Received: 18 August 2022 | Accepted: 19 September 2022

DOI: 10.1002/alz.12834

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

FEATURED ARTICLE

Herpes simplex virus alters Alzheimer's disease biomarkers - A hypothesis paper

Oliver Goldhardt[1] | **Robert Freiberger**[1] | **Tobias Dreyer**[2] | **Luisa Wilner**[1,3] | **Igor Yakushev**[3] | **Marion Ortner**[1] | **Hans Förstl**[1] | **Janine Diehl-Schmid**[1] | **Esther Milz**[4] | **Josef Priller**[1] | **Alfredo Ramirez**[4,5,6,7,8] | **Viktor Magdolen**[2] | **Markus Thaler**[9] | **Timo Grimmer**[1]

[1]Department of Psychiatry and Psychotherapy, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

[2]Department of Obstetrics and Gynecology, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

[3]Department of Nuclear Medicine, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

[4]Division of Neurogenetics and Molecular Psychiatry, Department of Psychiatry and Psychotherapy, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

[5]Department of Neurodegenerative Diseases and Geriatric Psychiatry, Medical Faculty, University Hospital Bonn, Bonn, Germany

[6]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

[7]Department of Psychiatry and Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, San Antonio, Texas, USA

[8]Cluster of Excellence Cellular Stress Responses in Aging-associated Diseases (CECAD), University of Cologne, Cologne, Germany

[9]Institute for Clinical Chemistry and Pathobiochemistry, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

Correspondence
Oliver Goldhardt, Department of Psychiatry and Psychotherapy, Klinikum rechts der Isar, Technische Universität München, Möhlstr. 26, 81675 München, Germany.
Email: oliver.goldhardt@tum.de

Abstract

Introduction: Human herpes simplex virus 1 (HSV1) is discussed to induce amyloid-β (Aβ) accumulation and neurofibrillary tangles of hyperphosphorylated tau (pTau) in Alzheimer's disease (AD) in cell culture and animal models. Aβ appears to be virostatic. We investigated the association between intrathecal antibodies against HSV or cytomegalovirus (CMV) and cerebrospinal fluid (CSF) AD biomarkers.

Methods: $A\beta_{42}/A\beta_{40}$ ratio, pTau, and tTau were measured in CSF of 117 patients with early AD positive for amyloid pathology (A+) and 30 healthy controls (A-). CSF-to-serum anti-HSV1/2-IgG antibody indices (AI-$IgG_{HSV1/2}$) and CMV (AI-IgG_{CMV}) were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Exclusively in HSV1-seropositive AD, pTau was positively and significantly predicted by AI-$IgG_{HSV1/2}$ and negatively by the $A\beta_{42}/A\beta_{40}$ ratio in both univariate and multivariate regression analyses. Furthermore, a significant and negative interaction between the AI-$IgG_{HSV1/2}$ and $A\beta_{42}/A\beta_{40}$ ratio on pTau was found.

Discussion: The results support the hypothesis that HSV infection contributes to AD.

KEYWORDS
Alzheimer's disease, amyloid, antibody index, Aβ42, Aβ40, ATN, cerebrospinal fluid, cytomegalovirus, Herpes simplex virus, FDG, immunoglobulin G, IgM, neuronal injury, PET, phospho tau, pTau, tau, tau pathology, total tau, tTau

Highlights

- HSV antibody index is positively associated with tau pathology in patients with AD.
- HSV antibody index is negatively associated with cerebral FDG metabolism.
- Amyloid modulates the association of HSV antibody index with CSF-pTau.
- HSV in AD offers a pathophysiological model connecting tau and amyloid.

1 | NARRATIVE

1.1 | Central scientific question

Alzheimer's disease (AD) is the most frequent cause of dementia and is histopathologically defined by intraneuronal fibrillary tangles of hyperphosphorylated tau (pTau) and extracellular fibrillary amyloid-β (Aβ) deposits in the brain.[1] Most evidence points to initial Aβ pathology preceding tau pathology; however, the pathophysiological interaction between Aβ and tau is yet not fully understood.[2] Deeper understanding of the pathophysiology of AD may be gained by investigating the physiological function of Aβ. Soluble Aβ-peptides have been shown to display antiviral activity against viruses.[3,4] It is of continuing scientific interest how viruses influence AD risk and whether they interact with AD pathology.

One of the pathogens that may be associated with Aβ- and tau-pathology is herpes simplex virus type 1 (HSV1). However, the causative relationship is debated with conflicting data.[5] We hypothesized that soluble Aβ inhibits the effect of HSV1 on neurons. In AD soluble Aβ levels are decreased. Therefore, HSV1 activity could be increased in AD and lead to neuronal dysfunction and cell loss.

1.2 | Current state of knowledge

The concept of interaction between HSV1 and AD has been first described by Ball et al. (1982) who proposed a functional link between the reactivation of the herpesvirus and its limbic predilection with brain regions also affected in AD.[6,7] HSV1 reactivation has been discussed to be more frequent in AD compared to controls,[8] and HSV1 DNA has been frequently found in amyloid plaques in the brain of patients with AD.[9] HSV1 infection is known to induce viral and human kinases such as glycogen synthase kinase 3β and protein kinase A,[10] leading to hyperphosphorylation of several intracellular molecules including tau in human and murine neuronal cells in vitro,[10–12] and in an AD Balb/c mouse model of HSV1 reactivation.[13]

Remarkably, Aβ exhibits antimicrobial peptide properties and appears to be involved in the inflammatory immune cascade against various pathogens by binding, blocking and inactivating various surface proteins of bacteria, Candida, and viruses.[14] Aβ also displays an inhibitory activity against herpes simplex virus type 1 (HSV1) infection in neuronal cell culture.[3] $Aβ_{42}$ stably binds to the envelope protein gD of HSV1, which interacts with the potential entry receptors of the host cell.[15] A neuroprotective effect of Aβ against herpesviruses was also confirmed in a 3D cell culture model using human neurons.[4] In neuronal cell culture experiments, HSV1 infection induced beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), resulting in increased amyloid turnover and elevated extracellular Aβ40 and Aβ42 levels in neuroblastoma cells and murine in vivo models.[16–19] Transgenic AD mice with amyloid overproduction showed a higher antiviral resistance to HSV1 than wildtype mice,[4] which was also observed as a trend by Bocharova et al. (2021).[20] In a 3D human neuronal stem cell model, HSV1 infection resulted in amyloidosis with increased presenilin expression, and amyloidosis was relatively reduced by valaciclovir treatment.[21] Taken together, cerebral amyloidosis and pTau pathology can be discussed as a consequence of infection or recurrent reactivation of HSV1.

Serum antibody levels generally reflect systemic rather than intrathecal HSV1 activity. CSF-to-serum antibody indices against HSV1 (AI-IgG_{HSV1}), that is, CSF-to-serum ratios controlled for blood-brain barrier disruption and nonspecific intrathecal antibody synthesis, are more likely to correlate with cerebral activity of neurotrophic pathogens. However, Wozniak et al showed that an elevated AI-IgG_{HSV1} of 1.5 or higher can be found with similar frequency in brain bank samples from patients with AD (14/27, 52%) and age-matched normal controls (NC; 9/13, 69%).[22] This suggests additional yet unknown factors that are necessary to understand the relationship between AD and HSV1. The association of elevated HSV1 antibodies with AD may also not be specific[14]: parallel infection with multiple herpesviruses, for example, HSV1 with cytomegalovirus (CMV),[23] or varicella zoster virus alone[24] have been associated with increased risk of AD.

1.3 | Knowledge gap

The unresolved question is whether HSV1 seropositivity affects AD pathology and whether intrathecal antibody synthesis in

HSV1-seropositive patients with AD is indeed indistinguishable from that of NC. Furthermore, it needs to be clarified whether intrathecal antibody synthesis is associated with CSF AD biomarkers. More specifically, is AI-IgG$_{HSV1}$ significantly associated with biomarker of neuronal damage, and if so, is this association modulated by soluble Aβ? Furthermore, it has to be elucidated whether such associations are found for HSV1 antibodies but not for antibodies against other neurotropic herpes virus like the CMV, especially against the background that parallel infection of HSV1 with CMV has been associated with an increased risk for AD.[23]

1.4 | Study approach

In this study, we aimed at elucidating whether cerebral HSV1 activity, measured indirectly by intrathecal antibody titers, is associated with CSF AD biomarkers for amyloid and tau pathology in humans. We also investigated the hypothesis that the association between HSV and pTau is mediated by the virus-static effect of soluble CSF-Aβ in HSV1-seropositive patients with AD. Using a cross-sectional design, the association between CSF AD biomarkers and clinical parameters was investigated in 117 patients with AD and 30 NC, stratifying by HSV1-seropositivity. In addition, intrathecal antibody synthesis against HSV1/2 (AI-IgG$_{HSV1/2}$) was analyzed for associations with CSF AD biomarkers. Anti-CMV antibodies were also measured to verify the specificity of findings with HSV1 antibodies. In a post-hoc analysis in 33 patients with AD, association of AI-IgG$_{HSV1/2}$ with cerebral glucose metabolism was investigated by FDG-PET.

1.5 | Findings

The AI-IgG$_{HSV1/2}$ was positively associated with CSF-pTau and CSF-tTau and negatively associated with cerebral FDG metabolism in patients with AD. Amyloid modulated both associations of AI-IgG$_{HSV1/2}$ with CSF-tTau and CSF-pTau but not with cerebral glucose metabolism in AD. No significant associations were found between AI-IgG$_{HSV1/2}$ and CSF-pTau, as well as with CSF-tTau, in NC and between the CSF-to-serum antibody index against CMV (AI-IgG$_{CMV}$) and CSF AD biomarkers.

1.6 | Limitations

The cross-sectional design does not allow for clear causal conclusions. Moreover, concentrations of CSF AD biomarkers do not necessarily correspond to the respective extracellular concentration in the brain. Since only antibodies against HSV1 and CMV were measured in addition to non-pathogenic factors, the influence of other neurotrophic agents including bacteria cannot be excluded.

RESEARCH IN CONTEXT

1. **Systematic Review**: Previous studies referenced in the manuscript have controversially discussed associations between infection or reactivation of human herpes simplex virus 1 (HSV1) and features of Alzheimer's disease (AD) in cell culture and animal models.
2. **Interpretation**: The positive association between cerebrospinal fluid (CSF) to serum anti-IgG$_{HSV1/2}$ antibody index (as a surrogate for HSV1-activity) and CSF phospho-Tau (pTau) in AD patients but not in healthy controls in our study supports the hypothesis that HSV1 infection enhances tau pathology in AD. This association was strongest in patients with low CSF amyloid ratios, consistent with the idea that amyloid inhibits HSV1. No such associations were found for cytomegalovirus, suggesting a specific effect of HSV1.
3. **Future Directions**: Intrathecal HSV1 activity needs to be followed in a longitudinal cohort of HSV1-seropositive patients with AD to elucidate causal relationships.

1.7 | Meaning of the study results

To our best knowledge, this is the first study to examine the association between intrathecal HSV antibody titers and CSF AD biomarkers in a well-characterized cohort of patients with predominately early AD and NC. The results of this manuscript essentially confirm a-priori hypotheses of this study and show similar associations of AI-IgG$_{HSV1/2}$ with two different biomarker categories (CSF-tau and cerebral glucose metabolism).

As hypothesized, a significant positive association was found between AI-IgG$_{HSV1/2}$ and CSF-pTau levels in HSV1-seropositive patients with sporadic AD. Furthermore, evidence is presented that the association between AI-IgG$_{HSV1/2}$ and tau pathology is modulated by amyloid, which is not found for AI-IgG$_{CMV}$. This is compatible with the view that HSV infection induces hyperphosphorylation of tau in neurons[11,12] and that this deleterious effect can be inhibited by the virostatic action of amyloid.[14]

1.7.1 | HSV1 and tau pathology

The significant association of AI-IgG$_{HSV1/2}$ with pTau in the CSF of humans with sporadic AD is consistent with previous studies finding associations of HSV1 infection with tau pathology in neuronal cell cultures and AD animal models.[10,11,13,25] AI-IgG$_{HSV1/2}$, but not serum IgG$_{HSV1}$ or IgM$_{HSV1}$, was found to be significantly and positively associated with CSF-pTau, indicating a CNS-specific process. In addition, AI-IgG$_{HSV1/2}$ was significantly associated with pTau only in T+. These

observations suggest a direct involvement of HSV1 in tau pathology rather than nonspecific viral comorbidity in AD-affected brains. The association of AI-IgG$_{HSV1/2}$ and CSF-pTau in AD was closer than between AI-IgG$_{HSV1/2}$ and CSF-tTau, suggesting hyperphosphorylation of tau as a dominant consequence of intrathecal HSV1 activity toward herpesvirus-associated neuronal injury. Besides, the effect of HSV1 on neuronal injury can also be observed by the associations between AI-IgG$_{HSV1/2}$ and cerebral glucose metabolism in a subgroup of AD patients.

1.7.2 | HSV1 and amyloid

In the CSF, a significant association between Aβ and pTau or tTau in AD, as found in this study, has been previously demonstrated.[26] However, a causal relationship between Aβ and tau pathology is controversial, especially because the spatial and temporal relationship between tau and Aβ in the brain is weak.[27] In the present study, the addition of the CSF-Aβ_{42}/Aβ_{40} ratio to the AI-IgG$_{HSV1/2}$, increased the predicted variability (as indicated by the adjusted R^2) of CSF-pTau and CSF-tTau: Both AI-IgG$_{HSV1/2}$ and CSF-Aβ_{42}/Aβ_{40} ratio resulted in significant similar moderate effects (as indicated by beta coefficients > 0.35) on CSF-pTau or CSF-tTau, respectively. Interestingly, there was no significant correlation between the CSF Aβ markers and AI-IgG$_{HSV1/2}$ in HSV1 seropositive patients with AD.

In addition, a modulating effect of the CSF-Aβ_{42}/Aβ_{40} ratio on the association of AI-IgG$_{HSV1/2}$ with CSF-pTau or CSF-tTau concentration was observed: in the group of patients with a high CSF-Aβ_{42}/Aβ_{40} ratio, the effect of HSV on pTau or tTau appears to be suppressed compared to the group displaying a low ratio (Figure 1.1 and Figure 1.2). This is consistent with the concept of an antiviral function of Aβ[14] by inhibition of the HSV1 replication through direct cell-virus receptor interactions[3,4] and agglutination to the viral protein corona.[34] Post-hoc analyses revealed that the modulating effect of the CSF-Aβ_{42}/Aβ_{40} ratio on the association of AI-IgG$_{HSV1/2}$ with CSF-pTau or with CSF-tTau is determined by CSF-Aβ_{40} rather than CSF-Aβ_{42} concentrations (Figure SA). One possible explanation could be that Aβ_{40} is less likely to accumulate into Aβ-fibrils in AD and is better cleared into CSF than Aβ_{42}.[26,29] This results in higher concentrations of Aβ_{40} mirroring processes in the brain more closely. Alternatively, a modulatory effect of Aβ_{42} on the association of CSF-pTau or CSF-tTau with AI-IgG$_{HSV1/2}$ may be overlooked because of the small proportion of Aβ_{42} relative to Aβ_{40}.

1.7.3 | Other pathogens and CMV

According to literature, no infectious pathogens other than the herpesvirus family have been positively associated with tau pathology. We also found no association between antibody levels against CMV and CSF AD biomarkers in AD. The lack of CMV-specific effects in this study points to a specific effect of HSV1. This is interesting because the antipathogenic function of soluble Aβ may not be specific for HSV1 alone[14]: parallel infections with multiple herpesviruses, for example HSV1 with CMV, have been associated with an increased risk of AD.[23] CMV may contribute to AD pathology indirectly via inflammatory pathways rather than via intracerebral interaction with Aβ and it is not known to reactivate in neurons but to erupt sporadically in lymphocytes. An alternative explanation for the epidemiologic risk of CMV and HSV1 seropositivity for AD could also be an increased susceptibility to active herpesvirus infection, as patients with AD with HSV1 seropositivity were also more likely to be CMV-seropositive and vice versa in this present study.

Pathogenic factors of microbes may be another chronic trigger for AD. For instance, the protease gingipain (GP), released by periodontal pathogenic *Porphyromonas gingivialis*, has been associated with increased cerebral Aβ and tau pathology in humans afflicted with AD.[30]

1.7.4 | NC and HSV1

No significant associations with CSF AD biomarker and AI-IgG$_{HSV1/2}$ were observed in HSV1-seropositive NC. By definition (inclusion criteria), the variability of CSF-pTau in this group is low (<60 ng/ml). In the HSV1-seropositive NC group, the positive and significant correlation of CSF-Aβ_{40} (as marker of Aβ turnover) with serum IgG$_{HSV1}$ (systemic viral activity) supports the hypothesis of virus-mediated increased Aβ production, whereas the negative and significant association of CSF-Aβ_{42} with AI-IgG$_{HSV1/2}$ indicates a possible interaction of reduced virostatic Aβ42 and intrathecal HSV1 activity, possibly representing a risk constellation for later development of AD.

1.7.5 | Clinical impact

The results of phase II drug trials using antiviral valaciclovir in HSV1-seropositive patients with mild AD (clinicaltrials.gov: NCT 03282916, NCT 02997982[31]) may help to clarify the involvement of herpesviruses in the course of AD. Our hypothesis would be strengthened if these trials show a reduction in tau pathology without altering amyloid pathology. These findings pave the way for further exploitation of the involvement of HSV1 infection in AD, as well as a potential therapeutic strategy for future management of the disease. It may turn out that patients with high CSF pTau, decreased soluble CSF Aβ, and elevated intrathecal antibody synthesis against HSV1 are most likely to benefit from anti-viral interventional therapy regimens in AD. Finally, vaccination trials with participants immunized decades before the onset of symptoms, that is, before the development of AD pathology, would be most promising to elucidate the role of HSV1 in AD etiology.

1.8 | Remaining questions and next steps

To confirm a contributing role of HSV1 activity, longitudinal cohorts are needed to investigate temporal changes in CSF AD biomarker levels

as a function of AI-IgG$_{HSV1/2}$. Intrathecal antibody synthesis against a variety of neurotrophic pathogens needs to be used to test specificity for HSV1. In addition, it would be highly recommended to compare Aβ-PET data with respect to intrathecal antibody synthesis with CSF AD biomarkers.

The presented hypothesis may explain why AI-IgG$_{HSV1/2}$ is associated with tau pathology in AD when soluble Aβ is decreased in the CSF. However, it remains unclear which mechanism mediates the actual link between HSV and AD pathology and whether this mechanism is mainly triggered by HSV1 or can be initiated independently. Additionally, HSV1-seronegatives could still be infected with HSV1 but do not show any viral reactivation or simply do not have a measurable antibody response at the time of testing.

Furthermore, it is important to demonstrate the functional relationship between HSV and AD biomarkers in defined in vitro and in vivo assays using neuronal cell culture and AD mouse models with and without HSV1 infection. Most valuable (but methodologically challenging) will be models that examine the effects of soluble amyloid concentration on the frequency of pathogenic infections, and in the case herpesviruses, on reactivation. More than that, the underlying regulatory mechanisms need to be uncovered: Several other biomarkers are involved in inflammation and correlate with tau pathology, such as the family of kallikrein-related peptidases, which may be linked to HSV1 in AD.[32]

1.9 | Pathophysiological model in line with associations

The data support the hypothesis that a reduced soluble Aβ concentration in the AD brain may facilitate HSV1 activity, which consequently triggers tau pathology and neuronal injury. The hypothesis suggests an impaired homeostasis of soluble amyloid affecting antiviral defenses. This is compatible with the concept of an anti-viral function of Aβ[14] through a direct inhibition of the HSV1 replication capacity via direct cell-virus receptor interactions[3,4,33] and agglutination to the viral protein corona.[34] Another indirect mechanism leading to an impaired virus-directed immune defense influenced by Aβ pathology may be an impaired phagocytic capacity of microglia of HSV1 or HSV1-infected neurons.[20]

The hypothesis may also explain why Aβ plaques formation is not necessarily correlated with HSV1 brain activity,[20] as only the soluble form of amyloid and not the accumulated plaque load might provide virus-directed effects.[4,34] This would explain, why a direct correlation of HSV1 DNA or reactivation in human trigeminal ganglions and brain of patients with herpes encephalitis or AD has not been observed in a recent histopathological study in humans,[35] and why amyloid plaque load alone does not provide significant protective effects against acute infection in murine models.[20]

2 | CONSOLIDATED RESULTS AND STUDY DESIGN

2.1 | Study design

For this study, patients with early AD were recruited from the Centre for Cognitive Disorders of the Technical University of Munich (TUM), Germany. A cohort of 117 amyloid-positive patients (CSF-Aβ42/Aβ40 ratio $\leq$0.05 or Aβ PET-positive) with evidence for neuronal injury (CSF-tTau >252 ng/ml) were selected using standard diagnostic criteria, with neuropsychological assessments available using the CERAD-Plus test battery, including the Mini-Mental State Examination (MMSE), and Clinical Dementia Rating scale (CDR). All patients were tested for their APOE genotype (Taqman Genotype assay). Anti-HSV1/2 and anti-CMV antibodies were determined in serum and CSF by enzyme-linked immunosorbent assay (ELISA) to calculate the intrathecal portion of antiviral IgG ratios. All of the above parameters were also assessed in a group of 30 healthy control subjects without CSF biomarker abnormalities (Aβ42/Aβ40 ratio >0.05 and CSF-tTau <252 ng/ml) and without subjective cognitive complaints or impairment on cognitive tests. The characteristics of the AD and NC cohorts are displayed in Table 1. Cerebral glucose metabolism, measured by FDG-PET, was provided in a subgroup of patients.

2.2 | Consolidated results

2.2.1 | Associations of AI-IgGHSV1/2 with CSF-pTau or CSF-tTau mediated by CSF-Aβ42/Aβ40 ratio in HSV1-seropositive patients with AD

(a) Univariate regression analyses with AI-IgG$_{HSV1/2}$ as the independent variable and the dependent variable CSF-pTau or CSF-tTau yielded significant models. AI-IgG$_{HSV1/2}$ was significantly and positively associated with CSF-pTau (β = 0.389) and with CSF-tTau (β = 0.362), respectively (see Figure 2.1, Figure 2.2, and Table 2). Univariate regression analyses with CSF-Aβ_{42}/Aβ_{40} ratio as the independent variable and CSF-pTau or CSF-tTau as the dependent variable also resulted in significant models. CSF-Aβ_{42}/Aβ_{40} ratio was significantly and negatively associated with CSF-pTau (β = −0.439) and with CSF-tTau (β = −0.433) (see Figure 2.3, Figure 2.4).

(b) Multivariate regression analysis with AI-IgG$_{HSV1/2}$ and CSF-Aβ_{42}/Aβ_{40} ratio as independent variables and CSF-pTau as dependent variable resulted in a significant model. AI-IgG$_{HSV1/2}$ was significantly and positively (β = 0.392) and CSF-Aβ_{42}/Aβ_{40} ratio was significantly and negatively (β = −0.452) associated with CSF-pTau (see Table 2). Regression analysis using tTau as the dependent variable instead of pTau also yielded in a significant model. AI-IgG$_{HSV1/2}$ was significantly and positively (β = 0.366) and CSF-Aβ_{42}/Aβ_{40} ratio was significantly and negatively (β = −0.424) associated with CSF-pTau (see Table 2).

TABLE 1 Characteristics of patients with AD and NC

Variable	AD *n* = 117	NC *n* = 30	AD vs. NC *p* value
Sex f:m	62 : 55	11 : 19	$p = 0.111$[b]
Age [years]	68.7 ± 8.44 (48-87)	60.1 ± 13.03 (41-82)	***p* = 0.002**
$T^+ : T^-$	88 : 29	1 : 29	**<0.001**[b]
ApoE ε4 allele frequency 0/1/2	46 / 49 / 20 [a]	24 / 6 / 0	**<0.001**[b]
MMSE	24.1 ± 3.67 (13-30)	29.7 ± 0.54 (28-30)	**<0.001**
Global CDR 0/0.5/1/2/3	0.77 ± 0.357 (0.5-2) 0/63/49/5	0 ± 0 30/0/0/0	**<0.001**[a]
CSF-Aβ_{42} [ng/l]	476 ± 141.2 (228-909)	1007 ± 309.9 (600-1982)	**<0.001**
CSF-Aβ_{40} [ng/l]	13,230 ± 4,300.5 (5,060-26,423)	12,614 ± 4,369.7 (6,548-26,800)	0.290
CSF-Aβ_{42}/Aβ_{40} ratio	0.038 ± 0.0081 (0.02-0.06)	0.081 ± 0.0148 (0.06-0.12)	**<0.001**
CSF-pTau [ng/l]	92.4 ± 43.84 (32-231)	42.9 ± 8.50 (28-62)	**<0.001**
CSF-tTau [ng/l]	792.1 ± 469.53 (191-3047)	251.8 ± 76.53 (130-437)	**<0.001**
Q_{alb}	6.93 ± 3.111 (2.6-26.5)	6.03 ± 1.860 (2.7-9.6)	0.246
Q_{unspec}	3.19 ± 1.547 (1.1-13.5)	2.66 ± 0.823 (1.2-4.4)	0.097
HSV1+ (IgG)	92 (78.6%)	24 (80.0%)	0.870[b]
HSV1+ (IgM)	2 (1.8%)	0 (0%)	0.471[b]
CMV+ (IgG)	50 (42.7%)	14 (46.7%)	0.698[b]

Note: Clinical parameters, CSF AD biomarkers and HSV biomarkers between patients with AD and cognitively normal controls.
Abbreviations: AD, Alzheimer's disease; Aβ42 or Aβ40, amyloid 1-42 or amyloid 1-40; ApoE, Apolipoprotein E; CDR, Clinical Dementia Rating Scale; CMV, cytomegalovirus; CMV+, anti-CMV-IgG seropositive (≥ 0.9 seropositivity index); CSF, cerebrospinal fluid; HSV, herpes simplex virus; HSV+, anti-HSV-seropositive (≥ 25 U/ml); IgG or IgM, immunoglobulin G or M; MMSE, Mini-Mental State Examination; MWU, Mann-Whitney-U test; n.a., not applicable; NC, normal control; Qalb, CSF/serum albumin ratio; Qunspec, unspecific CSF/serum IgG ratio; pTau, phospho Tau; T, tau pathology (assessed by CSF-pTau; ≥ 60 ng/l for T+); tTau, total Tau.
[a]ApoE genotype could not be measured in two cases.
[b]Chi-squared test.

The regression analyses in which the interaction term [AI-$IgG_{HSV1/2}$ * CSF-Aβ_{42}/Aβ_{40} ratio] was added to the independent variables AI-$IgG_{HSV1/2}$ and CSF-Aβ_{42}/Aβ_{40} ratio, as with CSF-pTau or CSF-tTau as dependent variable, respectively, resulted in significant models. The interaction term [AI-$IgG_{HSV1/2}$ * CSF-Aβ_{42}/Aβ_{40} ratio] was significantly and negatively associated with CSF-pTau ($\beta = -1.552$) and CSF-tTau ($\beta = -1.568$), and modulated the significant associations of AI-$IgG_{HSV1/2}$ with CSF-pTau or CSF-tTau, respectively, consistent with the expected negative effect of amyloid on HSV (compare Table 2). This model was also significant when heteroskedasticity was controlled by using post-hoc generalized (least square) linear regression models (GLM) (Table SA). These findings were also significant if patients with an AI-$IgG_{HSV1/2}$ above 2.0 (potential statistical outliers) were excluded from the analysis, and they were independent of age, sex, and APOE.

To further elucidate the effect of amyloid, the patient group was stratified into tertiles with low, intermediate, and high CSF-Aβ_{42}/Aβ_{40} ratios. Linear regression analyses of the associations between AI-$IgG_{HSV1/2}$ and CSF-pTau or CSF-tTau, respectively, in these tertile groups are displayed in Figure 1.1, Figure 1.2, which illustrate that the association between AI-$IgG_{HSV1/2}$ and CSF-pTau or CSF-tTau, respectively, is tighter in the group with low CSF-Aβ_{42}/Aβ_{40} ratios. In post-hoc analyses, high CSF-Aβ_{40} rather than low CSF-Aβ_{42} levels explained the interaction of CSF-Aβ_{42}/Aβ_{40} ratio with AI-$IgG_{HSV1/2}$ on CSF-pTau or CSF-tTau (Figure 1).

TABLE 2 Associations of AI-IgGHSV1/2 and CSF-Aβ42/Aβ40 ratio with CSF-pTau and CSF-tTau in HSV1 seropositive patients with AD

Dependent variable	Independent variables	Adjusted R^2 *p* value	AI-IgG$_{HSV1/2}$	CSF-Aβ$_{42}$/Aβ$_{40}$ ratio	[AI-IgG$_{HSV1/2}$ * CSF-Aβ$_{42}$/Aβ$_{40}$ ratio] interaction term
			Standardized β-coefficient, *p* value		
CSF-pTau	Aβ$_{42}$/Aβ$_{40}$ ratio	$r^2 = 0.191$ $p < 0.001$	$\beta = -0.449$ $p < 0.001$	-	-
	AI-IgG$_{HSV1/2}$	$r^2 = 0.140$ $p = 0.001$	$\beta = 0.389$ $p = 0.001$	-	-
	AI-IgG$_{HSV1/2}$ Aβ$_{42}$/Aβ$_{40}$ ratio	$r^2 = 0.339$ $p < 0.001$	$\beta = 0.392$ $p < 0.001$	$\beta = -0.452$ $p < 0.001$	-
	AI-IgG$_{HSV1/2}$ Aβ$_{42}$/Aβ$_{40}$ ratio [AI-IgG$_{HSV1/2}$ * CSF-Aβ$_{42}$/Aβ$_{40}$ ratio]	$r^2 = 0.389$ $p < 0.001$	$\beta = 1.650$ $p = 0.001$	$\beta = 0.417$ $p = 0.208$	$\beta = -1.552$ $p = 0.008$
CSF-tTau	Aβ$_{42}$/Aβ$_{40}$ ratio	$r^2 = 0.167$ $p = 0.001$	$\beta = -0.412$ $p = 0.001$	-	-
	AI-IgG$_{HSV1/2}$	$r^2 = 0.120$ $p = 0.001$	$\beta = 0.362$ $p = 0.001$	-	-
	AI-IgG$_{HSV1/2}$ Aβ$_{42}$/Aβ$_{40}$ ratio	$r^2 = 0.294$ $p < 0.001$	$\beta = 0.366$ $p < 0.001$	$\beta = -0.424$ $p < 0.001$	-
	AI-IgG$_{HSV1/2}$ Aβ$_{42}$/Aβ$_{40}$ ratio [AI-IgG$_{HSV1/2}$ * CSF-Aβ$_{42}$/Aβ$_{40}$ ratio]	$r^2 = 0.369$ $p < 0.001$	$\beta = 1.63$ $p = 0.001$	$\beta = 0.454$ $p = 0.186$	$\beta = -1.568.$ $p = 0.009$

Note: Linear regression analyses with the dependent variables CSF-pTau or CSF-tTau, respectively, and the independent variable AI-IgGHSV1/2 in 82 HSV1 seropositive patients with AD. The models were further controlled for CSF-Aβ42/Aβ40 ratio and consecutively for the interaction term [AI-IgGHSV1/2 * CSF-Aβ42/Aβ40 ratio]. Adjusted R^2 indicate the explained variability. The best significant model for the dependent variable CSF-pTau and for CSF-tTau include the variables AI-IgGHSV1/2, CSF-Aβ42/Aβ40 ratio and the interaction term [IgGHSV1/2 * CSF-Aβ42/Aβ40 ratio] in AD.

Abbreviations: Aβ42 or Aβ40, amyloid 1-42 or amyloid 1-40; AI-IgGHSV1/2, CSF to serum anti-HSV1/2-IgG antibody index; CSF, cerebrospinal fluid; HSV, herpes simplex virus; IgG, immunoglobulin G; pTau, phospho Tau; tTau, total Tau.

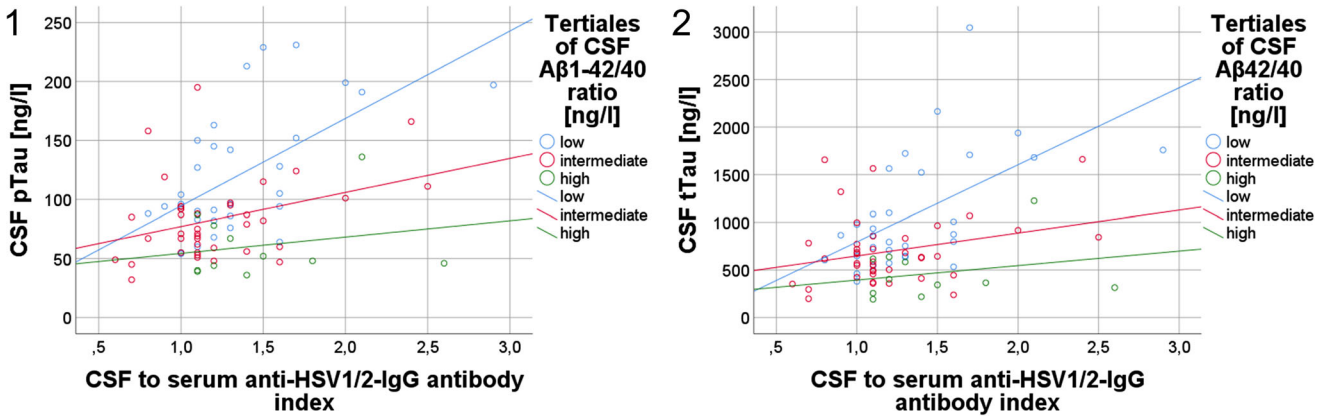

FIGURE 1 Associations of AI-IgG$_{HSV1/2}$ with CSF-pTau or CSF-tTau, respectively, in HSV1 seropositive patients with AD stratified into tertiles of CSF-Aβ42/Aβ40 ratio The HSV1 seropositive (s-IgG$_{HSV1}$ > 25 U/ml) patient group was stratified to tertile groups of low, intermediate and high CSF-Aβ$_{42}$/Aβ$_{40}$ ratio. Figure 1.1: Linear regression with the dependent variable CSF-pTau and the independent variable AI-IgG$_{HSV1/2}$: in the group with the lowest tertile of Aβ$_{42}$/Aβ$_{40}$ ratio the adjusted R^2 was 0.313 ($p = 0.001$) with $\beta_{AI\text{-}IgGHSV1/2} = 0.581$, in the group with the intermediate tertile of Aβ$_{42}$/Aβ$_{40}$ the adjusted R^2 was 0.164 ($p = 0.084$) with $\beta_{AI\text{-}IgGHSV1/2} = 0.478$, and in the group with the highest tertile of Aβ$_{42}$/Aβ$_{40}$ the adjusted R^2 was -0.015 ($p = 0.518$) with $\beta_{AI\text{-}IgGHSV1/2} = 0.107$. Figure 1.2: Linear regression with the dependent variable CSF-tTau and the independent variable AI-IgG$_{HSV1/2}$: in the group with the lowest tertile of Aβ$_{42}$/Aβ$_{40}$ ratio the adjusted R^2 was 0.278 ($p = 0.002$) with $\beta_{AI\text{-}IgGHSV1/2} = 0.551$, in the group with the intermediate tertile of Aβ$_{42}$/Aβ$_{40}$ the adjusted $R^2$was 0.149 (p = 0.149) with $\beta_{AI\text{-}IgGHSV1/2} = 0.406$, and in the group with the highest tertile of Aβ$_{42}$/Aβ$_{40}$ the adjusted R^2 was -0.024 ($p = 0.736$) with $\beta_{AI\text{-}IgGHSV1/2} = -0.056$. Aβ$_{42}$ or Aβ$_{40}$, amyloid 1-42 or amyloid 1-40; AI-IgG$_{HSV1/2}$, CSF to serum anti-HSV$_{1/2}$-IgG antibody index; CSF, cerebrospinal fluid; HSV, herpes simplex virus; IgG or IgM, immunoglobulin G or M; s-IgG$_{HSV1}$, serum anti-HSV1-IgG titer; pTau, phospho Tau; tTau, total Tau.

2.2.2 | CMV

Univariate and multivariate analyses examining associations between AD biomarkers in CSF and AI-IgG$_{CMV}$ instead of AI-IgG$_{HSV1/2}$ in CMV-seropositive patients with AD and in NC did neither yield statistically significant models nor a significant β coefficient for AI-IgG$_{CMV}$ (Table SB).

2.2.3 | NC and HSV1

Linear regression models with CSF-pTau or CSF-tTau as the dependent variable, and AI-IgG$_{HSV1/2}$ as the independent variable, controlled for Aβ_{42}/Aβ_{40} ratio and the interaction term [AI-IgG$_{HSV1/2}$ * CSF-Aβ_{42}/Aβ_{40} ratio], were not significant (Table SC).

2.2.4 | Cerebral glucose metabolism

FDG tracer uptake was associated with AI-IgG$_{HSV1/2}$ and AI-IgG$_{CMV}$ in AD. Cerebral glucose metabolism was negatively associated with AI-IgG$_{HSV1/2}$ in 20 HSV1-seropositive patients, who did not differ significantly from 10 HSV1-seronegative patients in global and voxel-wise FDG-PET signal (Figure 3), consistent with the finding between AI-IgG$_{HSV1/2}$ and CSF tTau. The association primarily involved the right temporal cortex, a region also affected in AD.

Interestingly, in a group comparison, CMV-seropositive patients with AD (n = 13) showed slightly lower local cerebral glucose metabolism in bilateral frontopolar and temporopolar cortices in voxel-based analyses compared with CMV-negative patients with AD (n = 20), whereas AI-IgG$_{CMV}$ was negatively and significantly associated with glucose metabolism in the precentral cortical area in CMV-seropositive patients. The cortical regions associated with CMV antibodies are largely different from those affected in AD (Figure 2).

3 | DETAILED METHODS AND RESULTS

3.1 | Methods

3.1.1 | Patient cohort and cognitively NC

A total of 117 patients with early AD were recruited from the Centre for Cognitive Disorders at the Technical University of Munich. Study participants had been referred by general practitioners, neurologists, psychiatrists, or other institutions for the evaluation of cognitive impairment, and had undergone a standardized diagnostic procedure including a detailed somatic, neurologic, and psychiatric examination. Patients with structural anomalies (contusion, tumor, or cyst), cerebral ischemia (≥ 1 cm in diameter), more than four microbleeds, or any subarachnoidal hemosiderosis on magnetic resonance imaging (MRI) were excluded. Standardized neuropsychological tests were administered, including the Mini-Mental State Examination (MMSE). To estimate clinical severity, the CDR was assessed, and the global score (global CDR) was calculated.[36]

The diagnosis of early AD, that is, mild cognitive impairment (MCI) or mild dementia due to probable AD, was made according to standard diagnostic criteria.[37,38] Cerebral Aβ pathology (A+) was demonstrated by increased specific tracer binding in position emission tomography (PET) and in cerebrospinal fluid (CSF) by decreased Aβ1-42 (Aβ_{42}) levels in relation to Aβ1-40 (Aβ_{40}) levels[39] (compare stratification to the ATN system in Table SD). The high likelihood of the underlying pathophysiological process was determined by the requirement of both a positive biomarker for amyloid pathology (A+: CSF-Aβ42/Aβ40-ratios ≤ 0.5 and/or amyloid PET positivity) and neuronal damage (N+: CSF-tTau ≥ 252 ng/ml and/or hippocampal atrophy on visual evaluation). Although patients were not selected for the presence of tau pathology (T+) as indicated by elevated CSF-pTau levels (≥ 60 ng/ml), CSF-pTau levels were also evaluated according to the ATN research framework.[40]

A total of 30 cognitively NC did not complain of any memory deficits (global CDR = 0) and scored in the normal range on psychometric tests using the same test battery as patients, including the MMSE.[41] NCs had to have a CSF-Aβ42/Aβ40-ratio > 0.05 to exclude amyloid pathology. Suspected or confirmed diagnosis of cancer, multiple sclerosis, or parkinsonism was excluded.

The local ethics committee approved the study protocol (538-19S), and all procedures were conducted in accordance with the Declaration of Helsinki, Sixth Revision. All participants provided written informed consent.

3.1.2 | APOE genotyping

Genotypes for rs7412 and rs429358, the single nucleotide polymorphisms (SNP) defining the ε-2, ε-3, and ε-4 alleles of APOE, were genotyped using commercially available TaqMan SNP Genotyping Assay (ThermoFisher Scientific). Both SNPs assays were amplified on genomic DNA using a QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific). Visual inspection of clusters formation was performed for each SNP before genotype data were further used to define the ε-2, ε-3, and ε-4 alleles in each sample.

3.1.3 | HSV ELISAs

Alegria 8-well-microstrips anti-HSV-1/2-IgG-CSF (ORG905GL), anti-HSV-1-IgG-Serum (ORG903G), and anti-HSV1-IgM (ORG903MX), which have been approved for European conformity (CE), were purchased from Orgentec Diagnostika GmbH (Mainz, Germany). ELISAs were performed according to manufacturer's instructions (www.orgentec.com), and test strips were measured photometrically at 650 nm on an Alegria instrument (Orgentec Diagnostika GmbH) using Sensotronic Memorized Calibration SMC. ORG903G was used

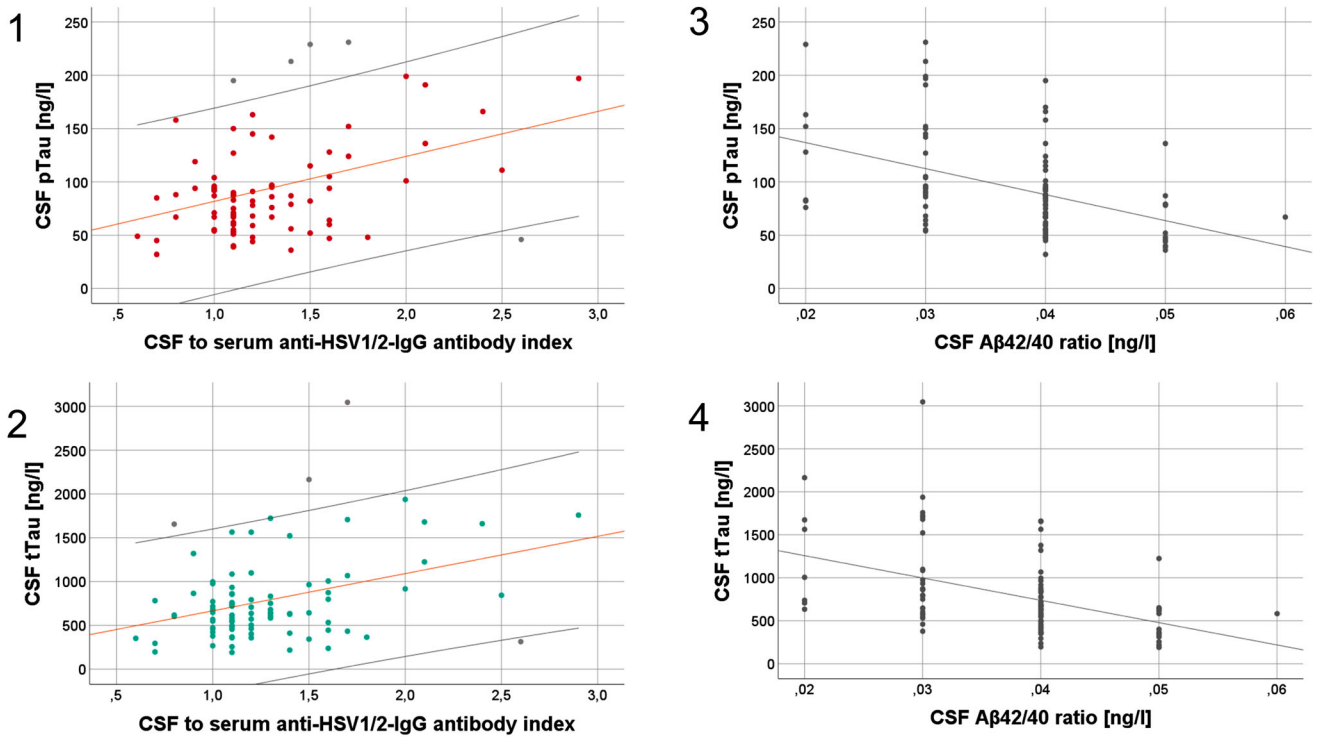

FIGURE 2 Associations of AI-IgGHSV1/2 or CSF-Aβ42/Aβ40 ratio with CSF-pTau or CSF-tTau, respectively, in HSV1 seropositive patients with AD. Univariate linear regression models in HSV1 seropositive (s-IgG_{HSV1} > 25 U/ml) patients with AD with the dependent variable CSF-pTau (Figure 2.1) or CSF-tTau (Figure 2.2), respectively, and the independent variable AI-$IgG_{HSV1/2}$, as well as the models with the dependent variable CSF-pTau (Figure 2.3) or CSF-tTau (Figure 2.4), respectively, and the independent variable CSF-$A\beta_{42}/A\beta_{40}$ ratio displayed as scatter plots with 95% confidence intervals (if statistically appropriate). AI-$IgG_{HSV1/2}$ was significantly and positively associated with pTau (Figure 2.1; adjusted R^2 = 0.140, $p = 0.001$, $\beta = 0.389$) and with tTau (Figure 2.2: adjusted $R^2 = 0.120$, $p = 0.001$, $\beta = 0.362$). CSF-$A\beta_{42}/A\beta_{40}$ ratio was significantly and negatively associated with CSF-pTau (Figure 2.3: adjusted $R^2 = 0.184$, $p < 0.001$, $\beta = -0.439$) and with CSF-tTau (Figure 2.4: adjusted $R^2 = 0.179$, $p < 0.001$, $\beta = -0.433$). $A\beta_{42}$ or $A\beta_{40}$, amyloid 1-42 or amyloid 1-40; AI-$IgG_{HSV1/2}$, CSF to serum anti-$HSV_{1/2}$-IgG antibody index; CSF, cerebrospinal fluid; HSV, herpes simplex virus; IgG or IgM, immunoglobulin G or M; s-IgG_{HSV1}, serum anti-HSV1-IgG titer; pTau, phospho Tau; tTau, total Tau.

exclusively to determine HSV1 seropositivity, as no cutoff for HSV-1 seropositivity is defined for ORG905L.

The detection limit of serum anti-HSV1-IgG (serum IgG_{HSV1}) is 5.9 U/ml when measured linearly, and for serum anti-HSV1-IgM (serum IgM_{HSV1}) 6.8 U/ml (non-linear). For serum IgG_{HSV1} and serum IgM_{HSV1}, results were predefined and interpreted according to the manufacturer's information as negative if < 20 U/ml, borderline if 20 to 25 U/ml, and positive if > 25 U/ml for HSV1 infection and reactivation, respectively.

The detection limit of anti-HSV-1/2-IgG-CSF was 10 U/ml. CSF and serum were diluted according to the protocol, and protein concentrations were assessed, including the ratio of albumin serum-to-CSF (Q_{alb}) and the ratio of nonspecific IgG (Q_{unspec}). No patient showed a polyspecific immune response.

The anti-HSV-1/2-IgG CSF-to-serum antibody index (AI-$IgG_{HSV1/2}$) was calculated by dividing the specific anti-HSV1/2-IgG CSF-to-serum titer (Q_{spec} = (IgG_{spec} CSF x CSF dilution factor) / (IgG_{spec} serum x serum dilution factor)) by the unspecific IgG CSF-to-serum titer (Q_{unspec} = IgG_{unspec} CSF / IgG_{unspec} serum) with reference to the upper discrimination line (Q_{lim}) of the reference range of the blood-derived protein fraction in CSF.[42] AI range from 0.5 to less than 1.5 was interpreted as normal and ≥ 1.5 as intrathecal synthesis of specific anti-HSV1/2 antibodies.

3.1.4 | CMV ELISA

Anti-CMV IgG antibodies in CSF and serum were measured in duplicate by ELISA (Abnova, KA1452) and repeated in adjusted dilutions if out of range. CSF was diluted 1:2 and serum 1:404. A standard curve with a polynomic (four-parameter) function was calculated using SPSS. According to the published specifications of the ELISA's protocol, semiquantitative positivity index on predetermined standard was used for the cutoff value. Sera that have absorbance value ≥ 90% cutoff were considered positive. Anti-CMV-IgG serum titers in AU/ml were calculated in CMV-seropositive patients and NCs. CSF-to-serum anti-CMV-IgG antibody indices (AI-IgG_{CMV}) were calculated similarly to AI-$IgG_{HSV1/2}$ specifications. Only valid values ≥ 0.5 were used for analyses, and values > 1.5 indicated intrathecal antibody production.

3.1.5 | CSF ELISA (Aβ42, Aβ40, phosphorylated Tau, total Tau)

CSF sampling and analyses have been described elsewhere.[43] CSF-$A\beta_{42}$ and CSF-$A\beta_{40}$ were measured in triplicate, and CSF-pTau, and CSF-tTau were measured in duplicate using commercially available ELISAs (IBL, amyloid beta (1-42) IBL RE 59661 and Amyloid beta (1-40) RE 59651; Fujirebio, Innotest P-TAU 81574 and hTAU Ag 81572). The coefficients of variation (CVs) were less than 6% for CSF-$A\beta_{42}$ and CSF-$A\beta_{40}$ and less than 3% for CSF-pTau and CSF-tTau. $A\beta_{42}/A\beta_{40}$ ratio was calculated.

3.1.6 | PET image processing

When available, [18F]-fluorodeoxyglucose-(FDG) PET and 3D T1 and 3D T2 data were used for analyses. Images were preprocessed using Statistical Parametric Mapping 12 (SPM12) software (Wellcome Trust Centre for Neuroimaging, London, UK) and sub-applications implemented in Matlab R2016b (Mathworks, Natick, Massachusetts, USA). Cerebral lesions were segmented using Lesion Growth Algorithm (LGA) as implemented in Lesion Segmentation Toolbox (LST).[44] Prior to gray matter and white matter segmentation, lesion probability maps were used to fill the white matter in T1 MPR using the lesion filling function of LST to minimize structurally induced errors in partial volume correction.[44] PET and T1 images were coregistered and partial volume effects (PVE) were corrected using the PETPVE12 toolbox.[45] Further processing included intensity normalization, spatial normalization and smoothing. For spatial normalization, the inverse deformation field information from the segmentation step was used to apply a Montreal Neurologic Institute (MNI) space mask to the patient image. The cerebellum and thalamus, defined by the Desikan-Killiany (DK) atlas served as reference regions for intensity scaling.[46] Images were then spatially normalized to MNI space and smoothed with a Gaussian kernel (8 mm). Mean intensity of the FDG PET signal of the cerebral cortex divided by the cerebellar cortex (C/cb FDG tracer uptake ratio), or the cerebral cortex divided by the thalamus (C/th FDG-PET tracer uptake ratio) were calculated using the DK atlas PVE-corrected FDG images.

3.1.7 | Statistical analyses

For statistical analyses, IBM SPSS Statistics Version 26 and R (2021, https://www.R-project.org/) was used. Mean differences were compared using t-test, Mann-Whitney U or chi-squared test depending on the distribution of the variables. Spearman-Rho or Pearson correlation analyses were calculated depending on the statistical distribution of the variables.

To identify HSV1 mediated effects, mean comparisons were performed in HSV1-seropositive (serum anti-HSV1-IgG ≥ 25 U/ml) versus HSV1-seronegative patients, and in HSV1-seropositive patients with and without intrathecal anti-$IgG_{HSV1/2}$ synthesis (AI-$IgG_{HSV1/2}$ ≥ 1.5).

Moreover, stratification of the patient group was performed by further dividing the cohort into T+ (pTau ≥ 60 ng/l) versus T- to investigate differences dependent on disease stage, as an elevation in pTau indicates an advanced disease stage.[14]

Similar comparisons were calculated with respect to CMV (anti-IgG_{CMV} seropositivity was defined by an index ≥ 0.9).

The main objective of this study was to investigate the association of AI-$IgG_{HSV1/2}$ with CSF-pTau, or CSF-tTau using linear regression. Univariate or multivariate regression analyses were performed at a significance level of 0.05. In the resulting models, clinical parameters with known association to AD pathology (age, sex, global CDR, APOE epsilon 4 allele frequency) were step-wise included as independent variables and remained in the model when the adjusted R^2 increased with an F likelihood of less than 0.05. In a second step, this association was controlled in a multivariate model for the CSF-Aβ42/Aβ40 ratio. Moderation effects of the independent variables and effect sizes were analyzed by linear regression and simple slope analyses. To reduce type I empirical error or false positive results, heteroskedasticity was tested using the White test. Therefore, post-hoc generalized (least square) linear regression models (GLM) were calculated.[47]

To identify confounding variables, correlations were made between clinical parameters (CDR, age, sex, and APOE), anti-viral antibodies and CSF AD biomarkers.

To evaluate the specificity of the results in HSV, we performed similar regression analyses in CMV-seropositive patients to find significant association between AI-IgG_{CMV} and CSF-pTau, or CSF-tTau, respectively.

Results from the AD patient group were then compared with results from similar analyses in the NC group.

Post-hoc, we investigated associations between AI-$IgG_{HSV1/2}$ and cerebral glucose metabolism measured by FDG-PET (another marker for neuronal dysfunction) in a subset of 33 patients with FDG-PET and structural MRI data. PET images were analyzed using SPM12 for voxel-based multivariate regression analyses, comparison of means, and for acquisition of means of regions of interest (ROI). C/cb and C/th FDG tracer uptake ratios were associated with AI-$IgG_{HSV1/2}$. For voxel-based and global analyses, cerebral masking based on Desikan-Killiany atlas was used.[46] Cluster were reported at a significance levels of 0.05 when cluster-level error was corrected family-wise. A threshold for voxel-level expansion was defined to indicate only significant clusters.

3.2 | Results

3.2.1 | Stratification by HSV1-seropositivity

For analyses investigating differences between HSV1 seropositive and negative patients with AD or NC, the cohorts were dichotomized based on IgG_{HSV1}-seropositivity (predefined: positive > 25 U/ml; negative <20 U/ml). Of 117 patients fulfilling the inclusion criteria from the local biobank cohort and 30 NCs, HSV1-seropositivity was found in 78.6% (n = 92) patients, and in 80.0% (n = 24) of NCs. Twenty-five patients

and 6 NCs were negative for s-IgGHSV1. None of the patients or NCs showed borderline values. Acute primary infection with HSV1 or significant acute reactivation was mainly excluded by serum anti-HSV1-IgM below a cutoff of 25 U/ml except in two patients. The composition of the cohort is depicted in Table 1. The descriptive data of the whole AD patient and NC cohort stratified to HSV1-seropositivity are presented in Table SE.

HSV1-seropositivity in both the AD and NC group is very similar to the world's average of about 80%.[48] In humans, HSV1 infects the peripheral ganglia cells and establishes a latent infection in neurons.[49] HSV1 can cyclically reactivate and provoke recurrent infections which are not limited to the ganglia cells but can also reach neurons of the hippocampus or temporal cortices in AD as well as in NC.[35,50,51] However, antibody titers against HSV1 in serum mostly indicate only chronic viral reactivation as the majority of humans initially were infected in their youth, and therefore are not specific for HSV1 activity in the central nervous system. This is one explanation why HSV1-seropositivity (anti-IgG and anti-IgM against HSV1) was not linked to clinical severity (MMSE, CDR), age, sex, APOE epsilon 4 allele frequency, or to the CSF biomarker Aβ42, Aβ40, pTau, tTau in this cross-sectional design.

However, other studies have shown an association of serum antibodies with AD: A meta-analysis, not corrected for age or the APOE genotype, revealed weak evidence that anti-HSV1 IgG- or anti-HSV1/2 IgG-seropositivity is associated with the risk of dementia or MCI. However, the quality of the included studies was very heterogeneous.[52] Later published studies showed that the interaction of APOE ε4 heterozygosity with anti-HSV1-IgG seropositivity increased the risk for AD.[53] Furthermore, in HSV1 IgG-positive patients with AD over 65 years of age, disease progression was found to be accelerated especially in APOE ε4 allele carriers as compared to HSV1 IgG-negative patients.[54] Lövheim et al. found an association between increased serum levels of specific anti-HSV1 antibodies and AD risk in a longitudinal study on 3432 Swedish elderly people.[55] Moreover, elevated serum HSV1 antibody titers have been connected to cortical grey matter volumes of AD patients compared to healthy control.[56] Another study connected a high anti-HSV1-IgG affinity index with symptoms of MCI in prodromal AD.[57] Therefore, a pathophysiological connection between HSV1 activity, as measured by antibody titers, and AD can be assumed.

In the present study, the APOE genotype was not linked with HSV1-seropositivity. This is interesting because the APOE epsilon 4 (ε-4) allele frequency is the most important genetic risk factor in sporadic AD, which correlates with the course and risk of the disease,[58] and was positively associated with HSV1 DNA load in the brain after infection in a mouse model.[59] In people carrying an ε-4 allele, orofacial efflorescences, as a sign of HSV1 reactivation, are more frequent.[60] The risk for AD is increased in HSV1-seropositive APOE ε4 carriers compared to noncarriers.[61] In serologically HSV1-positive AD patients, the APOE ε4 allele frequency is higher than in HSV1-negative ones (52.8% to 11%).[62] The associations of the APOE genotype with both AD and HSV1 infection suggest an etiological link between both pathologies.

Intrathecal anti-HSV1/2-IgG synthesis measured by CSF to serum antibody indices of IgG against HSV might be more suitable to display intrathecal HSV1 activity and to investigate its correlation with AD biomarkers.

3.2.2 | Stratification by intrathecal anti-HSV1/2-IgG synthesis in HSV1-seropositive patients

HSV1-seropositive patients with AD and NC were subdivided according to the presence of intrathecal synthesis of specific HSV1/2 antibodies (AI-IgGHSV1/2 <1.5 and ≥1.5), to investigate whether elevation in antibody indices is linked to CSF biomarkers and clinical severity (CDR, MMSE). Intrathecal antibody production against HSV1/2 (AI-$IgG_{HSV1/2} \geq 1.5$) was seen in 22 of 82 HSV1-seropositive patients with AD (10 of 92 HSV1-seropositive patients with invalid antibody indices <0.5 were excluded), and in 8 of 24 HSV1-seropositive NCs (Table SF). Frequency of intrathecal anti-HSV1/2-IgG synthesis did not differ between AD and NC ($p = 0.387$, Chi X^2), which had been proposed in histopathological confirmed patients with AD compared to NC.[46]

Patients with AD with AI-$IgG_{HSV1/2} \geq 1.5$ differed significantly from those with AI-$IgG_{HSV1/2} < 1.5$ with higher global CDR scores (0.89 ± 0.343 vs. 0.73 ± 0.338, $p = 0.029$) and CSF-pTau (121.7 ± 59.75 vs. 84.5 ± 37.16, $p = 0.013$) and CSF-tTau (1115 ± 717.0 vs. 701 ± 356.7, $p = 0.009$) in Mann-Whitney U tests indicating more severe disease stages. HSV1-positive patients with AD with intrathecal anti-HSV1-IgG synthesis (AI-$IgG_{HSV1/2} \geq 1.5$) had significant elevated levels of CSF-pTau ($p = 0.025$) and CSF-tTau ($p = 0.011$) when compared to HSV1-seronegative patients as well as HSV1-seropositive patients without intrathecal anti-HSV1-IgG synthesis. No difference of any parameter was observed in the NC. Distribution of AI-$IgG_{HSV1/2}$ can be depicted in Figure SC.

As no difference of any CSF AD biomarker linked to intrathecal anti-HSV1/2-IgG synthesis was observed in the NC group, an impact of HSV activity might only be relevant when AD progresses to more severe stages with increased tau pathology.

3.2.3 | Stratification by the ATN system

Based on this finding, we post-hoc investigated whether intrathecal HSV1/2-IgG synthesis is linked to the ATN system in which T+ represents a later pathological stage of AD than T-.[61] Using a cutoff of ≥ 60 ng/l pTau in CSF for T+, present in 71 of 92 HSV1-seropositive patients compared to 21 T- patients (biomarker concordance of the AD cohort is depicted in Table SD), we did not find any significant differences in AI-$IgG_{HSV1/2}$ levels ($p = 0.322$) or in the frequency of elevated (≥1.5) intrathecal HSV1/2-IgG synthesis ($p = 0.428$, Chi X^2) (depicted in Table SG). Even though T+ patients with AD did not differ from T- patients in clinical severity (age, sex, MMSE, global CDR, or HSV antibody titers), in accordance with the current concept of AD biomarker evolution,[63] in this sample also significantly lower Aβ_{42}/Aβ_{40} ratios were observed in T+. AI-$IgG_{HSV1/2}$ was significantly and positively correlated with CSF-tTau (Rho = 0.336) and with CSF-pTau (Rho = 0.364) in T+ but not in T-. AI-$IgG_{HSV1/2}$ was significantly and positively

correlated with CSF-Aβ_{42}/Aβ_{40} ratio in T- (Rho = 0.568) but not in T+. All other correlations were not significant (Table SH).

However, intrathecal anti-IgG$_{HSV1/2}$ antibody synthesis did not vary simply with the T biomarker classification due to the ATN system. This pointed to the relevance of other factors that influence the relation of CSF pTau with AI-IgG$_{HSV1/2}$., but can also statistically explained by the fact alone that the group size of the T-negative correspond to only a quarter of the T+, and the cutoff for CSF-pTau is ultimately not an absolute quantity, but has the effect of arbitrarily separating the distribution of the cohort. Nevertheless, the intrathecal soluble amyloid concentration, as amyloid shows antiviral properties,[3] has been a priori hypothesized to inhibit HSV1. The relevant question to support this hypothesis was whether associations of AI-IgG$_{HSV1/2}$, amyloid, and tau fit to the hypothesis that elevated CSF tau levels go along with higher HSV1 activity in the case of low CSF amyloid ratios.

3.2.4 | Associations of intrathecal anti-HSV1/2 IgG synthesis and the CSF-Aβ42/Aβ40 ratio with CSF pTau

After determining the composition of the AD and NC cohorts in detail, we tested the hypothesis that HSV is associated with tau pathology or neuronal damage, respectively, assuming a priori a positive virus-mediated effect on CSF-pTau and modulation of this effect by amyloid. Therefore, in a first step (a), we independently investigated the associations of AI-IgG$_{HSV1/2}$ and the CSF-Aβ_{42}/Aβ_{40} ratio, respectively, with CSF-pTau (or CSF-tTau, respectively) in univariate linear regression models. In a second step (b), we investigated the associations of both, AI-IgG$_{HSV1/2}$ and the CSF-Aβ_{42}/Aβ_{40} ratio, with CSF-pTau (or CSF-tTau) in a multivariate linear regression model. Inclusion of any of the factors age, sex, global CDR, or APOE epsilon 4 allele frequency as independent variables in the linear regression models below did not result in statistically significant estimates or increased variability (data not shown).

(a) Univariate regression analyses with AI-IgG$_{HSV1/2}$ as the independent variable and the dependent variable CSF-pTau or CSF-tTau yielded significant models. The AI-IgG$_{HSV1/2}$ was significantly and positively associated with CSF-pTau (β = 0.389, p = 0.001) and with CSF-tTau (β = 0.362, p = 0.001), respectively (see Figure 2.1, Figure 2.2, and Table 2). Univariate regression analyses with CSF-Aβ_{42}/Aβ_{40} ratio as the independent variable and CSF-pTau or CSF-tTau as the dependent variable also resulted in significant models. CSF-Aβ_{42}/Aβ_{40} ratio was significantly and negatively associated with CSF-pTau (β = −0.439) and with CSF-tTau (β = −0.433) (see Figure 2.3, Figure 2.4). Because AI-IgG$_{HSV1/2}$ and the CSF-Aβ_{42}/Aβ_{40} ratio were not correlated (β = −0.050), an independent effect on CSF-pTau can be assumed.

(b) Multivariate regression analysis with AI-IgG$_{HSV1/2}$ and CSF-Aβ_{42}/Aβ_{40} ratio as independent variables and CSF-pTau as dependent variable resulted in a significant model. AI-IgG$_{HSV1/2}$ was significantly and positively (β = 0.392) and CSF-Aβ_{42}/Aβ_{40} ratio was significantly and negatively (β = −0.452) associated with CSF-pTau (see Table 2). Regression analysis using tTau as the dependent variable instead of pTau also yielded in a significant model. AI-IgG$_{HSV1/2}$ was significantly and positively (β = 0.366) and CSF-Aβ_{42}/Aβ_{40} ratio was significantly and negatively (β = −0.424) associated with CSF-pTau (see Table 2).

To control for high outliers of AI-IgG$_{HSV1/2}$, that might significantly affect the linear model, we excluded post-hoc patients with AI-IgG$_{HSV1/2}$ ≥ 2.0, which still resulted in a significant model but with smaller effect size (adjusted R^2 = 0.244, p < 0.001, $\beta_{AI\text{-}IgGHSV1/2}$ = 0.210 (p = 0.043), $\beta_{CSF\text{-}A\beta42/A\beta40\ ratio}$ = −0.451 (p < 0.001). This suggests that the model is influenced by, but not based on, the highest AI-IgG$_{HSV1/2}$ values. In the present study, inclusion of CSF-Aβ_{42}/Aβ_{40} ratio together with AI-IgG$_{HSV1/2}$ in the regression analysis explained additional variability (as indicated by increased adjusted R^2) of CSF-pTau and CSF-tTau: both the AI-IgG$_{HSV1/2}$ and the CSF-Aβ_{42}/Aβ_{40} ratio resulted in significant similar moderate effects (as indicated by beta coefficients > 0.35) on CSF-pTau or CSF-tTau, respectively.

The association of (a) and (b) are in line with previous studies finding associations of HSV1 infection with tau pathology in neuronal cell cultures and AD animal models.[10,11,13,25] Since tTau, a marker of neuronal damage, is similarly associated with AI-IgG$_{HSV1/2}$ as pTau, this suggests virus-mediated neurotoxic effects leading to the release of different tau derivatives, despite the different categorization of these two biomarkers in the ATN system.[40]

3.2.5 | Associations of AI-IgGHSV1/2 with CSF-pTau or CSF-tTau modulated by CSF-Aβ42/Aβ40 ratio in HSV1 seropositive patients with AD

Although a virostatic role of soluble amyloid has been hypothesized, we did not find a correlation between the CSF-Aβ_{42}/Aβ_{40} ratio and AI-IgG$_{HSV1/2}$ but both variables explained the variability of tau. We therefore investigated whether the CSF-Aβ_{42}/Aβ_{40} ratio has a modulating effect on the association of AI-IgG$_{HSV1/2}$ with the CSF-pTau or CSF-tTau concentration, respectively. To this end, we added the interaction term [AI-IgG$_{HSV1/2}$ * CSF-Aβ_{42}/Aβ_{40} ratio] to the independent variables AI-IgG$_{HSV1/2}$ and the CSF-Aβ_{42}/Aβ_{40} ratio in the multivariate regression model with CSF-pTau or CSF-tTau, respectively, as dependent variable. The regression analyses in which the interaction term [AI-IgG$_{HSV1/2}$ * CSF-Aβ_{42}/Aβ_{40} ratio] was added to the independent variables AI-IgG$_{HSV1/2}$ and CSF-Aβ_{42}/Aβ_{40} ratio, and with CSF-pTau or CSF-tTau as dependent variable, respectively, resulted in significant models. The interaction term [AI-IgG$_{HSV1/2}$ * CSF-Aβ_{42}/Aβ_{40} ratio] was significantly and negatively associated with CSF-pTau (β = −1.552) and CSF-tTau (β = −1.568), and modulated the significant associations of AI-IgG$_{HSV1/2}$ with CSF-pTau or CSF-tTau, respectively, consistent with the expected negative effect of amyloid on HSV (Table 2).

There was a tendency for heteroskedasticity in the model with CSF-pTau (p = 0.06) and CSF-tTau (p = 0.08) using the White test, which could lead to type I empirical error (false positive results). Therefore, post-hoc generalized (least square) linear regression models (GLM) were calculated that resulted in significant β-coefficients of

comparable magnitude for the independent variables compared with those of the linear regression models. GLMs are depicted in Table SC.

The modulating effect of the CSF-Aβ_{42}/Aβ_{40} ratio on the association of AI-IgG$_{HSV1/2}$ with CSF-pTau or CSF-tTau concentration, respectively, was lowest in the group with high CSF-Aβ_{42}/Aβ_{40} ratios, where the effect of HSV on pTau or tTau appears to be suppressed compared to the group with low ratios. To further elucidate the effect of amyloid, the patient group was stratified into tertiles with low, intermediate, and high CSF-Aβ_{42}/Aβ_{40} ratios. Linear regression analyses of the associations between AI-IgG$_{HSV1/2}$ and CSF-pTau or CSF-tTau, respectively, in these tertile groups are displayed in Figure 1.1, Figure 1.2, which illustrate that the association between AI-IgG$_{HSV1/2}$ and CSF-pTau or CSF-tTau, respectively, is tighter in the group with low CSF-Aβ_{42}/Aβ_{40} ratios.

Post-hoc, we investigated whether the interaction effect of the CSF-Aβ_{42}/Aβ_{40} ratio was determined by CSF-Aβ_{42} or CSF-Aβ_{40} concentrations. Linear regression analyses with the dependent variable CSF-pTau or CSF-tTau, respectively, and with the independent variable AI-IgG$_{HSV1/2}$ in the tertiary groups with low, intermediate and high CSF-Aβ_{40} or CSF-Aβ_{42} concentrations, respectively, are illustrated in Figure SA. High CSF-Aβ_{40} rather than low CSF-Aβ_{42} levels explained the interaction of CSF-Aβ_{42}/Aβ_{40} ratio with AI-IgG$_{HSV1/2}$ on CSF-pTau or CSF-tTau.

Inclusion of any of the variables age, sex, global CDR, or the APOE epsilon 4 allele frequency as independent variables in the above linear regression models did not result in statistically significant estimates or increased explained variability (data not shown).

The interpretation of the results depends on the assumption that the soluble CSF Aβ fraction, in terms of the Aβ_{42}/Aβ_{40} ratio, tends to reflect the soluble nonagglutinated Aβ concentration in the brain. It will be critical to determine in brain whether only the fraction of soluble Aβ, as opposed to cerebrally deposited Aβ, modulates the relationship between intrathecal HSV activity and pTau in CSF. It may turn out that the soluble CSF amyloid concentration in relation to viruses such as HSV represents a separate biomarker for innate immune defense.

3.2.6 | Associations of AI-IgGHSV1/2 or AI-IgGCMV with the cerebral glucose metabolism

HSV

FDG PET images were available in a subset of 33 patients with AD, 20 of whom were HSV1-seropositive and had valid AI-IgG$_{HSV1/2}$ values ($\geq$ 0.5), 10 were HSV1-seronegative and three HSV1-seropositive without a valid AI-IgG$_{HSV1/2}$ value. We used FDG PET, which indicates neuronal dysfunction, as an alternative biomarker for neuronal damage because it also indicates neuronal damage that correlates with CSF pTau and tTau levels in AD.[64] Analogous to the mean comparison and linear regression analyses that investigated the association of AI-IgG$_{HSV1/2}$ with CSF-pTau, we compared global and voxel-based cerebral glucose metabolism between HSV1-seropositive and seronegative patients and then analyzed the association between AI-IgG$_{HSV1/2}$ and cerebral glucose metabolism in HSV1-seropositive patients with AD in a multivariate regression model.

HSV1-seropositive and HSV1-seronegative patients did not differ significantly in FDG tracer uptake at the voxel level (t-test, $p = 0.989$), global comparison with C/cb ($p = 0.706$) or C/th ratio ($p = 0.602$), or age, sex, global CDR, or APOE e4 allele frequency.

However, in HSV1-seropositive patients with AD, AI-IgG$_{HSV1/2}$ was significantly and negatively associated with the local C/th and C/cb FDG tracer uptake. Higher ratios were directly related to lower glucose metabolism in regions partly covering the right temporal and the inferior right parietal cortex (Figure 3). CSF-Aβ_{42}/Aβ_{40} ratio was also negatively associated with cerebral glucose metabolism but the association of the AI-IgG$_{HSV1/2}$ with the FDG-PET tracer uptake was not significantly affected by CSF-Aβ_{42}/Aβ_{40} ratio at the voxel-based level.

CMV

FDG-PET images were available in a subset of 13 CMV-seropositive patients with valid AI IgG$_{HSV1/2}$ values ($\geq$ 0.5) and 20 CMV-seronegative patients. Interestingly, the voxel-based C/cb FDG uptake signal was significantly lower in bilateral temporopolar and frontopolar cortices in CMV-seropositive compared with CMV-seronegative patients with AD (Figure SB), although it was not significantly different in either C/th or C/cb ratio in global mean comparison. In addition, AI-IgG$_{CMV}$ was significantly and negatively associated with precentral glucose metabolism (C/cb) in CMV-seropositive patients (Figure SB). Therefore, anti-CMV antibodies appear to be associated with different cortical regions compared to anti-HSV1 antibodies, and regions that are less typically affected in AD. However, these results have to be confirmed in studies with larger sample size, before any conclusions can be drawn.

3.2.7 | Neurotoxic effects by HSV1 in relation to soluble amyloid

Although this study is based on correlations, the common associations of different biomarkers for neuronal damage with AI-IgG$_{HSV1/2}$ support the hypothesis of a neurotoxic effect by HSV1 in AD. However, increased AI-IgG$_{HSV1/2}$ can also be found in the elder healthy individuals in our and previous studies.[61] Most importantly, the loss of antiviral function of amyloid in AD by reducing its soluble form could lead to higher HSV1-mediated tau pathology or neuronal damage and explain why HSV1-seropositive patients generally do not differ from HSV1-seronegative patients in terms of CSF AD biomarkers or FDG-PET. This suggests the possibility that increased HSV1 activity may be a secondary event in HSV1-seropositive patients with AD, which implies in other words that HSV1 is rather a pathological cofactor in advance stages of AD than a causative one. HSV1 may lead to worsening AD pathology, as supported by negative associations of AI-IgG$_{HSV1/2}$ with regional glucose metabolism and its positive association with pTau or tTau in CSF. The heterogeneity of the epidemiological data discussing HSV1 as a risk factor for AD may be explained in the way that HSV1

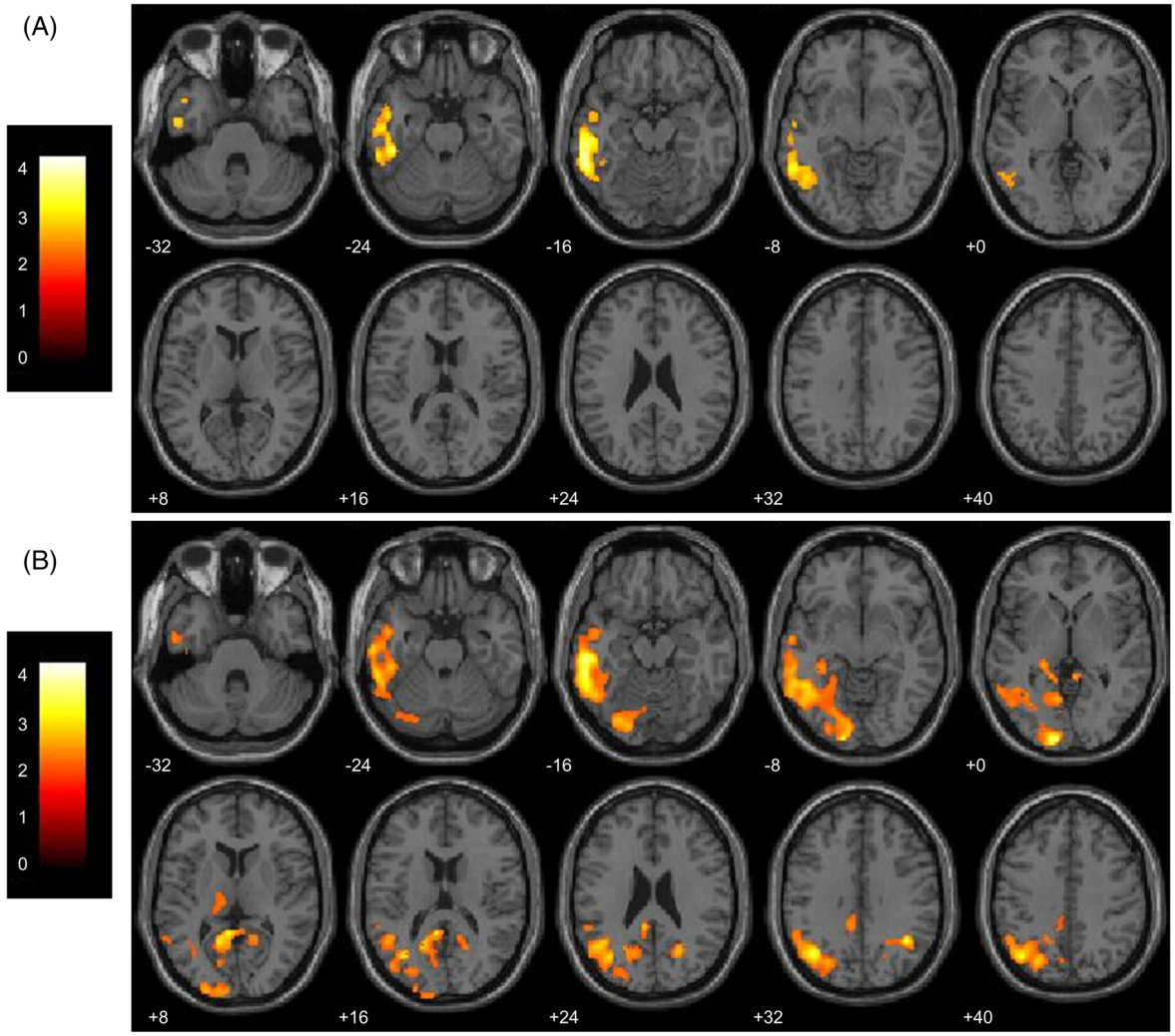

FIGURE 3 Associations of cerebral glucose metabolism with AI-IgG$_{HSV1/2}$ Voxel-based associations between the PVC corrected [F18] FDG uptake ratio in grey matter and the AI-IgG$_{HSV1/2}$ in 20 HSV1-seropositive (s-IgG$_{HSV1}$ > 25 U/ml) patients with AD. Significant ($p < 0.05$, threshold of 300, corrected for multiple comparisons using family wise error) inverse correlations are depicted in yellow and are projected on axial T1 MRI scans (average of 152 scans, implemented in SPM12), numbers indicate z-coordinates of slices in Talairach space in mm. Level of significance was then stepwise raised by 0.025, and remaining results are displayed using a threshold to only display significant clusters (FEW-corrected on cluster level). (A) AI-IgG$_{HSV1/2}$ is negatively associated with the FDG tracer uptake signal intensity normalized to cerebellum ($p = 0.01$) predominantly on the right temporal cortex. (B) AI-IgG$_{HSV1/2}$ is negatively associated with the FDG tracer uptake signal intensity normalized to thalamus ($p = 0.025$) on the right temporal cortex, partly the right cerebellum and the lower right parietal cortex. AI-IgG$_{HSV1/2}$, CSF to serum anti-HSV$_{1/2}$-IgG antibody index; CSF, cerebrospinal fluid; FDG, fluorodeoxyglucosis; HSV, herpes simplex virus; PET, position emission tomography; PVC, partial volume correction.

simply leads to faster AD progression. This hypothesis is also supported by higher clinical severity in HSV1-seropositive patients with AD and the increased intrathecal anti-IgG$_{HSV1/2}$-antibody synthesis equal to or higher than AI-IgG$_{HSV1/2}$ of 1.5 compared with patients with AI-IgG$_{HSV1/2}$ below 1.5 (compared Table SF).

In addition to this, our main finding of an association between AI-IgG$_{HSV1/2}$ and CSF-pTau was replicated by substituting CSF-pTau with CSF-tTau despite the different categorization of these two biomarkers in the ATN system.[40] Whether HSV1 activity triggers tau pathology initially or whether CSF-pTau is increased in terms of neuronal damage caused by HSV1 has to be elucidated in humans with AD. An in vitro model with primary adult hippocampal neurons suggested that pTau potentially acts as an acute response to any perceived danger-associated molecular pattern in primary adult hippocampal neurons, while Aβ aggregation is a long-term response to persistent threats, including HSV-1 infection.[25]

The observation that HSV1 encephalitis may be associated with AD-like biomarker concentrations of pTau and soluble Aβ in CSF,[65,66] could be interpreted that HSV1 reactivation is more likely to occur in patients with low amyloid levels in CSF. However, because most of these generally rare cases do not have prior or long-term follow-up CSF AD biomarker testing, this question remains unanswered. In addition,

increased viral activity could also occur in any other immunocompromised host state, initialized by HSV1 reactivation and promoted by decompensation of the host's anti-viral defense.

3.2.8 | Correlation analyses of HSV1 antibody levels, CSF AD biomarker, and clinical biomarkers in HSV1-seropositive patients with AD and NC

To find further evidence for the hypothesis of the involvement of HSV1 in advanced AD, correlation analyses of serum anti-HSV1-IgG, serum anti-HSV1-IgM, and AI-$IgG_{HSV1/2}$ with CSF AD biomarkers ($A\beta_{40}$, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$ ratio, pTau, tTau) and with clinical parameters (age, years of AD symptoms, MMSE, CDR, APOE epsilon 4 frequency) were performed in HSV1-seropositive patients with AD and NC and compared between groups (Table SI and SE). In NC, neither anti-HSV1-IgG, serum anti-HSV1-IgM nor AI-$IgG_{HSV1/2}$ were significantly correlated with each other, whereas in AD, serum anti-HSV1-IgG was positively correlated with AI-$IgG_{HSV1/2}$ (Rho = 0.253). The is likely due to different commercial ELISA for AI-$IgG_{HSV1/2}$ and serum anti-HSV1-IgG, as only the serum anti-HSV1-IgG ELISA provides cutoff values for seropositivity. There were no significant correlations between serological HSV biomarkers and clinical parameters, except for the correlation between years of AD symptoms and serum IgM_{HSV1} (Rho = −0.208) in AD, but this was no longer significant after Bonferroni correction. Frequent reactivation (serum anti-HSV1-IgG ≥ 25 U/l) was not observed.

In HSV1-seropositive patients with AD (n = 92), the only significant correlations between HSV antibody and AD CSF biomarkers were positive correlations of AI-$IgG_{HSV1/2}$ with CSF-pTau (Rho = 0.283) and with CSF-tTau (Rho = 0.264). The CSF-$A\beta_{42}/A\beta_{40}$ ratio was significantly and inversely correlated with CSF-pTau (Rho = −0.469) and CSF-tTau (Rho = −0.476). Whereas CSF-$A\beta_{40}$ was correlated with CSF-pTau both in AD (Rho = 0.578) and in NC (Rho = 0.462), a correlation of CSF-$A\beta_{42}$ with CSF-pTau was only present in AD (Rho = 0.280).

In the HSV1-seropositive NC (n = 24), AI-$IgG_{HSV1/2}$ was negatively correlated with CSF-$A\beta_{42}$ (Rho = −0.455, p = 0.029). Interestingly, only in HSV1-seropositive NC, serum IgG_{HSV1} tended to be positively correlated with CSF-$A\beta_{42}$ (β = 0.371, p = 0.074) and CSF-$A\beta_{40}$ (β = 0.436, p = 0.033). Serum IgM_{HSV1} as a marker for viral reactivation did not correlate with any anti-HSV1-IgG biomarker. All correlations are summarized in Table SI.

In another study involving 1222 nondemented patients, a significant inverse association was found between plasma $A\beta_{40}$ and $A\beta_{42}$ with anti-HSV1/2-IgM in the highest quartile of anti-HSV1/2-IgM levels but not with anti-HSV1/2-IgG.[28] IgG serotiter has not been shown to correlate with plasma Aβ levels.[28,67] These results would be compatible with the hypothesis that during HSV1-reactivation, the soluble amyloid fraction is altered by virus-mediated effects. However, in our study, Aβ (and anti-HSV Ig) has been measured in CSF of patients with AD dementia so the results cannot be directly compared.

3.2.9 | Correlation analyses of HSV1 antibody levels with CSF AD biomarker and T+ and T- HSV1-seropositive patients with AD

Because tau pathology (T+) classified patients with advanced AD according to the CSF-based ATN classification, we also performed correlations to compare them with the subgroup of T- patients. Correlations between AI-$IgG_{HSV1/2}$ and CSF AD biomarkers (Aβ42, Aβ42, CSF-Aβ42/Aβ40 ratio, pTau, tTau) in T+ (n = 71) and T- (n = 21) HSV1-seropositive patients with AD were investigated. In T+, AI-$IgG_{HSV1/2}$ was positively correlated with CSF-tTau (Rho = 0.336) and with CSF-pTau (Rho = 0.364). In contrast to the correlation analyses in the whole HSV1-seropositive patient group, in the T- sub group, AI-$IgG_{HSV1/2}$ was significantly and positively correlated with CSF-$A\beta_{42}/A\beta_{40}$ ratio in T- (Rho = 0.568, p = 0.009) but not in T+ (Table SH).

This finding is interesting, with respect of former reported mean comparisons between T+ and T- in HSV1-seropositive patients with AD. Despite lower CSF-Aβ42/Aβ40 ratios in T+, the levels of both CSF-Aβ42 (498 ng/l vs. 396 ng/l) and CSF-Aβ40 (14,506 ng/l vs. 9,520 ng/l) were significantly higher in T+.

In accordance with the current concept of AD biomarker evolution,[63] in which T+ represents a later stage of AD as compared to T-, significantly higher CSF levels of pTau and tTau and a significantly lower $A\beta_{42}/A\beta_{40}$ ratio were observed in T+ in this sample. Interestingly, despite the lower $A\beta_{42}/A\beta_{40}$ ratio, both $A\beta_{42}$ and $A\beta_{40}$ concentrations were significantly higher at T+ as compared with T-. This is compatible with previous observations in which autophagic Aβ clearance was reduced by HSV1 infection in human neuroblastoma cells, neurons, astrocytes, and macrophages,[68] and APP processing was increased in sporadic AD[65] and in HSV1-infected neurons,[16] resulting in higher Aβ levels. It is tempting to interpret these alterations as an attempt to mitigate the progressive HSV infection. This view is strengthened by the fact that in T- there is (still) a significant positive correlation between AI-$IgG_{HSV1/2}$ and CSF-$A\beta_{42}/A\beta_{40}$ ratio, which was not (anymore) observed in T+, and that the finding of a significant positive (causal?) association of AI-$IgG_{HSV1/2}$ on CSF-pTau as well as on CSF-tTau in the whole cohort was not (yet) retrieved in T- but in T+. This may mean that the harmful effects of the HSV infection can still be controlled by the increase in soluble CSF-Aβ at T-, but no longer at T+.

3.2.10 | CMV and patients with AD and NC

CMV seropositivity status was not linked to age and sex in AD and NC (compare Table SK). Interestingly, in the AD group, CMV-seropositives were significantly more likely (p = 0.013, odds ratio 1.30) to also be HSV1-seropositive compared with CMV seronegative, which was not observed in NCs. Conversely, patients with AD and HSV1 seropositivity also were significantly more likely to be CMV seropositive (p = 0.013, odds ratio 2.35) than HSV1-seronegative patients.

Moreover, intrathecal anti-CMV-IgG antibody synthesis was found only in the CMV-seropositive AD group but not in the CMV-seropositive NC group (compare Figure SB). This is interesting because parallel infections of various herpesviruses, including HSV1 with CMV, have been associated with an increased risk for AD.[23] An alternative explanation for the epidemiological risk for AD concerning CMV and HSV1 seropositivity could also be increased susceptibility for herpesvirus infection.

Post-hoc stratification of the AD group combining CMV and HSV1 seropositivity revealed no significant differences in clinical parameter or CSF AD biomarkers between groups (Table SL). Furthermore, we found no valid correlation between CMV antibody markers and CSF AD biomarkers in either of the AD or NC groups (Table SM), which, at least in this cross-sectional cohort, does not support an independent effect of CMV or a synergistic effect of both herpesviruses on AD biomarkers. AI-IgG_{CMV} was not associated with CSF pTau and tTau in CMV-seropositive patients with AD or NC (Table SB).

In addition, HSV2 is suspected to increase AD risk.[29,52] The ELISA used in the current study does not distinguish between anti-HSV1 and anti-HSV2 IgG in CSF. Therefore, the association of HSV1 with tau pathology may have been influenced by HSV2 in a few samples. However, HSV2 prevalence is generally only about 10%.[48]

3.2.11 | NC and HSV1

To answer the question of whether the significant results of the above analyses are specific to patients with AD, the analyses were repeated in comparison with NC using the same schema. In NC, the frequency of HSV1 or CMV seropositivity was similar to that in the AD group (compare Table 1). HSV1-seropositive NC were significantly younger than HSV1-seropositive patients with AD but did not differ by sex (Table 1), which was similar for CMV-seropositive patients with AD compared with the NC group (Table SK).

No significant associations with CSF AD biomarkers and AI-$IgG_{HSV1/2}$ were observed in the HSV1-seropositive NC, by definition (inclusion criteria) variability of CSF-pTau in this group (<60 ng/ml) is low. Linear regression models with CSF-pTau or CSF-tTau as dependent variable, and AI-$IgG_{HSV1/2}$ as independent variable, controlling for $A\beta_{42}/A\beta_{40}$ ratio and the interaction term [AI-$IgG_{HSV1/2}$ * CSF-$A\beta_{42}/A\beta_{40}$ ratio] were not significant (Table SC). In the HSV1-seropositive NC, CSF-$A\beta_{40}$ was positively correlated with serum anti-HSV1 IgG ($\beta = 0.436$, $p = 0.033$) and with CSF-pTau ($\beta = 0.415$, $p = 0.044$), and CSF-$A\beta_{42}$ was significantly and negatively correlated with AI-$IgG_{HSV1/2}$ ($\beta = -0.455$, $p = 0.029$) (Table SI). In the HSV1-seropositive NC group, the positive and significant correlation of CSF-$A\beta_{40}$ (as a marker of amyloid turnover) with serum anti-HSV1-IgG (as a marker for a general infectious status) supports the hypothesis of virus-mediated increased amyloid production, whereas the positive correlation of CSF-$A\beta_{42}$ with AI-$IgG_{HSV1/2}$ points to the proposed interaction of reduced virostatic $A\beta_{42}$ with intrathecal HSV1 activity, potentially as a risk constellation for later developing AD.

ACKNOWLEDGMENTS

The authors thank the medical-technical laboratory assistants Ms Sabine Creutzburg, Mrs Tamara Eisele, and Ms Ingrid Schwab for their contribution to this project. Preliminary results of this manuscript have already been presented previously in part at the virtual International Conference on Alzheimer's and Parkinson's Diseases 2021.[69]

Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors report no actual or potential conflict of interest with regards to the submitted work. Outside the submitted manuscript, T.G. received consulting fees from Abbvie, Alector, Anavex, Biogen, Eli Lilly, Functional Neuromodulation, Grifols, Iqvia/Quintiles, Novo Nordisk, Noselab, NuiCare, Roche Diagnostics, Roche Pharma, Toyama, UCB, and Vivoryon; lecture fees from Biogen, Life Molecular Imaging, Novo Nordisk, Roche Pharma, and Schwabe; and grants to his institution from Actelion and Novartis. Outside the submitted work, O.G. reports having received consulting fees from Eli Lilly, and grants to his institution from Actelion, and prescreening activities for Julius Clinical/Toyama. All further authors declare no competing interests.

REFERENCES

1. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82(4):239-259.
2. Kametani F, Hasegawa M. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Front. Neurosci*. 2018;12:25.
3. Bourgade K, Le Page A, Bocti C, et al. Protective effect of amyloid-β peptides against herpes simplex virus-1 infection in a neuronal cell culture model. *JAD*. 2016;50(4):1227-1241.
4. Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, et al. Alzheimer's disease-associated β-amyloid is rapidly seeded by herpesviridae to protect against brain infection. *Neuron*. 2018;99(1):56-63.e3.
5. Itzhaki RF. Overwhelming Evidence for a Major Role for Herpes Simplex Virus Type 1 (HSV1) in Alzheimer's Disease (AD); Underwhelming Evidence against. *Vaccines*. 2021;9(6):679.
6. Ball MJ, Nuttall K, Warren KG. Neuronal and lymphocytic populations in human trigeminal ganglia: implications for ageing and for latent virus. *Neuropathology and Applied Neurobiology*. 1982;8(3):177-187.
7. Yong SJ, Yong MH, Teoh SL, et al. The hippocampal vulnerability to herpes simplex virus type i infection: relevance to Alzheimer's disease and memory impairment. *Front. Cell. Neurosci*. 2021;15.
8. Readhead B, Haure-Mirande J-V, Funk CC, et al. Multiscale analysis of independent alzheimer's cohorts finds disruption of molecular, genetic, and clinical networks by human herpesvirus. *Neuron*. 2018;99(1):64-82.e7.
9. Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *The Journal of pathology*. 2009;217(1):131-138.
10. Wozniak MA, Frost AL, Itzhaki RF. Alzheimer's disease-specific tau phosphorylation is induced by herpes simplex virus type 1. *JAD*. 2009;16(2):341-350.
11. Zambrano Á, Solis L, Salvadores N, Cortés M, Lerchundi R, Otth C. Neuronal cytoskeletal dynamic modification and neurodegeneration induced by infection with herpes simplex virus type 1. *JAD*. 2008;14(3):259-269.
12. Álvarez G, Aldudo J, Alonso M, Santana S, Valdivieso F. Herpes simplex virus type 1 induces nuclear accumulation of hyperphosphorylated tau in neuronal cells. *J. Neurosci. Res*. 2012;90(5):1020-1029.

13. Chiara G de, Piacentini R, Fabiani M, et al. Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice. *PLoS pathogens*. 2019;15(3):e1007617.
14. Gosztyla ML, Brothers HM, Robinson SR. Alzheimer's amyloid-β is an antimicrobial peptide: a review of the evidence. *JAD*. 2018;62(4):1495-1506.
15. Wang H-C, Zhang Q-X, Zhao J, Wei N-N. Molecular docking and molecular dynamics simulations studies on the protective and pathogenic roles of the amyloid-β peptide between herpesvirus infection and Alzheimer's disease. *Journal of Molecular Graphics and Modelling*. 2022;113:108143.
16. Chiara G de, Marcocci ME, Civitelli L, et al. APP processing induced by herpes simplex virus type 1 (hsv-1) yields several app fragments in human and rat neuronal cells. *PloS one*. 2010;5(11):e13989.
17. Piacentini R, Civitelli L, Ripoli C, et al. HSV-1 promotes Ca2+-mediated APP phosphorylation and Aβ accumulation in rat cortical neurons. *Neurobiology of aging*. 2011;32(12):2323.e13-2323.e26.
18. Shipley SJ, Parkin ET, Itzhaki RF, Dobson CB. Herpes simplex virus interferes with amyloid precursor protein processing. *BMC microbiology*. 2005;5(1):48.
19. Wozniak MA, Itzhaki RF, Shipley SJ, Dobson CB. Herpes simplex virus infection causes cellular β-amyloid accumulation and secretase upregulation. *Neuroscience letters*. 2007;429(2-3):95-100.
20. Bocharova O, Pandit NP, Molesworth K, et al. Alzheimer's disease-associated β-amyloid does not protect against herpes simplex virus 1 infection in the mouse brain. *Journal of Biological Chemistry*. 2021;297(1):100845.
21. Cairns DM, Rouleau N, Parker RN, Walsh KG, Gehrke L, Kaplan DL. A 3D human brain–like tissue model of herpes-induced Alzheimer's disease. *Sci. Adv*. 2020;6(19):eaay8828.
22. Wozniak MA, Shipley SJ, Combrinck M, Wilcock GK, Itzhaki RF. Productive herpes simplex virus in brain of elderly normal subjects and Alzheimer's disease patients. *Journal of medical virology*. 2005;75(2):300-306.
23. Lövheim H, Olsson J, Weidung B, et al. Interaction between cytomegalovirus and herpes simplex virus type 1 associated with the risk of Alzheimer's disease development. *JAD*. 2018;61(3):939-945.
24. Schnier C, Janbek J, Lathe R, Haas J. Reduced dementia incidence after varicella zoster vaccination in Wales 2013-2020. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*. 2022;8(1).
25. Powell-Doherty RD, Abbott ARN, Nelson LA, Bertke AS. Amyloid-β and p-tau anti-threat response to herpes simplex virus 1 infection in primary adult murine hippocampal neurons. *Journal of virology*. 2020;94(9).
26. Tapiola T, Alafuzoff I, Herukka S-K, et al. Cerebrospinal fluid β-amyloid 42 and tau proteins as biomarkers of alzheimer-type pathologic changes in the brain. *Archives of neurology*. 2009;66(3):382-389.
27. Iaccarino L, Tammewar G, Ayakta N, et al. Local and distant relationships between amyloid, tau and neurodegeneration in Alzheimer's Disease. *NeuroImage. Clinical*. 2018;17:452-464.
28. Féart C, Helmer C, Fleury H, et al. Association between IgM anti-herpes simplex virus and plasma amyloid-beta levels. *PloS one*. 2011;6(12):e29480.
29. Hillen H. The Beta Amyloid Dysfunction (BAD) hypothesis for alzheimer's disease. *Front. Neurosci*. 2019;13:1154.
30. Dominy SS, Lynch C, Ermini F, et al. Porphyromonas gingivalis in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv*. 2019;5(1):eaau3333.
31. Weidung B, Hemmingsson E-S, Olsson J, et al. VALZ-Pilot: High-dose valacyclovir treatment in patients with early-stage Alzheimer's disease. *Alzheimers Dement (N Y)*. 2022;8(1):e12264.
32. Goldhardt O, Warnhoff I, Yakushev I, et al. Kallikrein-related peptidases 6 and 10 are elevated in cerebrospinal fluid of patients with Alzheimer's disease and associated with CSF-TAU and FDG-PET. *Translational neurodegeneration*. 2019;8(1):25.
33. Bourgade K, Garneau H, Giroux G, et al. β-Amyloid peptides display protective activity against the human Alzheimer's disease-associated herpes simplex virus-1. *Biogerontology*. 2015;16(1):85-98.
34. Ezzat K, Pernemalm M, Pålsson S, et al. The viral protein corona directs viral pathogenesis and amyloid aggregation. *Nature Communications*. 2019;10(1):2331.
35. Tran DN, Bakx ATCM, Dis V, Aronica E, Verdijk RM, Ouwendijk WJD. No evidence of aberrant amyloid β and phosphorylated tau expression in herpes simplex virus-infected neurons of the trigeminal ganglia and brain. *Brain Pathology*. 2021:e13044.
36. Morris JC. The Clinical Dementia Rating (CDR). *Neurology* 1993;43(11):2412.2-2412-a.
37. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia* 2011;7(3):270-279.
38. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia* 2011;7(3):263-269.
39. Müller EG, Edwin TH, Stokke C, et al. Amyloid-β PET—Correlation with cerebrospinal fluid biomarkers and prediction of Alzheimert's disease diagnosis in a memory clinic. *PloS one* 2019;14(8):e0221365.
40. Jack CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of alzheimer's disease. *Alzheimer's & Dementia*. 2018;14(4):535-562.
41. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". *Journal of Psychiatric Research*. 1975;12(3):189-198.
42. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clinica Chimica Acta*. 2001;310(2):173-186.
43. Tsolakidou A, Alexopoulos P, Guo L-H, et al. β-Site amyloid precursor protein–cleaving enzyme 1 activity is related to cerebrospinal fluid concentrations of sortilin-related receptor with A-type repeats, soluble amyloid precursor protein, and tau. *Alzheimer's & Dementia* 2013;9(4):386-391.
44. Schmidt P, Gaser C, Arsic M, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. *NeuroImage* 2012;59(4):3774-3783.
45. Gonzalez-Escamilla G, Lange C, Teipel S, Buchert R, Grothe MJ. PETPVE12: an SPM toolbox for Partial Volume Effects correction in brain PET - Application to amyloid imaging with AV45-PET. *NeuroImage*. 2017;147:669-677.
46. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*. 2006;31(3):968-980.
47. Schmidt AF, Finan C. Linear regression and the normality assumption. *Journal of clinical epidemiology*. 2018;98:146-151.
48. Korr G, Thamm M, Czogiel I, Poethko-Mueller C, Bremer V, Jansen K. Decreasing seroprevalence of herpes simplex virus type 1 and type 2 in Germany leaves many people susceptible to genital infection: time to raise awareness and enhance control. *BMC infectious diseases*. 2017;17(1):471.
49. Whitley RJ, Roizman B. Herpes simplex virus infections. *Lancet (London, England)*. 2001;357(9267):1513-1518.
50. Jamieson GA, Maitland NJ, Wilcock GK, Yates CM, Itzhaki RF. Herpes simplex virus type 1 DNA is present in specific regions of brain from aged people with and without senile dementia of the Alzheimer type. *The Journal of pathology*. 1992;167(4):365-368.
51. Jamieson GA, Maitland NJ, CRASKE J, Wilcock GK, Itzhaki RF. Detection of herpes simplex virus type 1 DNA sequences in normal and Alzheimer's disease brain using polymerase chain reaction. *Biochemical Society transactions*. 1991;19(2):122S-122S.

52. Warren-Gash C, Forbes HJ, Williamson E, et al. Human herpesvirus infections and dementia or mild cognitive impairment: a systematic review and meta-analysis. *Scientific reports*. 2019;9(1):4743.
53. Lopatko Lindman K, Weidung B, Olsson J, et al. A genetic signature including apolipoprotein Eε4 potentiates the risk of herpes simplex–associated Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*. 2019;5(1):697-704.
54. Lövheim H, Norman T, Weidung B, et al. Herpes Simplex Virus, APOE ε4, and cognitive decline in old age: results from the betula cohort study. *JAD*. 2019;67(1):211-220.
55. Lövheim H, Gilthorpe J, Adolfsson R, Nilsson L-G, Elgh F. Reactivated herpes simplex infection increases the risk of Alzheimer's disease. *Alzheimer's & Dement*. 2015;11(6):593-599.
56. Mancuso R, Baglio F, Cabinio M, et al. Titers of herpes simplex virus type 1 antibodies positively correlate with grey matter volumes in Alzheimer's disease. *JAD*. 2013;38(4):741-745.
57. Kobayashi N, Nagata T, Shinagawa S, et al. Increase in the IgG avidity index due to herpes simplex virus type 1 reactivation and its relationship with cognitive function in amnestic mild cognitive impairment and Alzheimer's disease. *Biochemical and biophysical research communications*. 2013;430(3):907-911.
58. Grimmer T, Tholen S, Yousefi BH, et al. Progression of cerebral amyloid load is associated with the apolipoprotein e ε4 genotype in Alzheimer's disease. *Biological psychiatry*. 2010;68(10):879-884.
59. Burgos JS, Ramirez C, Sastre I, Valdivieso F. Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. *Journal of virology*. 2006;80(11):5383-7.
60. Koelle DM, Magaret A, Warren T, Schellenberg GD, Wald A. APOE genotype is associated with oral herpetic lesions but not genital or oral herpes simplex virus shedding. *Sexually transmitted infections*. 2010;86(3):202-206.
61. Linard M, Letenneur L, Garrigue I, Doize A, Dartigues J-F, Helmer C. Interaction between APOE4 and herpes simplex virus type 1 in Alzheimer's disease. *Alzheimer's & Dementia*. 2020;16(1):200-208.
62. Itzhaki RF, Lin W-R, Shang D, Wilcock GK, Faragher B, Jamieson GA. Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *Lancet (London, England)*. 1997;349(9047):241-244.
63. Jack CR, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet Neurology*. 2010;9(1):119-128.
64. Yakushev I, J. Muller M, Buchholz H-G, et al. Stage-dependent agreement between cerebrospinal fluid proteins and FDG-PET findings in Alzheimer's disease. *CAR* 2012;9(2):241-247.
65. Krut JJ, Zetterberg H, Blennow K, et al. Cerebrospinal fluid Alzheimer's biomarker profiles in CNS infections. *Journal of neurology*. 2013;260(2):620-626.
66. Shinomoto M, Kasai T, Tatebe H, et al. Cerebral spinal fluid biomarker profiles in CNS infection associated with HSV and VZV mimic patterns in Alzheimer's disease. *Translational neurodegeneration*. 2021;10(1):2.
67. Lopatko Lindman K, Weidung B, Olsson J, et al. Plasma Amyloid-β in relation to antibodies against herpes simplex virus, cytomegalovirus, and chlamydophila pneumoniae. *ADR*. 2021;5(1):229-235.
68. Miszczak D, Cymerys J. A game of survival: herpesvirus strategies of autophagy manipulation. *Postepy Hig Med Dosw*. 2014;68:1406-1414.
69. Goldhardt O, Freiberger R, Ortner M, et al. Intrathecal antibodies against herpes simplex virus are associated with tau pathology in humans with Alzheimer's disease. *Alzheimer's & Dement*. 2020;16(S4).

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Goldhardt O, Freiberger R, Dreyer T, et al. Herpes simplex virus alters Alzheimer's disease biomarkers - A hypothesis paper. *Alzheimer's Dement*. 2022;1-18. https://doi.org/10.1002/alz.12834