Received: 23 January 2024 | Revised: 19 March 2024 | Accepted: 24 March 2024

DOI: 10.1002/dad2.12603

Alzheimer's & Dementia
Diagnosis, Assessment & Disease Monitoring

RESEARCH ARTICLE

Cerebrospinal fluid soluble insulin receptor levels in Alzheimer's disease

Peter Thomas[1] | **Manon Leclerc**[2,3] | **Kira Evitts**[4,5] | **Caitlin Brown**[1] | **Wyatt Miller**[1] | **Angela J. Hanson**[6] | **William A. Banks**[1,6] | **Laura Gibbons**[7] | **Kimiko Domoto-Reilly**[8] | **Suman Jayadev**[8] | **Ge Li**[1,9,10] | **Elaine Peskind**[9,10] | **Jessica E. Young**[5,11] | **the Consortium for the early identification of Alzheimer's disease-Quebec (CIMA-Q)** | **Frederic Calon**[2,3] | **Elizabeth M. Rhea**[1,6]

[1]Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, Washington, USA

[2]Faculty of Pharmacy, Laval University, Quebec, Quebec, Canada

[3]Neuroscience Axis, CHU de Québec Research Center – Laval University, Quebec, Quebec, Canada

[4]Department of Bioengineering, University of Washington, Seattle, Washington, USA

[5]Institute for Stem Cells and Regenerative Medicine, University of Washington, Seattle, Washington, USA

[6]Department of Medicine, Division of Gerontology and Geriatric Medicine, University of Washington School of Medicine, Seattle, Washington, USA

[7]Department of Medicine, University of Washington, Seattle, Washington, USA

[8]Department of Neurology, University of Washington, Seattle, Washington, USA

[9]Veterans Affairs Northwest Mental Illness Research, Education, and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, Washington, USA

[10]Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, Washington, USA

[11]Department of Laboratory Medicine and Pathology, University of Washington School of Medicine, Seattle, Washington, USA

Correspondence
Elizabeth M. Rhea, Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, 1660 S. Columbian Way, Seattle, WA 98108, USA.
Email: meredime@uw.edu

The data used in the preparation of this article were obtained thanks to the Consortium for the early identification of Alzheimer's disease – Quebec (CIMA-Q; cima-q.ca). A list of researchers involved in the design of CIMA-Q can be found on the cima-q.ca website. These researchers contributed to the establishment of protocols, the implementation of the research infrastructure, the recruitment and follow-up of participants, the obtaining of data, the maintenance of biological and ex-vivo samples, and certain derived data.

Abstract

INTRODUCTION: Brain insulin resistance and deficiency is a consistent feature of Alzheimer's disease (AD). Insulin resistance can be mediated by the surface expression of the insulin receptor (IR). Cleavage of the IR generates the soluble IR (sIR).

METHODS: We measured the levels of sIR present in cerebrospinal fluid (CSF) from individuals along the AD diagnostic spectrum from two cohorts: Seattle ($n = 58$) and the Consortium for the Early Identification of Alzheimer's Disease-Quebec (CIMA-Q; $n = 61$). We further investigated the brain cellular contribution for sIR using human cell lines.

RESULTS: CSF sIR levels were not statistically different in AD. CSF sIR and amyloid beta (Aβ)42 and Aβ40 levels significantly correlated as well as CSF sIR and cognition in the CIMA-Q cohort. Human neurons expressing the amyloid precursor protein "Swedish" mutation generated significantly greater sIR and human astrocytes were also able to release sIR in response to both an inflammatory and insulin stimulus.

DISCUSSION: These data support further investigation into the generation and role of sIR in AD.

KEYWORDS
Alzheimer's disease, amyloid beta 42, cognition, insulin receptor, soluble

Highlights

- Cerebrospinal fluid (CSF) soluble insulin receptor (sIR) levels positively correlate with amyloid beta (Aβ)42 and Aβ40.
- CSF sIR levels negatively correlate with cognitive performance (Montreal Cognitive Assessment score).
- CSF sIR levels in humans remain similar across Alzheimer's disease diagnostic groups.
- Neurons derived from humans with the "Swedish" mutation in which Aβ42 is increased generate increased levels of sIR.
- Human astrocytes can also produce sIR and generation is stimulated by tumor necrosis factor α and insulin.

1 | BACKGROUND

Brain insulin resistance and deficiency is now considered a consistent feature of Alzheimer's disease (AD).[1] Patients with AD have a decreased insulin cerebrospinal fluid (CSF)/serum ratio (indicating decreased availability), impaired brain insulin receptor (IR) signaling (indicating insulin resistance), and when insulin is delivered to the brain, cognition is improved.[1,2] Brain insulin resistance and deficiency is also being pursued as a therapeutic target for pre-clinical AD by multiple mechanisms including intranasal insulin administration[3–5] or incretin receptor agonist (IRA) treatment,[6] based on cognitive improvement after treatment. However, the cause of brain insulin resistance and deficiency is largely unknown.

The IR is the primary mediator of insulin signaling and thus, can contribute to insulin resistance. The IR consists of two subunits linked by disulfide bonds. The α subunit is completely present on the extracellular side of the cell membrane. The β subunit is primarily comprised of the transmembrane and intracellular tyrosine kinase signaling domains. This receptor is expressed in all major cell types, including those within the brain (neurons, astrocytes, endothelial cells, microglia). The ectodomain of the receptor, which encompasses the entire α subunit and the extracellular portion of the β subunit, can be shed to generate the soluble insulin receptor (sIR). Shedding of cell surface proteins has been postulated to serve multiple functions. First, it can be a way to regulate expression of a cell surface receptor, thus eliminating the intracellular signaling events. Second, the soluble protein can bind to the ligand, which not only prevents the ligand from interacting with the full-length cell surface protein but can also extend the lifespan of the ligand. The cleavage of sIR is known to occur through common enzymes implicated in AD, including β-secretase amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), γ-secretase, and calpain-2.[7–10] and most recently by matrix metalloproteinases.[11]

The sIR was first described in 1972 as a protein secreted from human lymphocytes.[12] Twenty years later, it was identified and characterized in human plasma and found to competitively bind insulin.[13] Twenty years after that, it was investigated in human CSF in patients with human immunodeficiency virus (HIV).[14] Despite the slow progress that has been made regarding this protein, it has been shown to be negatively implicated in multiple diseases or conditions including diabetes,[15] HIV-associated neurocognitive disorders (HAND),[14] and most recently in aging.[11] In the CSF of patients with HAND who are symptomatic, such as those with cognitive impairment, sIR levels are increased compared to patients without HAND.[14]

To investigate possible mechanisms for brain insulin resistance and deficiency in AD, we determined whether sIR levels are increased in the CSF of patients with AD or pre-diagnostic stages, whether CSF sIR levels correlate with dementia status or CSF amyloid beta (Aβ) biomarkers, and whether sIR is released from human induced pluripotent stem cells (hiPSC) neurons containing the APP "Swedish" mutation or human astrocytes.

2 | METHODS

2.1 | Human CSF samples

CSF samples were obtained from participants from three separate centers: University of Washington (UW) Alzheimer's Disease Research Center (ADRC) Clinical Core; the Veterans Affairs (VA) Northwest Mental Illness Research, Education, and Clinical Center (MIRECC)

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors reviewed the literature using traditional (e.g., PubMed) sources and online content provided by reputable organizations (e.g., Alzheimer's Association) to evaluate the accumulated knowledge relevant to the role of the soluble insulin receptor in disease and cognitive impairment.
2. **Interpretation**: Our findings identified that while insulin receptor signaling, especially in the brain, in Alzheimer's disease (AD) is an important component, the levels of cerebrospinal fluid (CSF) soluble insulin receptor have not been assessed, nor the brain cell type that can produce this protein.
3. **Future Directions**: This pilot study provides evidence the CSF soluble insulin receptor is relevant in the context of AD and cognitive impairment. The mechanisms involved in the generation of the soluble insulin receptor warrant further investigation.

Behavioral Neurosciences Group (BNG) Sample and Data Repository; and the Consortium for the Early Identification of Alzheimer's Disease-Quebec (CIMA-Q) 16. Samples were provided with clinical data including sex, age of CSF draw, education, Clinical Dementia Rating (CDR; in some cases), Mini-Mental State Examination (MMSE) score (in some cases), Montreal Cognitive Assessment (MoCA) score (in some cases), apolipoprotein E (*APOE*) ε4 allele carriage,[17,18] body mass index (BMI), and CSF Aβ42 or Aβ40 (in some cases) levels. Due to the nature of this pilot study and small sample size, we do not report race or ethnicity.

UW ADRC and VA MIRECC BNG samples were combined and identified as the Seattle cohort (Table 1). Data collection was similar between the UW ADRC and the VA MIRECC BNG. Samples used were from individuals with a clinical diagnosis of probable AD or cognitively intact controls. All participants in the Seattle cohort undergo physical exam, neuropsychological battery, CDR scale and participant and co-participant interviews per national ADRC standardized protocols, the Uniform Data Set (UDS). After the ADRC visit UDS the diagnosis was adjudicated by consensus among clinicians, neuropsychologist, and raters.[19,20] VA MIRECC BNG samples were provided with MMSE scores and were converted to MoCA scores using the conversion methods of Fasnacht et al.[21] (see Table 1 for mean). In the Seattle cohort, individuals with a history of diabetes and males were excluded based on published data investigating a role for sIR in cognition.[14] and to gain the most insight from this pilot study. Inclusion criteria for the current study were clinical or research diagnosis of AD and age-matched cognitively intact controls. For the Seattle cohort CSF collections, individuals fasted the morning of collection. CSF was immediately frozen on dry ice. Aβ42 levels were previously measured by the Lumipulse assay (Fujirebio) in the UW ADRC samples and by the Luminex (InnoGenetics) multiplex according to the manufacturer's instructions as previously described.[22] in the VA MIRECC BNG samples. Aβ40 levels were not measured in this cohort.

CIMA-Q samples were analyzed separately and split into four groups: no cognitive impairment (Control), subjective cognitive disorder (SCD), mild cognitive impairment (MCI), and early probable AD (Table 2). CIMA-Q is a multi-center collaborative research infrastructure that examines longitudinal AD progression in older adults at risk for the disease.[16] The main objective of CIMA-Q is the longitudinal characterization of an observational cohort of elderly women and men aged ≥ 65, cognitively healthy, with SCD, suffering from mild cognitive disorders, or suffering from dementia due to probable AD. CIMA-Q collects clinical, cognitive, biological, neuroimaging, and pathological data from these participants to (1) establish an early diagnosis of AD, (2) to provide the scientific community with a well-characterized cohort, (3) identify new therapeutic targets to prevent or slow down cognitive decline and AD, and (4) to support new clinical studies on these targets.[16] Further details on recruitment methods, exclusion and inclusion criteria (detailed in Supplementary Material B in Belleville et al.[16]), testing materials, and general methodology for the CIMA-Q project can be found in Belleville et al.[16] Diagnostic criteria for AD and MCI was based on the National Institute on Aging–Alzheimer's Association and were described in detail in Belleville et al.[16] Participants who had agreed to undergo lumbar puncture (LP, Visit 4) for CSF collection came for a last visit at which a neurologist or anesthesiologist obtained 10 to 15 mL of CSF. The CSF was aliquoted rapidly into 50 × 0.1 mL fractions rapidly frozen and stored at −80° C. Fasting was not required prior to the lumbar puncture. The levels of Aβ40 and Aβ42 were determined by enzyme-linked immunosorbent assays (ELISA, #K15200E, MesoScale Discovery).[16] CIMA-Q CSF was spun at 20,000 × g for 10 minutes at 4°C prior to sIR assay. *APOE* genotype was assessed by the restriction digest method.[16,17]

The study was approved by the institutional review board at VA Puget Sound Health Care System. All participants signed an informed consent for CSF donation and a repository consent allowing their data and biospecimens to be shared.

2.2 | Human neuronal cultures

Human iPSC lines with the APP "Swedish" mutation (APPKM670/671NL) introduced using CRISPR/Cas9 gene editing have been previously published and characterized.[23,24] Briefly, cells were cultured in feeder-free conditions on Matrigel with mTESR media. Neural progenitor cells (NPCs) were generated and further differentiated to cortical neurons using previously described protocols.[25] Cells used include those homozygous for the Swedish mutation, $APP^{Swe/Swe}$, heterozygous for the mutation, $APP^{Swe/WT}$, and isogenic wild-type (WT) controls, APPWT/WT. After 21 days of differentiation, neurons from each line were selected for the CD184/CD44 negative population by magnetic-activated cell sorting and cultured as previously described in Shin et al.[24] Cells were plated in Matrigel (#356231)–coated 96-well plates at a density of 200,000

TABLE 1 Seattle cohort characteristics. Demographics, performance on neuropsychological measures, *APOE* ε4 data, and CSF Aβ biomarker levels in cognitively health controls and participants with AD.

Characteristics	Control			AD			Stats	*P* value
N (with CSF)	43			15				
Sex (% female)	100			100				
Age	73.67	±	8.13	72.73	±	8.82	T	0.707
Education (years)[a]	15.93	±	2.64	15	±	2.66	T	0.258
Mean CDR[a]	0.0465	±	0.15	1.07	±	0.55*	T	<0.0001
Mean MMSE[b]	29.36	±	0.92	22.10	±	4.56*	T	<0.0001
Mean MoCA (converted)[c]	26.27	±	2.284	15.5	±	4.99*	MW	<0.0001
Mean MoCA[d]	27.44	±	1.90	14.00	±	6.68*	MW	<0.0001
Mean MoCA (combined)[e]	27.14	±	2.042	15.07	±	5.30*	MW	<0.0001
APOE ε4 allele carriage (%)	32.6			53.3			FC	0.153
BMI[f]	24.96	±	3.23	24.25	±	4.46	T	0.526
CSF $A\beta_{42}$[g]	699.5	±	359.4	223.8	±	132.6*	T	<0.0001

Notes: Means ± SD are reported. VA MIRECC BNG Control $n = 11$, AD $n = 11$; UW ADRC Control $n = 32$, AD $n = 4$. Statistical tests used are as indicated: T: unpaired *t* test, two tailed; MW: Mann–Whitney test, two tailed; FC: contingency, Fisher exact test. *P* values refer to the significant analysis of variance.
Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; *APOE*, apolipoprotein E; Age, age at draw; BMI, body mass index; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; UW ADRC, University of Washington Alzheimer's Disease Research Center; VA MIRECC BNG, Veterans Affairs Northwest Mental Illness Research, Education, and Clinical Center Behavioral Neurosciences Group.
[a]Data missing for one subject.
[b]VA MIRECC BNG only ($n = 21$; data missing for one subject).
[c]VA MIRECC BNG MMSE converted MoCA score ($n = 21$; data missing for one subject).
[d]UW ADRC MoCA only ($n = 36$).
[e]VA MIRECC BNG converted and UW ADRC MoCA combined ($n = 57$; data missing for one subject).
[f]Data missing for two subjects.
[g]Data missing for six subjects.
*$P < 0.0001$ vs. control.

TABLE 2 CIMA-Q cohort characteristics demographics, performance on neuropsychological measures, *APOE* ε4 data, and CSF Aβ biomarker levels in cognitively health controls and participants with SCD, MCI, AD.

Characteristics	Control			SCD			MCI			AD			Stats	*P* value
N (with CSF)	14			21			18			8			–	
Sex (% female)	57			57			45			13.0			PC	0.151
Age	72.6	±	6.7	73.9	±	6.4	75.2	±	5.3	74.8	±	6.8	A	0.6846
Education (years)[e]	14.5	±	4.1	14.2	±	3.1	14.6	±	2.5	15	±	4.6	A	0.9381
Mean MMSE	24.9	±	1.9	24	±	1.7	24.2	±	1.9	22.3	±	3.2[a]	A	0.0389
Mean MoCA	28.4	±	1.9	28	±	1.2	24.2	±	2.0[b]	18.9	±	4.3[c]	W	0.0001
APOE ε4 allele carriage (%)	21	±	21	19	±	19	39	±	39	50	±	50	PC	0.2702
BMI	25.2	±	2.6	26.6	±	3.7	27.5	±	3.6	24.5	±	3.9	A	0.1505
CSF $A\beta_{40}$	5864	±	1688	6103	±	1699	5828	±	1313	4468	±	1172	A	0.0888
CSF $A\beta_{42}$	415.6	±	156.2	488.8	±	217.2	363	±	172.5	210.4	±	131.0[d]	A	0.0045
CSF $A\beta_{42/40}$	0.07	±	0.02	0.08	±	0.02	0.06	±	0.03	0.05	±	0.02[d]	A	0.0083

Notes: Means ± SD are reported. Statistical tests used are as indicated: PC: Contingency, Pearson test; A: one-way analysis of variance followed by a Tukey post hoc test, W: Kruskal–Wallis followed by Wilcoxon post hoc test. *P* values refer to the significant analysis of variance.
Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; *APOE*, apolipoprotein E; Age, age at draw; BMI, body mass index; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; SCD, subjective cognitive disorder.
[a]$P < 0.05$ vs. control.
[b]$P < 0.0001$ vs. SCD and control.
[c]$P < 0.05$ vs. all.
[d]$P < 0.01$ vs. SCD.
[e]Data missing for two subjects.

cells per well. Media was collected 72 hours after seeding and was pooled from multiple wells. Four biological replicates were used. Conditioned media was collected and stored at −80°C. After thawing, conditioned media was centrifuged at 10,000 × g for 15 minutes at 4°C.

2.3 | Human astrocyte cultures

Human astrocytes were purchased from Sciencell (#1800) and cultured according to the manufacturer's recommendations using the manufacturer's complete media (#1801). Cells were plated on poly-L-lysine coated 24-well plates and allowed to grow to confluence at 37°C with media changes every 2 to 3 days. For tumor necrosis factor alpha (TNF-α)–stimulated conditions, cells were treated with increasing doses of human TNF-α (R&D Systems; vehicle, 5, 10, 20 ng/mL) in complete media for 24 hours. The study was performed on 1 to 2 separate days with two technical replicates per treatment group. For insulin-stimulated conditions, media was replaced with either base media or base media containing 100 nM human insulin for 24 hours. Base media was used as the complete media contains insulin. The study was performed on a single day with three technical replicates per treatment group. Media were collected and stored at −80°C.

2.4 | sIR assay

CSF and conditioned media samples were thawed on ice. A commercially available kit (Biovendor, LLC) was used to measure sIR levels per the manufacturer's recommendations. This kit was previously validated with intra-assay coefficient of variations (CVs) < 5% and inter-assay reliability (intra-class correlation coefficients, ICC > 0.75).[26] CSF samples were diluted 1:2 with kit diluent based on previous studies.[26] and conditioned media was diluted 2:1 (150 µL media in 75 µL diluent) for the neurons and 1:2 (75 µL media in 150 µL diluent) for the astrocytes. Samples were run in duplicate on each plate and the average value reported after subtracting for background. The Seattle cohort samples were assayed on separate days so our final analysis adjusts for this while the CIMA-Q samples were assayed all on the same day.

2.5 | Statistical analysis

Prism 8.0 (GraphPad Inc.) and JMP 17.0 (SAS Institute Inc.) were used for statistical analysis. For the Seattle cohort, an unpaired *t* test (two tailed) was used to assess differences between the two groups (Control vs. AD) in the cohort characteristics in Table 1. A Fisher exact test was used to assess *APOE* ε4 carrier status. A Mann–Whitney non-parametric test, two sided, was used to analyze differences in MoCA scores. For the CIMA-Q cohort, a Kruskal–Wallis non-parametric test, followed by a Wilcoxon post hoc test, was used to assess differences among the four groups (Control, SCD, MCI, AD) in Table 2. The association of disease status with sIR levels was compared using linear regression models adjusted for sex and age. In the Seattle cohort, we also adjusted for sIR assay run and in CIMA-Q, for sex. Model assumptions were tested and found to be tenable. Pearson correlations were used to assess associations between two continuous variables (sIR and Aβ). Spearman correlations were used with MoCA scores as one ordinal variable. Results in the tables are presented as mean ± standard deviation while figure results are expressed as mean ± standard error. Data Availability: Individual data points are presented in each figure.

3 | RESULTS

3.1 | Cohort characteristics

Table 1 shows the demographics for the Seattle cohort, including performance on neuropsychological measures, *APOE* ε4 carrier status, and CSF Aβ42 levels. We included 43 control samples and 15 AD samples in our analysis. In the Seattle cohort, all were female, with significant differences in the CDR ($P < 0.0001$), MMSE ($P < 0.001$), MoCA score ($P < 0.0001$), and CSF Aβ42 concentrations ($P < 0.0001$) between the control and AD groups. Age of CSF draw, education, and BMI were not statistically different between the groups. *APOE* ε4 carrier status was also not statistically different (32.6% in the Control group vs. 53.3% in the AD group).

Table 2 shows the demographics for the CIMA-Q cohort. We included 14 Control, 21 SCD, 18 MCI, and 8 AD samples in our analysis. In all groups except the AD group, 45% to 57% were female. The AD group consisted of one female. There were significant differences in the MoCA score ($P < 0.0001$), MMSE ($P = 0.0431$), CSF Aβ42 concentrations ($P = 0.0085$), and the CSF Aβ42/40 ratio within the four groups. Age of CSF draw, education, and BMI were not statistically different. *APOE* ε4 carrier status was enriched in the AD population but not significantly.

3.2 | CSF sIR concentration

In this pilot study, CSF sIR levels were not statistically significant between the groups in either the Seattle or CIMA-Q cohort (Figure 1). People with AD in the Seattle cohort had −0.18 ng/mL lower sIR levels, adjusting for age and assay run, but this difference was not statistically significant ($P = 0.70$; Figure 1A). Differences in CIMA-Q were also not statistically significant, compared to controls, adjusting for age and sex (Figure 1B).

3.3 | CSF sIR correlations with CSF Aβ biomarkers

Levels of CSF sIR positively correlated with CSF Aβ species (Figure 2). In the Seattle cohort, there was a significant association between CSF sIR levels and $A\beta_{42}$ levels (Figure 2A). As $A\beta_{42}$ levels were measured using different assays between the two Seattle centers, we separately analyzed whether there was a significant association within each cen-

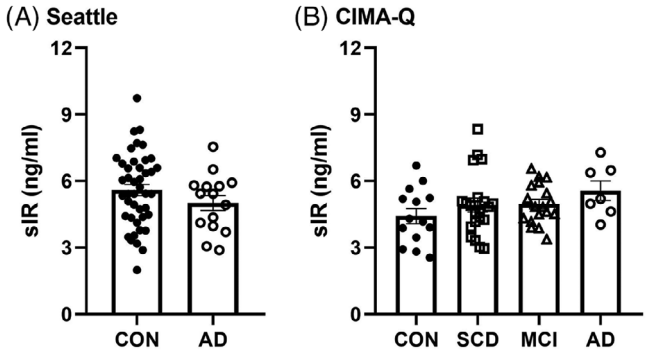

FIGURE 1 Levels of sIR in the CSF. There were no statistical differences in the levels of CSF sIR in the (A) Seattle or (B) CIMA-Q cohort. Linear regression modeling was used to assess statistical differences, adjusting for age in both cohorts, assay in Seattle, and sex in CIMA-Q. Data represent mean ± standard error of the mean. AD, Alzheimer's disease; CIMA-Q, Consortium for the Early Identification of Alzheimer's Disease-Quebec; CON, control; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; SCD, subjective cognitive disorder; sIR, soluble insulin receptor

ter (UW ADRC: open symbols; VA MIRECC: closed symbols). In both centers, there was a significant association between CSF sIR and Aβ_{42} (UW ADRC: F[1,30] = 5.777; r = 0.4018; P = 0.0226 and VA MIRECC: F[1,18] = 5.848; r = 0.4952; P = 0.0264). In the CIMA-Q cohort, there was a significant association (F[1,59] = 4.830; r = 0.2751; P = 0.0319) between CSF sIR and Aβ_{42} (Figure 2B). As the CIMA-Q cohort also had Aβ_{40} levels available, we were able to identify a significant association between CSF Aβ_{40} and sIR (F[1,59] = 8.881; r = 0.3617; P = 0.0042; Figure 2C). There was no association between CSF sIR and the A$\beta_{42/40}$ ratio (F[1,59] = 0.0758; r = 0.0358; P = 0.7841; Figure 2D).

3.4 | CSF sIR correlations with cognitive performance

Because brain insulin resistance correlates with cognition,[27] we assessed whether CSF sIR levels associated with cognitive performance (Figure 3). In the Seattle cohort, MoCA scores were not significantly associated with CSF sIR levels when groups were combined (Spearman r = –0.0204; P = 0.8801; Figure 3A). When diagnostic groups were split, there were still no associations between CSF sIR and MoCA score (Control: Spearman r = –0.2074; P = 0.1821; AD: Spearman r = –0.2746; P = 0.3390; Figure 3B). Alternatively, there was a significant association between CSF sIR and MoCA score in the CIMA-Q cohort (Spearman r = –0.2758; P = 0.0329; Figure 3C). There was one sample, a female, in the AD group that was removed from this analysis (red). When this sample was included in the analysis, the association trends toward significance (Spearman r = –0.2223; P = 0.085). This association was largely driven by the AD group as when the groups were split, with the same sample excluded, the AD group remained the only one with the significant association (Spearman r = –0.8524; P = 0.0238; Figure 3D). The association between MoCA and sIR remained significant after adjusting for age and/or years of education. When the sample was included, the association in the AD group was not significant (Spearman r = –0.3904; P = 0.3440).

3.5 | Neuronal AD production of sIR

Due to the positive association of sIR levels with Aβ_{42} and to further understand the central nervous system (CNS) cell type production of sIR in AD, we wanted to identify whether levels of sIR were increased in hiPSC-derived neurons with the "Swedish" (Swe) mutation in the human APP gene. In neurons expressing a double mutation in the APP gene (APP$^{Swe/Swe}$), Aβ_{42} levels in the media are significantly increased compared to the other two lines.[24] In APP$^{Swe/Swe}$ cells, sIR was increased in the culture media compared to the isogenic control (F[2,9] = 48.49; P < 0.0001; Figure 4).

3.6 | Astrocyte production of sIR

To further identify which brain cell types could produce sIR, we assessed the ability for human astrocytes to generate sIR. Based on previous published results suggesting an inflammatory stimulus could generate sIR in a neuronal cell line,[28] we cultured human astrocytes with increasing amounts of human TNF-α for 24 hours. The level of sIR present in the conditioned media was dose-dependently increased and significantly increased with 20 ng/mL stimulation (F[3,9] = 8.195; P = 0.0061; Figure 5A). We additionally tested whether insulin could self-regulate levels of sIR and found that 100 nM insulin was able to increase sIR significantly in the culture media (P = 0.0269; Figure 5B).

4 | DISCUSSION

In this small pilot study, despite CSF sIR levels in humans remaining similar across diagnostic groups, we are reporting for the first time that CSF sIR levels positively correlated with CSF Aβ_{42} and Aβ_{40} and is elevated in the culture media of neurons derived from humans with the "Swedish" mutation where Aβ_{42} is increased. In one cohort (CIMA-Q), CSF sIR levels negatively correlated with MoCA scores, again supporting a relationship between brain insulin resistance and deficiency and cognition. We found that in addition to neurons, human astrocytes can also produce sIR and generation is stimulated by TNF-α and insulin.

Several circulating forms of receptor molecules and their fragments have been identified, including the growth hormone receptor,[29] leptin receptor,[30] transferrin receptor,[31] and the receptor for advanced glycation end products (RAGE).[32] Studies have found these soluble receptors are normal constituents of body fluids that can extend the half-life of a ligand, interact with G-protein coupled receptors on the cell membrane to induce intracellular signaling, sequester the ligand from acting on the full-length receptor, or allow the intramembrane portion of the receptor to translocate to the nucleus, where it can act as a transcription factor.[33]

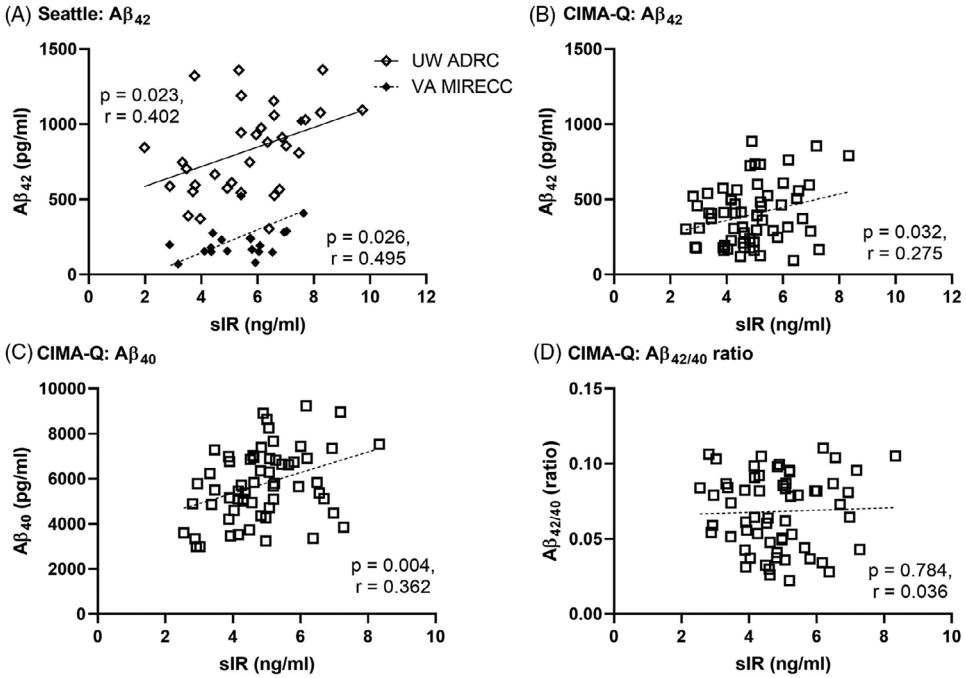

FIGURE 2 Association between CSF Aβ species and sIR. A, There was a significant association between Aβ42 and sIR in the Seattle cohort when samples were split between the two centers due to the differences in Aβ42 measurement methods (UW ADRC [solid line, $P = 0.023$]: Lumipulse; VA MIRECC [dotted line, $p = 0.026$]: Luminex). B, There was a significant association ($P = 0.032$) in the CIMA-Q cohort. C, There was a significant association between Aβ40 and sIR in the CIMA-Q cohort ($P = 0.004$). D, There was no association between the Aβ42/40 ratio and sIR in the CIMA-Q cohort ($P = 0.78$). Pearson correlations were used to assess univariate associations. Aβ, amyloid beta; CIMA-Q, Consortium for the Early Identification of Alzheimer's Disease-Quebec; CSF, cerebrospinal fluid; sIR, soluble insulin receptor; UW ADRC, University of Washington Alzheimer's Disease Research Center; VA MIRECC, Veterans Affairs Northwest Mental Illness Research, Education, and Clinical Center

While the exact function of the sIR cleaved protein is unknown, one possibility is that sIR can act as a reservoir for insulin. This can serve two functions, one potentiating insulin's effects and one reducing it. Positively, it can extend the lifespan of short-lived substrates, such as insulin. Indeed, ≈ 80% of the total circulating insulin is bound to the liver membrane-bound IR, contributing to the measurable half-life for insulin.[34] In states in which CSF insulin is decreased, as occurs in AD, an increase in CSF sIR could be a compensatory mechanism, extending the signaling capabilities of insulin present in the CSF. Given the indication that insulin blood–brain barrier transport, the primary source of brain insulin, is reduced in AD, extending the lifespan of the insulin once in the brain would prolong its ability to signal. Alternatively, and more likely, sIR binding insulin would not only reduce the amount of free, or unbound, insulin, which is the fraction of insulin that best interacts with the membrane-bound IR, but also generation of sIR would eliminate membrane-bound IR reducing intracellular signaling. This is more likely the dominant function of the sIR in this case, as in AD, brain insulin signaling is reduced.[1,27]

In our study, we observed cohort differences between some of the associations with sIR. Specifically, we observed a significant association between MoCA score and CSF sIR levels in the CIMA-Q cohort. We did not find this in the Seattle cohort. These cohorts display considerable differences, particularly in the proportion of samples present in each diagnostic group represented. The CIMA-Q cohort was more evenly distributed between the groups while the Seattle cohort was heavily weighted in the controls. The Seattle cohort has more people at the MoCA ceiling to likely find an association and we would need a broader distribution of scores, as was the case in the CIMA-Q cohort. These observations in the CIMA-Q cohort could be due to the spread in the disease progression, rather than heavily weighting the control group, which was the case for the Seattle cohort. Last, fasting was not required in the CIMA-Q cohort. As fasting is known to affect IR signaling and increase insulin binding in peripheral tissues, it is possible fasting may affect generation of the sIR, which requires further investigation.

As the AD diagnosis for each individual was defined clinically, and not neuropathologically, there could be an undetermined number of participants with cognitive impairment due to other etiologies. However, the presence of *APOE* ε4 carriers in both cohorts (53% carriage in the Seattle cohort and 50% carriage in the CIMA-Q cohort) is in line with those typically neuropathologically classified as AD,[35] indicating a high likelihood of the clinical diagnosis matching with neuropathology. Indeed, CSF sIR levels have been shown to correlate in non-AD cases of cognitive impairment.[14] and our findings in the CIMA-Q cohort support this relationship with cognition. While this etiologic heterogeneity may have impacted our results, we observed significant correlations between CSF sIR and Aβ peptides, indicating an association with this critical, pathological AD hallmark.

Although the IR is expressed in various organs in the periphery such as the muscle and liver, it is expected that a significant fraction of sIR detected in the CSF comes from the brain. Previous work has

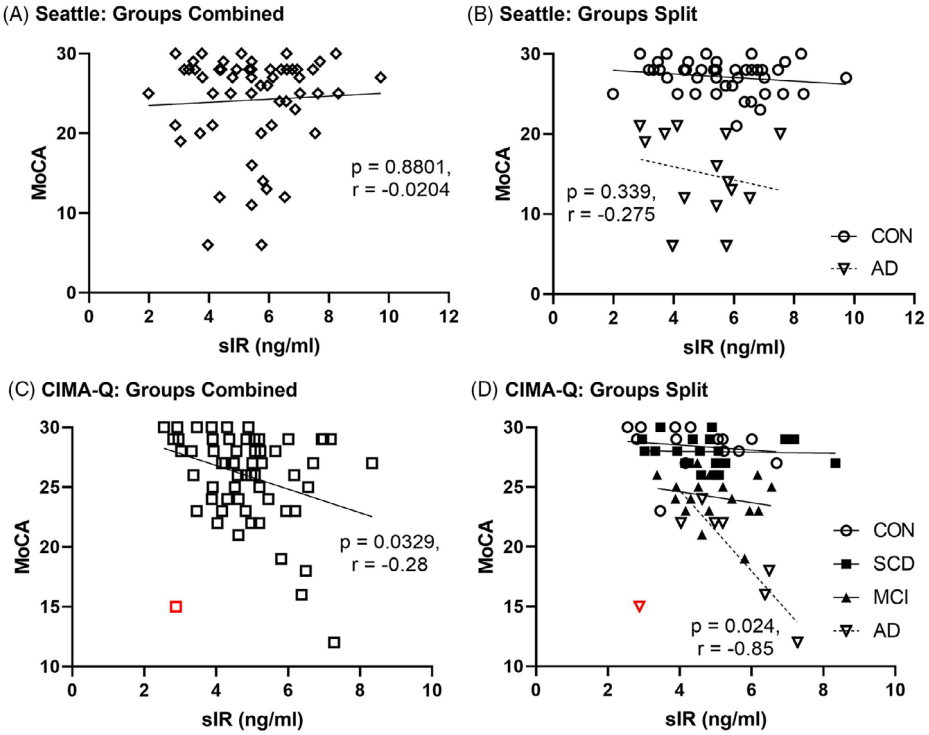

FIGURE 3 Association between CSF sIR and cognitive performance. A, In the Seattle cohort, there was not a significant association between CSF sIR levels and MoCA score ($P = 0.8801$) or (B) in the AD group when the diagnostic groups were split ($P = 0.339$). C, There was a significant association in the CIMA-Q cohort when groups were combined ($P = 0.0329$) and (D) in the AD group when the diagnostic groups were split ($P = 0.024$). The sample in red was removed from the reported results. Spearman correlations were used to assess univariate associations. AD, Alzheimer's disease; CIMA-Q, Consortium for the Early Identification of Alzheimer's Disease-Quebec; CON, control; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; SCD, subjective cognitive disorder; sIR, soluble insulin receptor

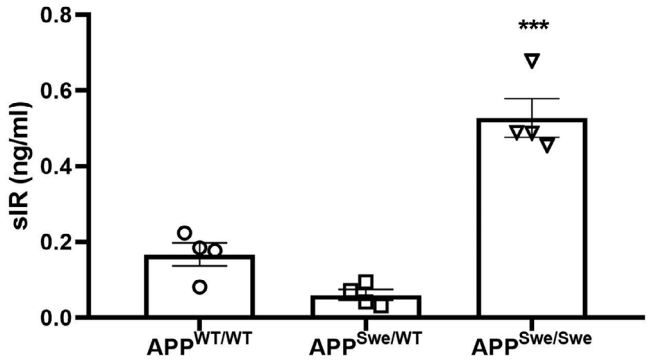

FIGURE 4 Levels of sIR in conditioned media from hiPSC-neurons containing the "Swedish" mutation. There was a significant increase in sIR levels in the media from APPSwe/Swe neurons compared to hiPSC-neurons derived from wild-type (APPWT/WT) and heterozygous (APPSwe/WT) for the APP Swedish mutation as identified by a one-way analysis of variance followed by a Tukey post hoc test (***$P < 0.0001$). Data represent mean ± standard error of the mean. hiPSC, human induced pluripotent stem cells; sIR, soluble insulin receptor

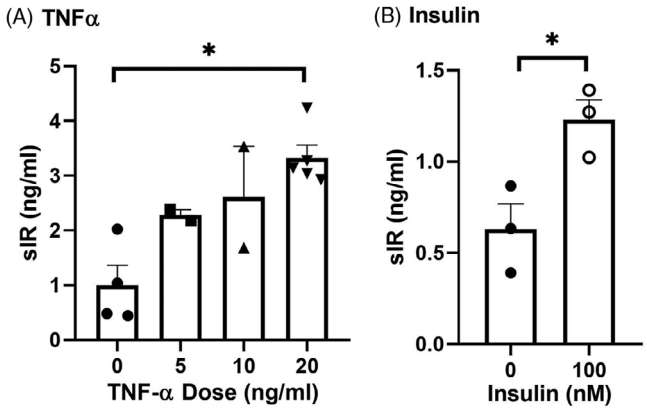

FIGURE 5 Levels of sIR in conditioned media from human astrocytes. There was a significant increase in sIR levels in the media from (A) TNF-α–treated astrocytes (one-way analysis of variance, Tukey post hoc; *$P < 0.05$) and (B) insulin-stimulated astrocytes (Student *t* test; *$P < 0.05$). Data represent mean ± standard error of the mean. sIR, soluble insulin receptor; TNF-α, tumor necrosis factor alpha

shown lower levels of IR in the brain of AD patients, particularly IRα-B in endothelial cells.[1,36–39] Indications of reduced IR signaling have been reported.[1,27] A significant correlation between lower IRα-B and lower cognitive scores.[36] is particularly interesting and supports the presently observed association of sIR with MoCA scores. Lower levels of IRα-B have also been shown in the 3xTg-AD model, which express the APP^{Swe}/PS1 mutation.[36] BACE1 activity in the liver has been implicated in a rise in plasma sIR.[40] Brain cells also express BACE1 and

enzymes that cleave IR. For instance, higher BACE1 activity has been repeatedly reported in the brain of AD patients, without mutation (including Leclerc et al.[36] but many others). Hence, the logical scenario is that a cleavage of IR in the brain, possibly by BACE1, would then lead to an enhanced release of sIR into the CSF, coinciding with increased $A\beta_{42}$ and $A\beta_{40}$ levels as we observed in our current study. These findings help explain why CSF sIR positively correlates with $A\beta_{42}$ but does not explain why sIR levels negatively correlate with cognition, as lower CSF $A\beta_{42}$ levels are often indicative of cognitive impairment. Therefore, we sought to further define the relationship between $A\beta_{42}$ and sIR generation using in vitro models.

In neurons derived from isogenic hiPSCs with different gene doses of the APP "Swedish mutation," we found significant differences in the levels of sIR in the conditioned media. In the $APP^{Swe/Swe}$ neuronal cultures, there was significantly more sIR. This mutation increases $A\beta$ secretion by enhancing amyloidogenic APP cleavage by BACE1.[24,41] Inhibition of BACE1 results in decreased $A\beta_{42}$ levels.[24] $A\beta_{42}$ levels secreted by $APP^{Swe/Swe}$ neurons are approximately 5-fold greater than levels secreted by $APP^{Swe/WT}$ neurons.[24] While our studies do not reveal the mechanism for increased shedding of sIR in the $APP^{Swe/Swe}$, the increases in $A\beta_{42}$ and sIR in the conditioned media support a similar relationship to that observed in our CSF samples. There is no increase in cytokine secretion from $APP^{Swe/Swe}$ neurons.[24] In our unpublished studies, RNA sequencing of these cells suggests that matrix metalloproteinase (MMP) 14 is increased in the $Swe^{WT/WT}$ cells. As MMP14 has recently been shown to generate sIR,[11] MMP14 could be the mechanism for increased sIR present in the conditioned media from these cells. It is also possible $A\beta_{42}$ could act on the cells to indirectly increase the amount of sIR cleavage. Future studies can assess proteomics in the conditioned media and cell lysates to identify proteins that may be altered to induce cleavage of the IR. Additionally, other iPSC derived neurons that contain mutations in the presenilin 1 (*PS1*) gene could be used to assess the impact on sIR generation, because these enzymes are also the enzymes shown to generate sIR.[7–10]

Neurons, astrocytes, and even brain endothelial cells express many of the enzymes found to cleave IR to generate sIR. Previous studies have found neurons can specifically generate sIR, with changes linked to inflammatory cytokines present in CSF.[28] Specifically, it was identified that TNF-α regulates secretion of sIR from the SH-SY5Y human neuronal cell line.[28] Astrocytes are important regulators of IR signaling and are highly implicated in AD pathology.[42] Genetic deletion of the astrocytic IR impairs $A\beta_{42}$ uptake, indicating a causal relationship.[42] CSF TNF-α is an inflammatory marker most consistently implicated in AD and is associated with conversion in individuals who are cognitively normal at baseline.[43] TNF-α is known to increase $A\beta$ burden through upregulation of BACE1.[44] Therefore, we investigated whether another brain cell type, astrocytes, could generate sIR and whether TNF-α could induce secretion of sIR, similar to what had been observed in neurons. We found 20 ng/mL TNF-α was able to increase sIR levels > 3-fold. While we did not test brain endothelial cell generation of sIR, these cells express the greatest amount of the IR of brain cell types.[45] and thus, could also be a source of sIR. These data support sIR can arise from brain cell types other than neurons and the CSF AD milieu may contribute to generation of sIR.

While sIR levels are increased in the plasma of individuals with type 2 diabetes, a condition in which insulin levels are often elevated,[15] a direct effect of insulin on sIR generation has not been tested. In our study, insulin incubation with human astrocytes was shown to increase sIR in the media 24 hours later. There is direct evidence showing PS1 is functionally modulated by insulin signaling.[46] Insulin signaling decreases phosphorylation of *PS1*, enhancing the interaction with N-cadherin and β-catenin, re-localizing *PS1* to the cell surface. Future studies could be designed to investigate *PS1* activity after insulin stimulation in human astrocyte cultures using a novel biosensor.[47] Insulin has been shown to induce cleavage of other surface proteins, including Klotho, an antiaging transmembrane protein, likely through stimulating proteolytic activity of ADAM10 and ADAM17.[48] Finally, CSF insulin levels are decreased in AD.[49] and we have shown loss of brain IR signaling slows insulin transport into the brain in mice.[50] Therefore, it is possible the dysregulated processing of the sIR by insulin could perpetuate a vicious cycle, leading ultimately to brain insulin resistance and deficiency.

Other groups have shown sIR levels are increased in the plasma of individuals with diabetes.[15] or throughout aging.[11] While we focused our current investigations on the CSF levels of sIR and CNS cell type contribution of sIR, it would be worth identifying whether there are relationships between the plasma and CSF sIR level in subjects with AD or more importantly pre-clinical AD. Correlations between plasma and CSF levels of sIR may provide insight into brain insulin resistance and deficiency, a feature that has, so far, proven to be difficult to assess in living subjects[1].

Limitations to our pilot study not only include the small sample size, but also the exclusion of males and those with clinically diagnosed type 2 diabetes in the Seattle cohort. The CIMA-Q cohort did not have these same exclusion criteria, but also had limited numbers of samples due to the increased separation by diagnostic group. Future studies are warranted to not only investigate sex differences in the levels of CSF sIR and relationship with AD pathology but also the effect of type 2 diabetes. As type 2 diabetes is a clear risk factor for AD, and circulating sIR levels are increased in individuals with type 2 diabetes[15] it is possible levels of sIR, both in the circulation and the CSF, may help explain the relationship between type 2 diabetes and AD. Additionally, due to the lack of standardization in sample repositories around the world, our different findings between the US-based cohort and Canadian cohort highlight the need to repeat the study in other cohorts to fully understand the link between MoCA scores and CSF sIR levels. Last, as we have focused on the levels of sIR in the CSF, follow-up studies should be conducted to identify any associations with systemically circulating sIR.

The data presented here for the first time investigate the levels of sIR present in the CSF in cognitively intact individuals compared to those that have been clinically diagnosed with AD, or the spectrum of prodromal to probable AD. Although there are not overall statistical differences in CSF sIR levels between the groups, there was a significant association with global cognitive score via the MoCA and

CSF $A\beta_{42}$ or $A\beta_{40}$ levels. Neurons and astrocytes can contribute to the generation of sIR and astrocyte sIR production can be simulated by inflammation or insulin. Further understanding about the regulation of sIR is also needed and whether there are cell-specific processes. Finally, the biological mechanism for sIR in AD and whether increased levels directly contribute to brain insulin resistance and deficiency remain to be elucidated.

ACKNOWLEDGMENTS

We would like to thank Elizabeth Colasurdo and Aimee Schantz, who assisted in identifying the VA MIRECC BNG samples and UW ADRC samples, respectively. This work was funded by a NIH-sponsored UW ADRC Development Project (P30 AG066509 to E.M. Rhea), NIH T32AG066574 (to K.M. Evitts), and the VA MIRECC (G. Li and E.R. Peskind). The CIMA-Q is supported by the Fonds de recherche du Québec – Santé (FRQS) grant, Fonds de recherche du Québec (FRQ) cohort funds (279261), Quebec Network for Research on Aging (RQRV), and the Fondation Famille Lemaire.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the supporting information.

REFERENCES

1. Rhea EM, Leclerc M, Yassine HN, et al. State of the science on brain insulin resistance and cognitive decline due to Alzheimer's Disease. *Aging Dis*. 2023. doi:10.14336/ad.2023.0814
2. Rhea EM, Banks WA, Raber J. Insulin resistance in peripheral tissues and the brain: a tale of two sites. *Biomedicines*. 2022;10(7). doi:10.3390/biomedicines10071582
3. Kellar D, Register T, Lockhart SN, et al. Intranasal insulin modulates cerebrospinal fluid markers of neuroinflammation in mild cognitive impairment and Alzheimer's disease: a randomized trial. *Sci Rep*. 2022;12(1):1346. doi:10.1038/s41598-022-05165-3
4. Craft S, Raman R, Chow TW, et al. Safety, efficacy, and feasibility of intranasal insulin for the treatment of mild cognitive impairment and Alzheimer disease dementia: a randomized clinical trial. *JAMA Neurol*. 2020. doi:10.1001/jamaneurol.2020.1840
5. Reger MA, Watson GS, Green PS, et al. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. *Neurology*. 2008;70(6):440-448. doi:10.1212/01.wnl.0000265401.62434.36
6. Nowell J, Blunt E, Edison P. Incretin and insulin signaling as novel therapeutic targets for Alzheimer's and Parkinson's disease. *Mol Psychiatry*. 2023;28(1):217-229. doi:10.1038/s41380-022-01792-4
7. Yuasa T, Amo-Shiinoki K, Ishikura S, et al. Sequential cleavage of insulin receptor by calpain 2 and gamma-secretase impairs insulin signalling. *Diabetologia*. 2016;59(12):2711-2721. doi:10.1007/s00125-016-4102-5
8. Meakin PJ, Mezzapesa A, Benabou E, et al. The beta secretase BACE1 regulates the expression of insulin receptor in the liver. *Nat Commun*. 2018;9(1):1306. doi:10.1038/s41467-018-03755-2
9. Ahmad F, Das D, Kommaddi RP, et al. Isoform-specific hyperactivation of calpain-2 occurs presymptomatically at the synapse in Alzheimer's disease mice and correlates with memory deficits in human subjects. *Sci Rep*. 2018;8(1):13119. doi:10.1038/s41598-018-31073-6
10. Evin G, Hince C. BACE1 as a therapeutic target in Alzheimer's disease: rationale and current status. *Drugs Aging*. 2013;30(10):755-764. doi:10.1007/s40266-013-0099-3
11. Guo X, Asthana P, Gurung S, et al. Regulation of age-associated insulin resistance by MT1-MMP-mediated cleavage of insulin receptor. *Nat Commun*. 2022;13(1):3749. doi:10.1038/s41467-022-31563-2
12. Gavin JR 3rd, Buell DN, Roth J. Water-soluble insulin receptors from human lymphocytes. *Science (New York, NY)*. 1972;178(4057):168-169. doi:10.1126/science.178.4057.168
13. Pezzino V, Papa V, Costantino A, et al. Identification and initial characterization of insulin receptor-like immunoreactivity in human plasma. *J Clin Endocrinol Metab*. 1992;74(5):1116-1121. doi:10.1210/jcem.74.5.1314845
14. Gerena Y, Skolasky RL, Velez JM, et al. Soluble and cell-associated insulin receptor dysfunction correlates with severity of HAND in HIV-infected women. *PLoS One*. 2012;7(5):e37358. doi:10.1371/journal.pone.0037358
15. Soluble Insulin Receptor Study G. Soluble insulin receptor ectodomain is elevated in the plasma of patients with diabetes. *Diabetes*. 2007;56(8):2028-2035. doi:10.2337/db07-0394 PMID:17563065
16. Belleville S, LeBlanc AC, Kergoat M-J, et al. The Consortium for the early identification of Alzheimer's disease – Quebec (CIMA-Q). *Alzheimers Dement*. 2019;11(1):787-796. doi:10.1016/j.dadm.2019.07.003
17. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J lipid res*. 1990;31(3):545-548.
18. Peskind ER, Li G, Shofer J, et al. Age and apolipoprotein E*4 allele effects on cerebrospinal fluid beta-amyloid 42 in adults with normal cognition. *Arch Neurol*. 2006;63(7):936-939. doi:10.1001/archneur.63.7.936
19. Morris JC, Weintraub S, Chui HC, et al. The Uniform Data Set (UDS): clinical and cognitive variables and descriptive data from Alzheimer Disease Centers. *Alzheimer Dis Assoc Disord*. 2006;20(4):210-216. doi:10.1097/01.wad.0000213865.09806.92
20. Besser L, Kukull W, Knopman DS, et al. Version 3 of the National Alzheimer's Coordinating Center's Uniform Data Set. *Alzheimer Dis Assoc Disord*. 2018;32(4):351-358. doi:10.1097/WAD.0000000000000279
21. Fasnacht JS, Wueest AS, Berres M, et al. Conversion between the Montreal Cognitive Assessment and the Mini-Mental Status Examination. *J Am Geriatr Soc*. 2023;71(3):869-879. doi:10.1111/jgs.18124
22. Mattsson N, Andreasson U, Persson S, et al. The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers Dement*. 2011;7(4):386-395.e6. doi:10.1016/j.jalz.2011.05.2243
23. Young JE, Fong LK, Frankowski H, Petsko GA, Small SA, Goldstein LSB. Stabilizing the retromer complex in a human stem cell model of Alzheimer's disease reduces TAU phosphorylation independently of amyloid precursor protein. *Stem Cell Reports*. 2018;10(3):1046-1058. doi:10.1016/j.stemcr.2018.01.031
24. Shin YJ, Evitts KM, Jin S, et al. Amyloid beta peptides (Abeta) from Alzheimer's disease neuronal secretome induce endothelial activation in a human cerebral microvessel model. *Neurobiol Dis*. 2023;181:106125. doi:10.1016/j.nbd.2023.106125
25. Mishra S, Knupp A, Szabo MP, et al. The Alzheimer's gene SORL1 is a regulator of endosomal traffic and recycling in human neurons. *Cell Mol Life Sci*. 2022;79(3):162. doi:10.1007/s00018-022-04182-9
26. Trombetta BA, Carlyle BC, Koenig AM, et al. The technical reliability and biotemporal stability of cerebrospinal fluid biomarkers for profiling multiple pathophysiologies in Alzheimer's disease. *PLoS One*. 2018;13(3):e0193707. doi:10.1371/journal.pone.0193707
27. Talbot K, Wang HY, Kazi H, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest*. 2012;122(4):1316-1338. doi:10.1172/jci59903
28. Gerena Y, Menendez-Delmestre R, Delgado-Nieves A, et al. Release of soluble insulin receptor from neurons by cerebrospinal fluid from

patients with neurocognitive dysfunction and HIV infection. *Front Neurol*. 2019;10:285. doi:10.3389/fneur.2019.00285
29. Herington AC, Elson D, Ymer S. Water soluble receptors for human growth hormone from rabbit liver. *J Recept Res*. 1981;2(2):203-220. doi:10.3109/10799898109039261
30. Rock FL, Peterson D, Weig BC, Kastelein RA, Bazan JF. Binding of leptin to the soluble ectodomain of recombinant leptin receptor: a kinetic analysis by surface plasmon resonance. *Horm Metab Res*. 1996;28(12):748-750. doi:10.1055/s-2007-979892
31. Cook JD, Baynes RD, Skikne BS. The physiological significance of circulating transferrin receptors. *Adv Exp Med Biol*. 1994;352:119-126. doi:10.1007/978-1-4899-2575-6_9
32. Yamagishi S, Imaizumi T. Serum levels of soluble form of receptor for advanced glycation end products (sRAGE) may reflect tissue RAGE expression in diabetes. *Arterioscler Thromb Vasc Biol*. 2007;27(6):e33-34.e32. doi:10.1161/ATVBAHA.107.139923
33. Lin SY, Makino K, Xia W, et al. Nuclear localization of EGF receptor and its potential new role as a transcription factor. *Nat Cell Biol*. 2001;3(9):802-808. doi:10.1038/ncb0901-802
34. Hovorka R, Powrie JK, Smith GD, Sonksen PH, Carson ER, Jones RH. Five-compartment model of insulin kinetics and its use to investigate action of chloroquine in NIDDM. *Am J Physiol*. 1993;265(1 Pt 1):E162-175. doi:10.1152/ajpendo.1993.265.1.E162
35. Alzheimer's Association. Is Alzheimer's Genetic? Accessed March 19, 2024. https://www.alz.org/alzheimers-dementia/what-is-alzheimers/causes-and-risk-factors/genetics#:~:text=APOE%2De4%20is%20the%20first,%2De2%20and%20APOE%2De3
36. Leclerc M, Bourassa P, Tremblay C, et al. Cerebrovascular insulin receptors are defective in Alzheimer's disease. *Brain*. 2022. doi:10.1093/brain/awac309
37. Frolich L, Blum-Degen D, Bernstein HG, et al. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J Neural Transm (Vienna)*. 1998;105(4-5):423-438. doi:10.1007/s007020050068
38. Steen E, Terry BM, Rivera EJ, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease – is this type 3 diabetes? *J Alzheimers Dis*. 2005;7(1):63-80.
39. Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *J Alzheimers Dis*. 2005;8(3):247-268.
40. Bao H, Liu Y, Zhang M, et al. Increased beta-site APP cleaving enzyme 1-mediated insulin receptor cleavage in type 2 diabetes mellitus with cognitive impairment. *Alzheimers Dement*. 2021;17(7):1097-1108. doi:10.1002/alz.12276
41. Haass C, Lemere CA, Capell A, et al. The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway. *Nat Med*. 1995;1(12):1291-1296. doi:10.1038/nm1295-1291
42. Chen W, Huang Q, Lazdon EK, et al. Loss of insulin signaling in astrocytes exacerbates Alzheimer-like phenotypes in a 5xFAD mouse model. *Proc Natl Acad Sci U S A*. 2023;120(21):e2220684120. doi:10.1073/pnas.2220684120
43. Contreras JA, Aslanyan V, Albrecht DS, Mack WJ. Alzheimer's Disease Neuroimaging I, Pa J. Higher baseline levels of CSF inflammation increase risk of incident mild cognitive impairment and Alzheimer's disease dementia. *Alzheimers Dement (Amst)*. 2022;14(1):e12346. doi:10.1002/dad2.12346
44. Liao YF, Wang BJ, Cheng HT, Kuo LH, Wolfe MS. Tumor necrosis factor-alpha, interleukin-1beta, and interferon-gamma stimulate gamma-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent MAPK pathway. *J Biol Chem*. 2004;279(47):49523-49532. doi:10.1074/jbc.M402034200
45. Zhang Y, Chen K, Sloan SA, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014;34(36):11929-11947. doi:10.1523/JNEUROSCI.1860-14.2014
46. Maesako M, Uemura K, Kubota M, et al. Insulin regulates Presenilin 1 localization via PI3K/Akt signaling. *Neurosci Lett*. 2010;483(3):157-161. doi:10.1016/j.neulet.2010.07.055
47. Houser MC, Hou SS, Perrin F, et al. A novel NIR-FRET biosensor for reporting PS/gamma-secretase activity in live cells. *Sensors (Basel)*. 2020;20(21). doi:10.3390/s20215980
48. Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. *Proc Natl Acad Sci U S A*. 2007;104(50):19796-19801. doi:10.1073/pnas.0709805104
49. Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology*. 1998;50(1):164-168.
50. Nguyen V, Thomas P, Pemberton S, et al. Central nervous system insulin signaling can influence the rate of insulin influx into brain. *Fluids Barriers CNS*. 2023;20(1):28. doi:10.1186/s12987-023-00431-6

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: Thomas P, Leclerc M, Evitts K, et al. Cerebrospinal fluid soluble insulin receptor levels in Alzheimer's disease. *Alzheimer's Dement*. 2024;16:e12603. https://doi.org/10.1002/dad2.12603