

**TITLE:** Infection with *Chlamydia Pneumoniae* alters calcium-associated gene regulation and processes in neuronal cells and monocytes: Implications for Alzheimer's Disease.

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## **ABSTRACT:**

## **BACKGROUND**

First proposed by Khachaturian in 1994, the calcium hypothesis postulates that sustained disturbance of intracellular calcium is the leading cause of neurodegenerative disorders. Studies showing alteration in calcium signaling in both sporadic and familial Alzheimer's disease (AD) support this hypothesis. Intracellular calcium signaling is tightly regulated in time, intensity, and space, and is responsible for a variety of neuronal functions. Calcium influx from the extracellular environment modulates calcium levels, as do intracellular stores in the endoplasmic reticulum. The focus of this study was to test various calcium related genes in both monocytes and neuronal cells. Previous studies have shown that cells infected with *Chlamydia pneumoniae* (Cpn) exhibit altered protein processing, such as amyloid and tau modification, consistent with those found in AD. We expect to see significant alterations in calcium genes, as well as their protein products in Cpn infected cells. Every calcium gene has a unique function in the cell. Determining which genes are up or down regulated following infection may provide insight into how the neurodegeneration process observed in AD is initiated by Cpn infections.

## **METHODS**

Using the AR39 strain of Cpn at a MOI of one, both THP-1 monocytes and SKNMC (ATCC) neuronal cells were infected for 48 hours. Cells were analyzed using Real-time PCR microarrays (SABiosciences) for calcium related genes. Protein regulation was recorded using fast western blotting with three calcium protein antibodies. Monocytes and neuronal cells were also labeled with 61C75 directly conjugated antibody to FITC (Fitzgerald, Inc.) for verification of infection with Cpn.

## **RESULTS**

Calcium genes with a two fold increase or decrease in Cpn-infected cells were noted. Twelve genes exhibited this behavior in both THP-1 and SKNMC cells. Of the twelve genes, S100A12 (S100 Calcium Binding Protein) had the greatest degree of up-regulation (approx. 35x) in THP-1 monocytes. NF-1 (Neurofibromin-1) had the highest degree of down-regulation in both THP-1 and SKNMC cells. CHGA (Chromogranin A) was up-regulated two fold in THP-1 cells and down-regulated two fold in SKNMC. In western blot protein analysis, S100A12 was up-regulated in SKNMC cells and showed no protein product in THP-1 monocytes; NF-1 was up-regulated in both THP-1 and SKNMC cells; and CHGA was down-regulated in both THP-1 and SKNMC cells. Western blot analysis was confirmed by immunofluorescence for NF-1 and S100A12 in SKMNC neuronal cells.

## **CONCLUSIONS**

Our data suggest that Cpn alters calcium-related gene regulation and levels of protein products of at least three calcium genes, perhaps to maintain an environment beneficial to its survival. These changes may be associated with the disturbances in intracellular calcium previously observed in AD, and may elucidate how Cpn may affect normal cellular processes, thereby contributing to the neuropathology seen in AD.