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Chlamydia pneumoniae infection of neuronal cells induces changes in calcium-associated gene expression consistent with Alzheimer's disease

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Chlamydia pneumoniae infection of neuronal cells induces changes in calcium-associated gene expression consistent with Alzheimer's disease

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Abstract

Background and Significance: Previous studies have shown that cells infected with *Chlamydia pneumoniae* (Cpn) exhibit altered gene expression consistent with that observed in Alzheimer's disease (AD). Furthermore, AD neurodegeneration has been linked to dysregulation of intracellular calcium and calcium-related processes. Therefore, we hypothesize that one mechanism by which pathogenesis evolves in AD is through infection-induced changes in expression of calcium-related genes.

Objectives: To determine if infection of neuronal cells with Cpn alters expression of calcium-related genes associated with neurodegeneration.

Methods: SK-N-MC neuronal cells were infected with Cpn (AR39 strain; MOI=1) for 3 to 72 hours, then calcium-related genes were screened with real-time PCR microarrays (SABiosciences PAHS-066).

Results: Following infection, approximately 29 genes displayed regulation changes of 2-fold or greater, including genes pertaining to neurotransmitters, cell cycle and immune regulators, and other calcium-responsive elements. Genes involved in synaptic function and memory such as AREG, ATF3, EGR2 and GEM were initially up regulated, then fell to baseline or below by 72 hours. Many of the affected genes have been implicated in AD pathogenesis.

Conclusions: Our data suggest that Cpn alters calcium-related gene expression in host neurons consistent with calcium dysfunction previously documented in AD. This study may elucidate how, in its effort to establish a favorable environment, Cpn could affect cellular processes that contribute to AD pathogenesis.

Introduction

Alzheimer's disease (AD) is considered to be the most common form of late-life dementia affecting memory, cognition, personality, and behavior (1). The cause(s) of AD are thought to involve both environmental and genetic factors. There are two main forms of the disease: familial or early-onset that typically affects patients below the age of 60 and sporadic or late-onset affecting those older than 60 years. The presence of a combination of mutated genes, coding for apparently abnormally functioning proteins, is a determining factor in early-onset AD (1). The specific risk factors/causes of late-onset AD remain to be elucidated, but environmental factors including trauma, diet, and infection may play a role.

The accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles leading to degeneration of specific populations of neurons are the two key histopathological entities characterizing AD. Beta-amyloid peptide (Aβ) is derived from the amyloid precursor protein (APP) and is a major component of the extracellular deposits in AD brains. One mechanism by which Aβ may contribute to the overall pathogenesis of AD is through the disruption of calcium homeostasis in neurons. Studies have shown that resting free calcium levels are lower in cells from Alzheimer's patients than in controls (2). Furthermore, alteration in calcium signaling has been observed in both sporadic and familial AD (3).

Calcium signaling is tightly regulated in time, intensity, and space, and is responsible for a variety of neuronal functions. In a healthy neuronal cell, calcium levels are regulated at both the gene and protein level. Calcium influx from the extracellular environment modulates calcium levels, as do intracellular stores in the endoplasmic reticulum. First proposed by Khachaturian in 1994, the calcium hypothesis postulates that sustained disturbances of intracellular calcium are a leading cause of neurodegenerative disorders (3).

Previously, we detected infection with *Chlamydia pneumoniae* (Cpn) in late-onset AD brains (4). This infection was found in glial and neuronal cells, and may initiate specific damage resulting in neurodegeneration. In this regard, we hypothesize that calcium homeostasis is altered by the presence of Cpn and possibly other pathogens, thereby affecting both acute and long-term events. To test this hypothesis, we used RT-PCR to evaluate the regulation of 84 calcium-related genes in neuronal cells over the duration of 3 to 72hrs following Cpn infection.

Materials & Methods

Neuronal cells, SK-N-MC (ATCC, HTB-10), were infected with ATCC's AR39 strain of *Chlamydia pneumoniae* at a MOI of 1 for 3-72 hours. The RT^2 Profiler PCR Human cAMP/Ca^{2+} PathwayFinder Array from Qiagen (SABiosciences) was used to analyze the expression of calcium-related genes. All experiments were performed in triplicate.

References

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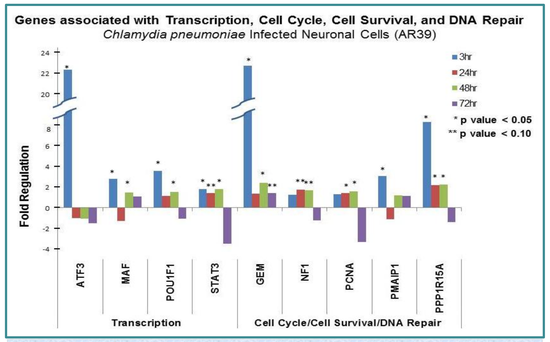
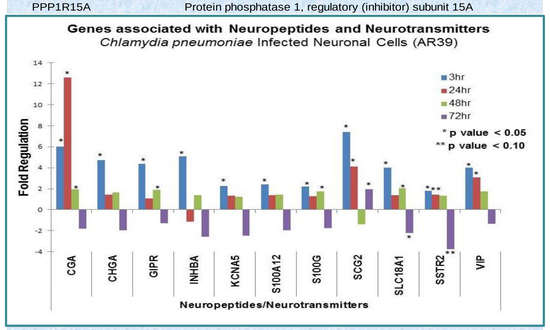
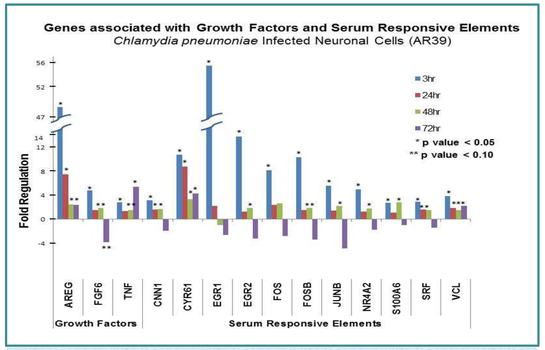
Results



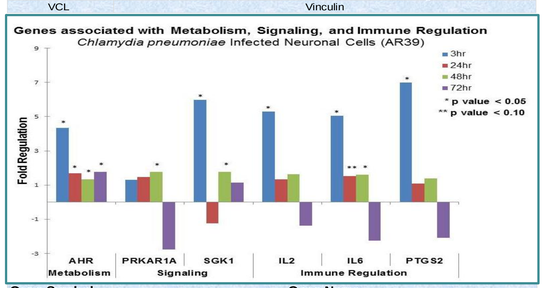
Gene Symbol	Gene Name
Transcription	
ATF3	Activating transcription factor 3
MAF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog
POU1F1	POU class 1 homeobox 1
STAT3	Signal transducer and activator of transcription 3
Cell Cycle / Cell Survival / DNA Repair	
GEM	GTP binding protein overexpressed in skeletal muscle
NF1	Neurofibromin 1
PCNA	Proliferating cell nuclear antigen
PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1
PPP1R15A	Protein phosphatase 1, regulatory (inhibitor) subunit 15A



Gene Symbol	Gene Name
Neuropeptides / Neurotransmitters	
CGA	Chorionic gonadotropin A
CHGA	Chromogranin A
GIPR	Gastric inhibitory polypeptide receptor
INHBA	Inhibin, beta A
KCNA5	Potassium voltage-gated channel, shaker-related subfamily, member 5
S100A12	S100 calcium binding protein A12
S100G	S100 calcium binding protein G
SCG2	Secretogranin II
SLC18A1	Solute carrier family 18 (vesicular monoamine), member 1
SSTR2	Somatostatin receptor 2
VIP	Vasoactive intestinal peptide

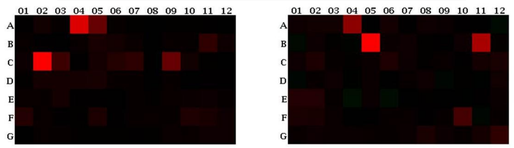


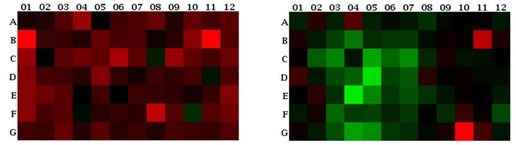
Gene Symbol	Gene Name
Growth Factors	
AREG	Amphiregulin
FGF6	Fibroblast growth factor 6
TNF	Tumor necrosis factor
Serum Responsive elements	
CNN1	Calponin 1
CYR61	Cysteine-rich, angiogenic inducer, 61
EGR1	Early growth response 1
EGR2	Early growth response 2
FOS	FBJ murine osteosarcoma viral oncogene homolog
FOSB	FBJ murine osteosarcoma viral oncogene homolog B
JUNB	Jun B proto-oncogene
NR4A2	Nuclear receptor subfamily 4, group A, member 2
S100A6	S100 calcium binding protein A6
SRF	Serum response factor (c-fos serum response element-binding transcription factor)
VCL	Vinculin



Gene Symbol	Gene Name
Metabolism	
AHR	Aryl hydrocarbon receptor
Signaling	
PPKAR1A	Protein kinase, cAMP-dependent, regulatory, type I, alpha
SGK1	Serum/glucocorticoid regulated kinase 1
Immune Regulation	
IL2	Interleukin 2
IL6	Interleukin 6
PTGS2	Prostaglandin-endoperoxide synthase 2

Heat Map Analysis of 84 Calcium-Related Genes

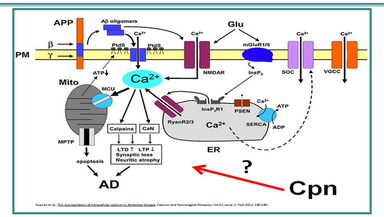




Layout	1	2	3	4	5	6	7	8	9	10	11	12
A	ADRB1	AHR	AMD1	AREG	ATF3	BCL2	BDNF	BRCA1	CALB1	CALB2	CALM1	CALR
B	CCNA1	CCND1	CDK5	CDKN2B	CGA	CHGA	CNN1	CREB1	CREM	CTF1	CYR61	DDIT3
C	DUSP1	EGR1	EGR2	ENO2	FGF6	FOS	FOSB	GCG	GEM	GIPR	HK2	HSPA4
D	HSPA5	IL2	IL6	INHBA	JUNB	JUND	KCNA5	LDHA	MAF	MIF	NCAM1	NF1
E	NOS2	NPY	NR4A2	PCK2	PCNA	PENK	PER1	PLAT	PLN	PMAIP1	POU1F1	POU2AF1
F	PPP1R15A	PPP2CA	PRKAR1A	PRL	PTGS2	RB1	S100A12	S100A6	S100G	SCG2	SGK1	SLC18A1
G	SOD2	SRF	SST	SSTR2	STAT3	TACR1	TGFB3	TH	THBS1	TNF	VCL	VIP

Conclusions

***Chlamydia pneumoniae* alters several genes and proteins associated with calcium signaling in neuronal cells. The disruption of calcium signaling is congruent with neurodegeneration as observed in Alzheimer's disease.**



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