and the interaction as factors.

Results: Levels of IL-1β were 109% higher in African Americans ($p<0.01$) and levels of IL-8 were 35% lower in African Americans ($p<0.03$). Neither of those levels was significantly affected by or interacted with sex. Levels of IL-16, CCL25, CCL26, and CX3CL1 were decreased 19-51% in women relative to men ($p<0.05$ for all).

Conclusions: IL-8 may play a protective role in AD pathology in that it appears to inhibit Aβ-induced apoptosis and increase BDNF (7). Increased levels of IL-1β may contribute to increased AD severity in African Americans by resulting in increased deposition and decreased clearing of Aβ plaques. These results implicate increased neuroinflammation in African Americans. More specifically, the NLRP3 inflammasome controls levels of IL-1β and previous studies have implicated this specific inflammasome in AD pathogenesis. Our future studies include measurement of neurodegenerative endpoints in this brain region as well as investigation of potential mechanisms, including potential race-related SNP differences in NLRP3 genes.

BACKGROUND

- African Americans are disproportionately affected by Alzheimer's Disease (AD), exhibiting a higher incidence of dementia and AD and increased symptom severity.
- That increased incidence/risk remains high even after adjustments for education, family dementia histories, and hypertension co-morbidity (10).
- Increased knowledge of AD in African Americans and other minority populations is crucial to 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 (8, 12) and has been shown to be significantly affected by AD (2, 6, 11).

METHODS AND RESULTS

Tissue preparation: AD-confirmed tissue samples of BA21 (middle temporal gyrus) (see Figure 1) were obtained from U.S. brain banks (3-6/gender/ethnicity). Age at death, Braak Stage, and health status (where available) were similar across ethnicities. Tissue was pulverized under liquid nitrogen and maintained at -80ºC.

A Bio-Plex Cell Lysis kit was used for protein assays according to the manufacturer's protocol. Tissue and buffer were homogenized using a MP FastPrep, spun in a microcentrifuge for 20 min at 15,000 RPM and 4ºC. The supernatant was aliquoted and stored at -80ºC.

Protein assays were conducted using a Pierce BCA protein assay kit according to the manufacturer's protocol. A 96 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 immunoassay system with an automated Bio-Plex Pro wash station. Standard curves for all assays were fitted using logistic 5PL regression. Protein content for each sample was normalized to the recommended protein range.

Cytokines were assayed using the Bio-Plex Pro Human Chemokine panel. Lysate samples were normalized to a final protein concentration of 600 µg/ml. Only those analytes which were above the limit of detection for at least 90% of the subjects were 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.

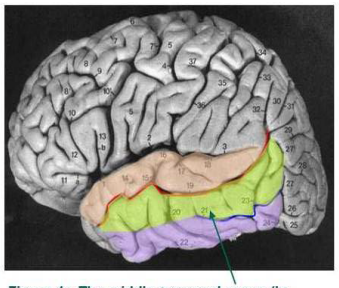


Figure 1: The middle temporal gyrus (in green) illustrating the BA21 region (image from: www.culhamlab.ssc.uwo.ca/fmri4newbies/PrimeronCorticalSulci.html)

Significant Ethnicity Effects: IL-1β levels were elevated in African Americans ($p<0.01$) (Figure 2); IL-8 levels were decreased in African Americans ($p<0.03$) (Figure 3); and CCL3 levels were elevated in African Americans ($p<0.04$) (Figure 4).

Figure 2: IL-1β levels are increased 109% in African Americans

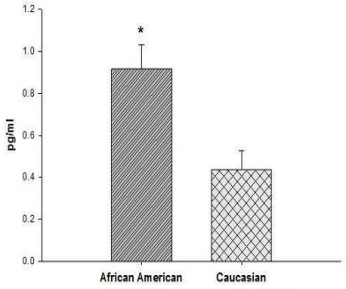


Figure 3: IL-8 levels are decreased 35% in African Americans

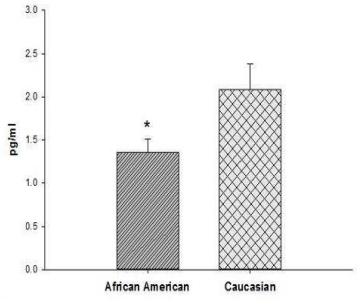
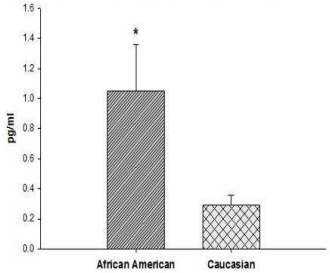


Figure 4: CCL3 levels are increased 262% in African Americans



Gender Effects: IL-16, CXC3CL1, CCL25 and CCL26 levels were significantly decreased in females relative to males. (all $p<0.05$). There were no significant ethnicity x gender interactions.

Figure 5: IL-16 levels are decreased 51% in females

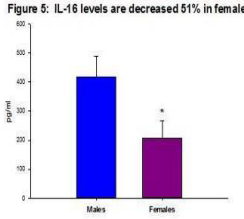


Figure 6: CCL25 levels are decreased 21% in females

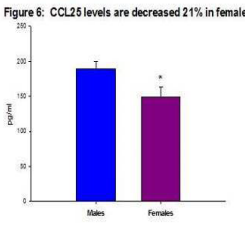


Figure 7: CCL26 levels are decreased 19% in females

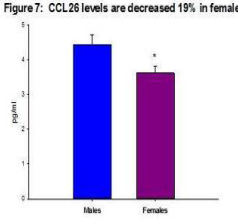
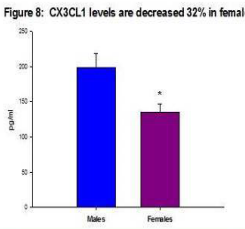


Figure 8: CX3CL1 levels are decreased 32% in females



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-1β are commonly described in AD (5) and can result from activation of the NLRP3 inflammasome which is now believed to be critically involved in AD (9).
- There is some evidence that CCL3 levels are increased in a *APOEε4* or *APOEε2* background (3); however, APOE status was not available for our subjects.
- The ethnicity-related differences in IL-8, IL-1β, 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.

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