in vivo *36*: 2650-2653 (2022)
doi:10.21873/invivo.12999

Circulating *Chlamydia Trachomatis* Antigens in Subjects With Alzheimer's Disease

STEVEN LEHRER[1] and PETER H. RHEINSTEIN[2]

[1]*Department of Radiation Oncology, Icahn School of Medicine at Mount Sinai, New York City, NY, U.S.A.;*
[2]*Severn Health Solutions, Severna Park, MD, U.S.A.*

Abstract. *Background/Aim: Chlamydia pneumoniae (C. pneumoniae) is implicated in the pathogenesis of Alzheimer's disease (AD). Chlamydial elementary and reticulate bodies have been identified in tissues from afflicted AD brain regions by electron and immunoelectron microscopy, whereas similar tests of non-AD brains were negative for the bacterium. Studies in mice have shown that C. pneumoniae can rapidly penetrate the central nervous system by entering glia and causing beta amyloid deposition via the nerves between the nasal cavity and the brain, which serve as invasion pathways. Materials and Methods: We used data from the UK Biobank (UKBB) to assess the relationship of chlamydia and AD. Circulating C. pneumoniae antigen measurements were not available, but UKBB data field 23037 held measurements of PorB antigen for Chlamydia trachomatis (C. trachomatis). We used C. trachomatis as a surrogate for C. pneumoniae since serum cross-reactivity to C. trachomatis and C. pneumoniae antigens occurs in patients with documented infection and in healthy children as revealed by microimmunofluorescence and immunoblotting techniques. Single nucleotide polymorphism (SNP) data for rs429358 and rs7412 were used to impute ApoE genotypes. Results: PorB antigen levels for C. trachomatis were significantly higher in subjects with AD (p=0.007). PorB antigen levels were not related to ApoE genotype (e3e3, e3e4, e4e4) p=0.783. To control for the effects of age, sex, educational level, and apoE genotype, logistic regression analysis was performed. AD was the dependent variable. Independent variables were sqrt PorB antigen for C. trachomatis, age, sex, educational level, apoE genotype. AD odds ratio (OR) increased 1.156 for each unit increase of sqrt PorB antigen for C. trachomatis and the effect was significant (p=0.004). Conclusion: PorB antigens for C. trachomatis being significantly higher in subjects with AD, corroborates previous studies demonstrating that C. pneumoniae inflammation appears to play a role in AD development. AD may result from the reactivation of embryologic processes and pathways silenced at birth. A trigger for the reactivation may be bacterial or viral infections. Further studies are warranted.*

Correspondence to: Dr. Steven Lehrer, Box 1236 Radiation Oncology, Mount Sinai Medical Center, 1 Gustave L. Levy Place, New York 10029, NY, U.S.A. Tel: +1 2127657132, e-mail: steven.lehrer@mssm.edu

Key Words: Neurodegeneration, infectious disease, amyloid, apoE.

Alzheimer's disease (AD) is a progressive inflammatory brain disease that affects over 45 million people worldwide and is caused by a mix of inherited and environmental factors that produce brain inflammation, neuronal cell death, and dementia. Nearly 30 years ago, infectious organisms in the brain were postulated to have a role in Alzheimer's disease (AD). Recently, several new findings have rekindled interest in the microbial explanation of AD (1). One of these is the detection of *Chlamydia pneumoniae* (*C. pneumoniae*) in brain tissues of AD patients (2, 3).

Chlamydial elementary and reticulate bodies have been identified in tissues from afflicted AD brain regions by electron and immunoelectron microscopy, whereas similar tests of non-AD brains were negative for the bacterium. *C. pneumoniae* culture tests of a subgroup of afflicted AD brain tissues were strongly positive, but equivalent investigations of non-AD brain tissues were negative (4). Transcripts from two essential *C. pneumoniae* genes were found in afflicted areas of AD brains, but not in controls, according to reverse transcription (RT)-PCR experiments using RNA from affected areas of AD brains. *C. pneumoniae* was found in pericytes, microglia, and astroglia in AD brains but not in controls, according to a study involving immunohistochemical investigation (5). Further immunolabelling experiments, notably in areas of neuropathology in the AD brain, identified intracellular *C. pneumoniae*. *C. pneumoniae* is thus prevalent, alive, and transcriptionally active in areas of neuropathology in the AD brain, implying that infection with the organism is

Table I. *Demographics of subjects included in the study.*

	Control	AD
N	9,412	17
Age (years)	56±8.1	65±3.5
Sex	57% Females	67% Females
Years of education	14.6±4.8	13.5±4.8
Race	95% White	100% White

AD: Alzheimer's disease.

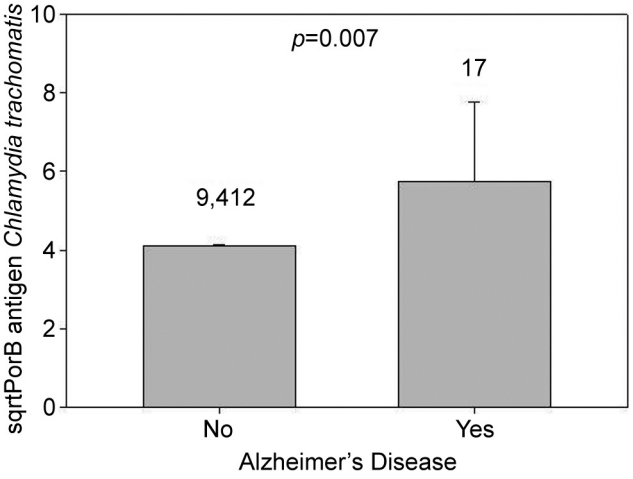

Figure 1. *Sqrt PorB antigens Chlamydia trachomatis in subjects without (no) and with (yes) Alzheimer's disease (mean+SEM). Number of subjects in each group above corresponding bar. Output from the Luminex reader produced quantitative data expressed in median fluorescence intensity (MFI) values per pathogen-specific antigen and serum.*

a risk factor for AD (5). In addition, studies in mice have shown that *C. pneumoniae* can rapidly penetrate the central nervous system by entering glia and causing beta amyloid deposition *via* the nerves between the nasal cavity and the brain, which serve as invasion pathways (6).

In the current study, we used data from the UK Biobank (UKBB) to assess the relationship of chlamydia and AD.

Materials and Methods

The UKBB is a large prospective observational study of men and women. Participants have been recruited from across 22 Centers located throughout England, Wales, and Scotland between 2006 and 2010 and continue to be longitudinally followed for capture of subsequent health events (7). This methodology resembles that of the ongoing Framingham Heart Study (8), with the exception that the UKBB program collects postmortem samples, which Framingham did not. Our UKBB application was approved as UKBB project 57245 (S.L., P.H.R.). Our analysis included subjects with Alzheimer's disease, designated by ICD10 G30.

Table II. *Logistic regression with 95% confidence intervals, lower bound (L.B.), upper bound (U.B.).*

	95% L.B.	O.R.	95% U.B.	*p*-Value
Sex	0.205	0.612	1.823	0.379
Age	1.110	1.261	1.432	<0.001
Education	0.906	1.005	1.115	0.922
e3e3 *vs*. e4e4	0.017	0.154	1.364	0.093
e3e3 *vs*. e3e4	0.084	0.692	5.681	0.732
Sqrt chlamydia	1.048	1.156	1.275	0.004

Dependent variable: Alzheimer's disease (AD); Independent variables: sex, age, years education, e3e3 *vs*. e4e4, e3e3 *vs*. e3e4, sqrt chlamydia. Odds ratio (O.R.)=1.156 associated with chlamydia indicates that AD odds ratio increased 1.156 for each unit increase of sqrt PorB antigen for *Chlamydia trachomatis* and the effect was significant (p=0.004). The overall association of apoE e4 carriers and homozygotes with AD was significant (p=0.004).

Ethics approval. UKBB has approval from the Northwest Multicenter Research Ethics Committee (MREC), which covers the UK. It also sought the approval in England and Wales from the Patient Information Advisory Group (PIAG) for gaining access to information that would allow it to invite people to participate. PIAG has since been replaced by the National Information Governance Board for Health & Social Care (NIGB). In Scotland, UK Biobank has approval from the Community Health Index Advisory Group (CHIAG).

UKBB phenotype data are organized in categories that are divided into data fields. With the search function on the UK Biobank site (https://www.ukbiobank.ac.uk/) phenotype information can be located. Information on single nucleotide polymorphisms can be retrieved from the 24 individual chromosome files, ukb22828. Sex, age, and education information was obtained from UK Biobank category 100094, baseline characteristics.

UKBB selected 20 pathogens for a panel of antigen measurements. The antigens were chosen because they are either established risk factors for outcomes, such as cancer, and cardiovascular or neurodegenerative diseases, or are of novel scientific interest. The final selection of antigens was based on their known biological functions and/or existing assays (9).

Circulating *C. pneumoniae* antigen measurements were not selected, but UKBB data field 23037 held measurements of PorB antigen for *Chlamydia trachomatis* (*C. trachomatis*). PorB is the major outer membrane protein from Neisseria meningitidis, a TLR2/1 ligand, which can significantly increase co-stimulatory ligand expression and cytokine production in antigen presenting cells (10). We used *C. trachomatis* as a surrogate for *C. pneumoniae* since serum cross-reactivity to *C. trachomatis* and *C. pneumoniae* antigens occurs in patients with documented infection and in healthy children by microimmunofluorescence and immunoblotting techniques (11). Therefore, *C. trachomatis* measurements can serve as an acceptable substitute for *C. pneumoniae* measurements.

Output from the Luminex reader produced quantitative data expressed in median fluorescence intensity (MFI) values per pathogen-specific antigen and serum (9). Single nucleotide polymorphism (SNP) data for rs429358 and rs7412 were used to

determine *ApoE* genotypes (12) since we wished to exclude the effect of *ApoE4* in our analysis. The *ApoE4* isoform significantly increases risk of AD and would confound our results if no correction were made.

Statistical analysis was performed with the SPSS 26 (IBM, Armonk, NY, USA) software. Multivariate analysis was performed with logistic regression.

Results

Demographics of subjects are presented in Table I. We retrieved porB antigen data from the UKBB data field 23037, field designation blood antigens — biological samples. To normalize PorB antigen for *C. trachomatis* measured values, square root transformation was performed.

PorB antigen levels *versus* AD are shown in Figure 1. Levels are significantly higher in subjects with AD (p=0.007, two tailed t-test). PorB antigen levels were unrelated to the *ApoE* genotype (e3e3, e3e4, e4e4) p=0.783. The lack of relationship to *ApoE* genotype is important because genotype apoE4 increases the risk of AD (13).

To control for the effects of age, sex, educational level, and *ApoE* genotype, logistic regression analysis was performed. AD was the dependent variable. Independent variables were sqrt PorB antigen for *C. trachomatis*, age, sex, educational level, apoE genotype. Sqrt transformation of PorB antigen for *C. trachomatis* was performed to normalize the values. Results are shown in Table II. AD odds ratio (OR) increased 1.156 for each unit increase of sqrt *PorB* antigen for *C. trachomatis* and the effect was significant (p=0.004).

Discussion

According to our findings, the PorB antigen for *C. trachomatis* being significantly higher in subjects with AD, corroborates previous studies demonstrating that *C. pneumoniae* inflammation appears to play a role in AD development (14). *C. pneumoniae* has been shown to induce the production of IL-6 and TNF-alpha, which are responsible for neuronal death in microglial cells and astrocytes, as well as the synthesis of IL-1 and IL-8, which are known to cause neurodegeneration in Alzheimer's disease by activating nitric oxide synthase in persistently infected monocytes (15). Other mechanisms by which *C. pneumoniae* may contribute to the development and progression of AD have been documented (16-19).

A weakness in our study is that we used PorB antigens for *Chlamydia trachomatis* as a surrogate for *C. pneumoniae* antigens. Thus, we are not able to say with absolute certainty that the association of PorB antigens for *Chlamydia trachomatis* with AD we identified confirms the previously documented association of AD with *C. pneumoniae*. Another weakness is that *C. trachomatis*, a sexually transmitted disease (STD), might have been acquired with other STDs such as syphilis, and tertiary syphilis could have been misdiagnosed as AD. We were not able to confirm interaction of PorB antigen for *C. trachomatis* and *ApoE* isoform, but our sample size was small. An interaction of PorB antigen for *C. trachomatis* and *ApoE* isoform would be important to confirm since the mechanism whereby *ApoE* isoforms change the risk of AD is not understood. AD may result from reactivation of embryologic processes and pathways silenced at birth. A trigger for the reactivation may be bacterial or viral infections (20, 21), such as *C. trachomatis*. Further studies are warranted.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Dr. Lehrer and Dr. Rheinstein contributed equally to the conception, writing, and data analysis of this study.

Acknowledgements

This work was supported in part through the computational resources and staff expertise provided by Scientific Computing at the Icahn School of Medicine at Mount Sinai. Research reported in this paper was supported by the Office of Research Infrastructure of the National Institutes of Health under award numbers S10OD018522 and S10OD026880. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1 Itzhaki RF, Golde TE, Heneka MT and Readhead B: Do infections have a role in the pathogenesis of Alzheimer disease? Nat Rev Neurol *16(4)*: 193-197, 2020. PMID: 32152461. DOI: 10.1038/s41582-020-0323-9

2 Gérard HC, Dreses-Werringloer U, Wildt KS, Deka S, Oszust C, Balin BJ, Frey WH 2nd, Bordayo EZ, Whittum-Hudson JA and Hudson AP: Chlamydophila (Chlamydia) pneumoniae in the Alzheimer's brain. FEMS Immunol Med Microbiol *48(3)*: 355-366, 2006. PMID: 17052268. DOI: 10.1111/j.1574-695X.2006.00154.x

3 Dreses-Werringloer U, Bhuiyan M, Zhao Y, Gérard HC, Whittum-Hudson JA and Hudson AP: Initial characterization of Chlamydophila (Chlamydia) pneumoniae cultured from the late-onset Alzheimer brain. Int J Med Microbiol *299(3)*: 187-201, 2009. PMID: 18829386. DOI: 10.1016/j.ijmm.2008.07.002

4 Balin BJ and Appelt DM: Role of infection in Alzheimer's disease. J Am Osteopath Assoc *101(12 Suppl Pt 1)*: S1-S6, 2001. PMID: 11794745.

5 Balin BJ, Gérard HC, Arking EJ, Appelt DM, Branigan PJ, Abrams JT, Whittum-Hudson JA and Hudson AP: Identification and localization of Chlamydia pneumoniae in the Alzheimer's brain. Med Microbiol Immunol *187(1)*: 23-42, 1998. PMID: 9749980. DOI: 10.1007/s004300050071

6 Chacko A, Delbaz A, Walkden H, Basu S, Armitage CW, Eindorf T, Trim LK, Miller E, West NP, St John JA, Beagley KW and Ekberg JAK: Chlamydia pneumoniae can infect the central nervous system *via* the olfactory and trigeminal nerves and contributes to Alzheimer's disease risk. Sci Rep *12(1)*: 2759, 2022. PMID: 35177758. DOI: 10.1038/s41598-022-06749-9

7 Arthur RS, Wang T, Xue X, Kamensky V and Rohan TE: Genetic factors, adherence to healthy lifestyle behavior, and risk of invasive breast cancer among women in the UK Biobank. J Natl Cancer Inst *112(9)*: 893-901, 2020. PMID: 31899501. DOI: 10.1093/jnci/djz241

8 Mahmood SS, Levy D, Vasan RS and Wang TJ: The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. Lancet *383(9921)*: 999-1008, 2014. PMID: 24084292. DOI: 10.1016/S0140-6736(13)61752-3

9 Brenner N and Waterboer T: Serological measurement of infectious agents in UK biobank: A pilot study in 10,000 samples. Available at: https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/infdisease.pdf [Last accessed on August 29, 2022]

10 Lisk C, Yuen R, Kuniholm J, Antos D, Reiser ML and Wetzler LM: Toll-like receptor ligand based adjuvant, PorB, increases antigen deposition on germinal center follicular dendritic cells while enhancing the follicular dendritic cells network. Front Immunol *11*: 1254, 2020. PMID: 32636846. DOI: 10.3389/fimmu.2020.01254

11 Gonen R, Shemer-Avni Y, Csángó PA, Sarov B and Friedman MG: Serum reactivity to Chlamydia trachomatis and C. pneumoniae antigens in patients with documented infection and in healthy children by microimmunofluorescence and immunoblotting techniques. APMIS *101(9)*: 719-726, 1993. PMID: 8240791.

12 Kuo CL, Pilling LC, Atkins JL, Masoli JAH, Delgado J, Kuchel GA and Melzer D: APOE e4 genotype predicts severe COVID-19 in the UK Biobank community cohort. J Gerontol A Biol Sci Med Sci *75(11)*: 2231-2232, 2020. PMID: 32451547. DOI: 10.1093/gerona/glaa131

13 Mortimer JA, Snowdon DA and Markesbery WR: The effect of APOE-epsilon4 on dementia is mediated by Alzheimer neuropathology. Alzheimer Dis Assoc Disord *23(2)*: 152-157, 2009. PMID: 19484916. DOI: 10.1097/wad.0b013e318190a855

14 Di Pietro M, Filardo S, Romano S and Sessa R: *Chlamydia trachomatis* and *Chlamydia pneumoniae* interaction with the host: latest advances and future prospective. Microorganisms *7(5)*: 140, 2019. PMID: 31100923. DOI: 10.3390/microorganisms7050140

15 Balin BJ, Hammond CJ, Little CS, Hingley ST, Al-Atrache Z, Appelt DM, Whittum-Hudson JA and Hudson AP: *Chlamydia pneumoniae*: an etiologic agent for late-onset dementia. Front Aging Neurosci *10*: 302, 2018. PMID: 30356749. DOI: 10.3389/fnagi.2018.00302

16 Appelt DM, Roupas MR, Way DS, Bell MG, Albert EV, Hammond CJ and Balin BJ: Inhibition of apoptosis in neuronal cells infected with Chlamydophila (Chlamydia) pneumoniae. BMC Neurosci *9*: 13, 2008. PMID: 18218130. DOI: 10.1186/1471-2202-9-13

17 Bu XL, Yao XQ, Jiao SS, Zeng F, Liu YH, Xiang Y, Liang CR, Wang QH, Wang X, Cao HY, Yi X, Deng B, Liu CH, Xu J, Zhang LL, Gao CY, Xu ZQ, Zhang M, Wang L, Tan XL, Xu X, Zhou HD and Wang YJ: A study on the association between infectious burden and Alzheimer's disease. Eur J Neurol *22(12)*: 1519-1525, 2015. PMID: 24910016. DOI: 10.1111/ene.12477

18 Carter C: Alzheimer's disease: APP, gamma secretase, APOE, CLU, CR1, PICALM, ABCA7, BIN1, CD2AP, CD33, EPHA1, and MS4A2, and their relationships with herpes simplex, C. Pneumoniae, other suspect pathogens, and the immune system. Int J Alzheimers Dis *2011*: 501862, 2011. PMID: 22254144. DOI: 10.4061/2011/501862

19 Loeb MB, Molloy DW, Smieja M, Standish T, Goldsmith CH, Mahony J, Smith S, Borrie M, Decoteau E, Davidson W, McDougall A, Gnarpe J, O'DONNell M and Chernesky M: A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer's disease. J Am Geriatr Soc *52(3)*: 381-387, 2004. PMID: 14962152. DOI: 10.1111/j.1532-5415.2004.52109.x

20 Lehrer S and Rheinstein PH: Alzheimer's disease and Parkinson's disease may result from reactivation of embryologic pathways silenced at birth. Discov Med *31(163)*: 89-94, 2021. PMID: 34965376.

21 Lehrer S and Rheinstein PH: Herpes zoster vaccination reduces risk of dementia. In Vivo *35(6)*: 3271-3275, 2021. PMID: 34697158. DOI: 10.21873/invivo.12622

Received June 22, 2022
Revised August 18, 2022
Accepted August 29, 2022