Autotaxin is Related to Metabolic Dysfunction and Predicts Alzheimer's Disease Outcomes

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Abstract

Background—Obesity and insulin resistance are associated with neuropathology and cognitive decline in Alzheimer's disease (AD).

Objective—Ecto-nucleotide pyrophosphatase/phosphodiesterase 2, also called autotaxin, is produced by beige adipose tissue, regulates metabolism, and is higher in AD prefrontal cortex (PFC). Autotaxin may be a novel biomarker of dysmetabolism and AD.

Methods—We studied Alzheimer's Disease Neuroimaging Initiative participants who were cognitively normal (CN; n=86) or had mild cognitive impairment (MCI; n=135) or AD (n=66). Statistical analyses were conducted using SPSS software. Multinomial regression analyses tested if higher autotaxin was associated with higher relative risk for MCI or AD diagnosis, compared to the CN group. Linear mixed model analyses were used to regress autotaxin against MRI, FDG-PET, and cognitive outcomes. Spearman correlations were used to associate autotaxin and CSF biomarkers due to non-normality. FreeSurfer 4.3 derived mean cortical thickness in medial temporal lobe and prefrontal regions of interest.

Results—Autotaxin levels were significantly higher in MCI and AD. Each point increase in log-based autotaxin corresponded to a 3.5 to 5 times higher likelihood of having MCI and AD, respectively. Higher autotaxin in AD predicted hypometabolism in the medial temporal lobe [$R^2=0.343$, $p<0.001$] and PFC [$R^2=0.294$, $p<0.001$], and worse performance on executive function and memory factors. Autotaxin was associated with less cortical thickness in PFC areas like

orbitofrontal cortex [R^2=0.272, $p<0.001$], as well as levels of total tau, p-tau[181], and total tau/$A\beta_{1-42}$.

Conclusions—These results are comparable to previous reports using insulin resistance. CSF autotaxin may be a useful dysmetabolism biomarker for examining AD outcomes and risk.

Keywords

diabetes mellitus; fluorodeoxyglucose F18; insulin resistance; mild cognitive impairment; MRI; positron emission tomography

INTRODUCTION

Recent studies link obesity and metabolic dysfunction to decreased gray matter volume, less glucose metabolism, and other factors that contribute to Alzheimer's disease (AD)[1–4]. Obesity may give rise to cerebral ischemia, which in turn is associated with brain tissue degeneration [5]. Additionally, obese individuals are more likely to have higher insulin resistance (IR), defined as the progressive inability for insulin to bind to its receptor [6]. IR is correlated with temporal and frontal amyloid deposition and brain atrophy in late middle-aged participants at risk for AD [2, 7], as well as less glucose metabolism in cognitively normal (CN) elders [8] and AD participants [1]. Resulting oxidative stress may induce cell damage in the brain [9].

Numerous studies have also linked obesity with neuroinflammation and subsequent neurodegeneration, as induced by blood-brain barrier damage or microglial activation in the hippocampi of mice [9, 10]. Rats fed a high fat diet showed increased microglial activation as well as higher levels of the chaperone Hsp72, which are neuroprotective responses to brain injury [11]. Such high fat feeding also upregulates the expression of proinflammatory cytokines in the hypothalamus of rodents [12]. High levels of C-reactive protein, an inflammation marker, have been associated with increased white matter damage in individuals free of dementia [13]. Amylin oligomers and plaques were present in diabetic patients' temporal lobe gray matter and absent in controls [14]. In a subsequent study, rats that overexpressed human amylin in the pancreas had elevated levels of brain pro-inflammatory cytokines, as well as suppressed anti-inflammatory expression [15].

While it is increasingly clear that metabolic dysregulation may play a role in AD, there are currently no established biomarkers in cerebrospinal fluid (CSF) that may reflect central metabolic dysfunction. CSF insulin may not be a reliable biomarker because individuals with type 2 diabetes show reduced insulin uptake in the brain, potentially due to reduced transport across the blood-brain barrier when an individual maintains constant high blood insulin levels [8]. Ecto-nucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2), also known as autotaxin, is an enzyme derived from beige adipose that aids in glucose metabolism regulation and adipose tissue expansion [16] and has been shown to be elevated in the brains of AD subjects versus controls [17]. Autotaxin expression is an outcome of the accumulation of triglycerides in the adipocyte [18]. Autotaxin is mostly expressed in the human brain, placenta, ovary, and small intestine, and stimulates the formation of lysophosphatidic acid (LPA) [19].

In the central nervous system, leptomeningeal cells in the pia mater, as well as astrocytes and oligodendrocytes, are hypothesized to secrete autotaxin [20]. LPA is a lipid-based growth factor and signaling molecule found in all mammalian cells, which promotes the generation of tissue fibrosis *in vivo* and *in vitro*, as well as neurotransmitter release and cell contraction and aggregation via utilization of G protein-coupled receptors [21, 22]. Rat neuronal cell culture work indicates that LPA administration leads to cortical folding and premature differentiation of cells [22]. Normal weight mice who were injected with LPA showed deficits in insulin response to glucose, and pancreatic beta-cells showed impaired insulin release when exposed to LPA [23]. Intriguingly, rats administered LPA showed increased blood-brain barrier permeability, suggesting that the effects of elevated autotaxin levels may be due to central and systemic factors [24]. EDG-2, an LPA receptor gene, is overexpressed in growing preadipocytes and may be an important regulator of adipose tissue development [25]. Obese women display positive correlations between serum autotaxin and fasting glucose [26]. Curiously, body mass index (BMI) and autotaxin showed no significant correlation in this cohort, indicating that autotaxin may be more of a product of IR, as opposed to the extent of obesity in an individual.

Importantly, some evidence suggests that autotaxin may play a role in AD, whereas the role of other metabolic biomarkers may be less clear. Individuals with AD-like dementia showed significantly higher gene expression of autotaxin in the frontal cortex, as compared to control brains [17]. Additional research is warranted to elucidate the association of high autotaxin levels with established AD biomarkers, cognitive function, and prefrontal cortex (PFC) and medial temporal lobe (MTL) brain volume and glucose metabolism in AD. PFC was a target area because our previous associations with IR are most consistent in that region [7, 8, 27–29], and that obesity across the lifespan is consistently associated with prefrontal atrophy [3]. Additionally, autotaxin in AD has only been examined in frontal cortices [17]. MTL is relevant to AD onset and progression, and we have shown that it is also sensitive to metabolic dysfunction.

In this study, data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were used to examine CSF autotaxin peptide levels among 287 individuals (75.06 ± 6.89 years of age) who were diagnosed as being CN or having late mild cognitive impairment (MCI) due to AD or early AD. Autotaxin was regressed against cortical thickness (CT) and fluorodeoxyglucose (FDG) values in PFC and MTL, to examine if autotaxin was related to frontal and temporal atrophy and hypometabolism akin to our findings with IR [7, 8, 27, 28]. Relationships with neuropsychological function and traditional CSF biomarkers were also ascertained. The purpose of this study was to determine if CSF-derived autotaxin may be a biomarker for metabolic dysfunction in the brain and could be associated with AD neurological outcomes.

MATERIALS AND METHODS

Participants

Data from 287 adults aged 56 to 89 were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W.

Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see http://www.adni-info.org. Written informed consent was obtained from all ADNI participants at their respective ADNI sites. The ADNI protocol was approved by site-specific institutional review boards. Baseline mass spectrometry data for autotaxin was available for 86 CN, 135 MCI, and 66 AD participants. By month 24, MCI participants were classified as either remaining stable (MCI-S, n=80) or progressing to AD (MCI-P, n=55). CN subjects had MMSE scores between 24 and 30, a CDR score of 0, and no dementia. Participants were classified as MCI if they had an MMSE score between 24 and 30, a CDR score of 0.5, complaints of memory loss, and objective memory loss measured on the Wechsler Memory Scale Logical Memory II. Probable AD was defined as MMSE scores between 20 and 26, a CDR score of 0.5 or 1.0, and meeting NINCDS/ADRDA criteria for probable AD. For additional information, see ADNI Procedures Manual (http://adni.loni.usc.edu). Details of the consensus procedure by the ADNI Conversion Committee are described elsewhere[1]. Other baseline data included demographics, structural MRI for PFC and MTL CT, neural glucose metabolism as determined by FDG uptake, APOE4 genotype, CSF and serum biomarkers, BMI, and neuropsychological performance. While amyloid imaging was also of interest, baseline scans were not analyzed due to small sample size (n=20).

Mass spectrometry and fasting glucose

Data were downloaded from the Biomarkers Consortium CSF Proteomics MRM dataset. As described previously[29], the ADNI Biomarkers Consortium Project investigated the extent to which selected peptides, measured with mass spectrometry (MS), could discriminate among disease states. Briefly, Multiple Reaction Monitoring-MS (MRM-MS) was used for targeted quantitation of 567 peptides representing 221 proteins in a single run (Caprion Proteome Inc., Montreal, QC, Canada). Analyte values of ENPP2 are in arbitrary signal units on a natural log scale. Fasting insulin was assayed using a plasma multiplex immunoassay panel (http://adni.loni.usc.edu/). Fasting glucose was derived from a standard laboratory test. Insulin and glucose were used to calculate the homeostatic model assessment, HOMA-IR [30]. Analyses for this report focused on the autotaxin peptide WWGGQPLWITATK. This peptide performed better in predicting glucose metabolism and cortical thickness than ENPP2 SYPEILTLK in preliminary stepwise regression analyses. To confirm that autotaxin reflected central dysmetabolism, established metabolic and inflammatory biomarkers in CSF were regressed against autotaxin, including insulin-like growth factor binding protein 2 (IGFBP2) peptides, pyruvate kinase isozymes M1/M2, neurosecretory protein VGF, fructose-bisphosphate aldolase A, cholecystokinin and IL-6 receptor [31, 32]. These were the only metabolic or metabolic-related indices available in the CSF for ADNI.

APOE genotype

The ADNI Biomarker Core at the University of Pennsylvania conducted APOE ε4 genotyping. We characterized participants as being "non-APOE4" (i.e., zero APOE ε4 alleles) or "APOE4" (i.e., one to two APOE ε4 alleles).

Amyloid and tau CSF biomarkers

CSF sample collection, processing, and quality control of p-tau$_{181}$, total tau, and Aβ$_{1-42}$ are described in the ADNI1 protocol manual (http://adni.loni.usc.edu/) and Shaw et al. [33].

Clinical and cognitive assessments

Baseline global cognition and assessment scores included the Mini-Mental State Examination (MMSE), clinical dementia rating-sum of boxes (CDR-sob), and AD assessment scale-cognitive subscale 11 (ADAS-cog11). The ADNI1 procedures manual describes diagnostic criteria (http://adni.loni.usc.edu/). The various tests that comprise executive function and memory factor are described elsewhere[34].

MRI

T1-weighted images, collected on 1.5T MR imaging units with a resolution of $1.25 \times 1.25 \times 1.25$ mm [35], were pre-processed using FreeSurfer 4.3 [36]. As described previously [2], this software corrects for motion, deskulls, bias corrects, segments, and parcellates gray and white matter into labeled cortical areas. We chose to examine CT instead of volume, as CT is typically considered a more sensitive index of gray matter pathology in participants who are at-risk [37] or have AD [38]. To contain type 1 error, we first explored one broad region of interest (ROI) in bilateral prefrontal cortex and one ROI in bilateral medial temporal lobe, followed by finer grained associations in sub-ROI if an initial omnibus analysis in a given broad ROI was significant. In the PFC, bilateral sub-ROIs included: 1) pars orbitalis; 2) pars triangularis; 3) caudal middle frontal; 4) rostral middle frontal; 5) superior frontal; 6) frontal pole; 7) lateral orbitofrontal; and 8) medial orbitofrontal. These areas are very similar to ROI chosen in previous reports on metabolic dysfunction with regional amyloid [2] and FDG [1]. In the MTL, bilateral sub-ROI included hippocampal volume (where CT is not calculated by FreeSurfer) and CT in: 1) medial temporal gyrus; 2) parahippocampal gyrus; and 3) entorhinal cortex. Bilateral pre-central gyrus was chosen as a control ROI, an area that shows little association with dysmetabolism and brain atrophy in middle-aged participants [7] or glucose metabolism in AD[1].

FDG-PET

Acquisition is described elsewhere [27, 39]. Images were resliced to a 1.5 mm^3 voxel resolution in a $160 \times 160 \times 96$ spatial matrix, intensity normalized to pons to derive the standardized uptake volume ratio (SUVR), smoothed using an 8mm Gaussian kernel, and normalized to Montreal Neurological Institute space. Due to the poorer native resolution of the FDG-PET images and smoothing to reduce noise, only the two broad ROI in PFC and medial temporal lobe were used. Similarly broad ROI have been used to examine relationships between dysmetabolism and FDG-PET [27].

Statistical analyses

All statistical analyses were conducted using SPSS 23.0 software (IBM Corp., Armonk, NY). All analysis models except for cognition included the following covariates: age at baseline, sex, BMI, APOE ε4 genotype, and either baseline diagnosis or MCI conversion. The random effect of Subject was also covaried. For executive function, memory and global

assessments, education was added as a covariate but not baseline diagnosis or MCI conversion, because cognitive assessments directly inform how participants are clinically classified. Linear mixed models, followed by least significant differences (LSD) post-hoc tests, were used to test if autotaxin levels differed by baseline diagnosis (CN, MCI, AD) or MCI conversion (MCI-S or MCI-P). Multinomial regression analyses tested if higher autotaxin was associated with higher relative risk ratios for an MCI or AD diagnosis, with CN as the reference group. Logistic regression was similarly used to assess increased risk of being MCI-P relative to MCI-S based on autotaxin.

Mixed model analyses were also performed to regress autotaxin against metabolic, MRI, FDG-PET, and cognitive outcomes. The main effect of autotaxin and its interaction with baseline diagnosis or MCI conversion were tested in a given model. Previous work has suggested that systemic IR is associated with neural outcomes like less regional FDG-PET in AD, but show different relationships in MCI-S or MCI-P depending on the brain region [27]. For the MRI analyses, to robustly contain type 1 error, an initial multivariate repeated measures omnibus was initially conducted for a broad PFC or medial temporal ROI, as described elsewhere [40], where each ROI was composed of several sub-ROI selected a priori based on our prior work [1, 2]. Briefly, this technique determines if there is an overall significant association with all sub-ROIs, allowing subsequent analyses to investigate each sub-ROI to determine where omnibus signal was derived without Bonferroni or similar corrections [41]. Even so, as a further type 1 error check described by Willette et al. [27], Holm-Bonferroni correction [42] was used to adjust the family-wise error rate to 0.05. Specifically, for a significant follow-up interaction for a given sub-ROI, autotaxin levels were regressed against CT for CN, MCI, and AD separately, or 3 null hypotheses per ROI. A p value of 0.017 was needed among one of the groups to achieve significance, followed by 0.025 and 0.050.

Lastly, as described by the ADNI Biomarker Core [29], CSF and plasma analyte values were log-transformed to achieve normality. With the exception of AD biomarkers, all variables had homoscedastic variance and a normal distribution. Due to violations in regression diagnostics for p-tau$_{181}$, total tau, and Aβ_{1-42} (see below), the non-parametric Spearman's statistic was used to correlate autotaxin and CSF biomarker values.

RESULTS

Demographics and data summary

Summary information is listed in Table 1. All variables were normally distributed except for Aβ_{1-42} (D=0.130, p<0.001), Total tau (D=0.121, p<0.001), and p-tau (D=0.104, p<0.001), as fit against normal Q-Q plots was non-linear. Data transforms did not resolve non-normality. Non-parametric correlations were therefore conducted between autotaxin and CSF amyloid and tau biomarkers.

Baseline diagnosis: differences in autotaxin levels

Linear mixed models showed a main effect of baseline clinical diagnosis on autotaxin [F(2,286)=3.100, p=0.047]. There was a significant increase in log-scaled autotaxin from

CN to either MCI [Mean Difference±SE=0.078±0.036, p=0.029] or AD [Mean Difference ±SE=0.094±0.043, p=0.029], but not between MCI and AD [Mean Difference ±SE=0.016±0.038, p=0.665].

As a follow-up analysis, multinomial logistic regression was then used to examine if CSF autotaxin expression predicted an increased likelihood of being MCI or AD. The reference group was CN. The likelihood ratio statistic [χ^2=5.990, p=0.050] indicated that higher autotaxin levels predicted a higher Odds Ratio for being MCI [β±SE=1.248±0.605, OR=3.485, 95% CI=1.065 to 11.397, Wald=4.264, p=0.039] or AD [β±SE=1.597±0.747, OR=4.940, 95% CI=1.134 to 21.351, Wald=4.574, p=0.032]. These results suggest that autotaxin levels were higher in memory impaired versus CN participants, and that per point increase in log-based autotaxin values corresponded to a roughly 3.5 to 5 times increase in the odds of having some degree of clinically relevant memory impairment. For MCI conversion by 24 months, there was no significant difference between MCI-S and MCI-P. Baseline autotaxin levels also did not predict likelihood of MCI conversion [χ^2=0.177, p=0.674].

Associations of autotaxin with metabolic and inflammatory indices

Next, to validate autotaxin as an energy metabolism biomarker, CSF autotaxin levels were regressed against total body mass, peripheral and CSF metabolic and inflammatory biomarkers (Table 2), as well as used to predict likelihood of having pre-diabetes or type 2 diabetes. We found that higher autotaxin was related to higher fasting glucose, several established biomarkers of metabolism in the CSF, CSF interleukin-6, and that higher levels per point increase reflected a 300% greater likelihood of having pre-diabetes or type 2 diabetes.

ROI analysis: PFC and MTL CT

For bilateral PFC CT, a multivariate omnibus was conducted in one broad PFC ROI incorporating several sub-ROI from FreeSurfer areas, to minimize type 1 error [41]. The main effect of autotaxin and its interaction with baseline diagnosis were investigated. The interaction tested if autotaxin differentially predicted less CT among CN, MCI, or AD participants. There was a marginal, within-subject Regions × Baseline Diagnosis × Autotaxin interaction after Huynh-Feldt correction [F(14,1200)=1.784, p=0.066], which became significant after removing one outlier with a high autotaxin value (18.31) [F(14,1197)=2.031, p=0.032]. As indicated in Table 3, even after applying Holm-Bonferroni correction, higher autotaxin was associated with thinner CT in most PFC sub-ROI for AD participants, such as medial orbitofrontal cortex (Fig. 1A) and only in Pars Orbitalis for MCI, while the CN group showed negative and positive associations. By contrast, the omnibus for the one broad medial temporal ROI was non-significant, where further analyses were not pursued to guard against type 1 error. In the pre-central gyrus control region, there was no significant main effect of autotaxin or interaction with baseline diagnosis.

ROI analysis: PFC and MTL glucose metabolism

For FDG-PET, an Autotaxin * Baseline Diagnosis interaction [F(2,146)=3.106, p=0.048] showed that higher autotaxin predicted less glucose uptake in the broad PFC ROI for AD

subjects [β±SE=−0.127±0.027, t(1,35)= −4.708, p<0.001], marginally for CN [β ±SE=-0.074±0.041, t(1,35)= −1.822, p=0.077] and not for MCI [β±SE=0.043±0.029, t(1,70)=1.497, p=0.139]. Removal of the same autotaxin outlier from the PFC CT analysis did not affect the association in the AD group [β±SE=−0.116±0.032, T(1,34)= −3.610, p=0.001] (Fig. 1B). In addition, the Autotaxin * MCI Conversion interactions were non-significant for PFC.

Similar relationships were seen in the broad temporal ROI. A Baseline Diagnosis * Autotaxin interaction [F(2,146)=14.175 p<0.001] revealed that higher autotaxin was associated with less MTL glucose metabolism in AD [β±SE=−0.586±0.026, t(1,37)= −4.334 p<0.001] and CN participants [β±SE=−0.429±0.049, t(1,35)= −2.773 p=0.009], but not in MCI [β±SE=0.189±0.027, t(1,72)=1.619 p=0.110].

FDG uptake in the pre-central gyrus control region did not show a main effect of autotaxin or interaction with baseline diagnosis or MCI conversion.

Cognition: global and executive function factor scores

As shown in Table 4, separate linear mixed models indicated that per point increase in autotaxin, scores were lower for the executive function and memory factors, but not for global cognition and function scores.

CSF biomarkers: $A\beta_{1-42}$, total tau, and p-tau

Across all participants, the Spearman's statistic showed that higher autotaxin was associated with higher levels of total tau, p-tau$_{181}$, and total tau/$A\beta_{1-42}$ (Table 5).

DISCUSSION

In this study, we hypothesized that CSF-derived autotaxin may be a biomarker for brain metabolic dysfunction relevant to AD. Strikingly, higher autotaxin predicted less bilateral PFC and MTL glucose uptake in individuals with AD, with similar effects seen in bilateral PFC CT. Results also showed that participants who had MCI or AD had significant increased log-scaled autotaxin. IR shows a similar relationship with PFC and MTL FDG-PET in late middle-aged participants [28], and among aged CN adults with type 2 diabetes [8] or AD [27], where the ADNI AD group for the previous report and this cohort show similar results.

Higher autotaxin correlated with established metabolic biomarkers and higher risk for pre-diabetes and type 2 diabetes. CSF ENPP2 was moderately associated with peripheral fasting glucose, but not BMI, where others have noted similar findings [26]. Furthermore, per point increase in autotaxin, executive function and memory factor scores were lower. Similarly, a decreased insulin sensitivity index in rhesus monkeys [43] was associated with less PFC gray matter and mediated worse motor-planning executive function performance. Higher HOMA-IR in aged humans with AD was also related to less MTL and PFC glucose metabolism [27], respectively mediating worse memory and executive function factor scores (unpublished data). Several studies have found similar associations between metabolic dysfunction, PFC outcomes, and executive function. Diabetes was significantly associated

with worse cognitive performance and attention deficit in young adults without dementia [44]. In a cohort of bariatric surgery patients, as HOMA-IR levels decreased following the surgery, cognition scores improved [45]. Taken together, autotaxin shows a pattern of relationships with established metabolism biomarkers, MTL and PFC-specific outcomes, and related cognitive dysfunction that may reflect central dysmetabolism and is similar to our previous findings with IR.

Autotaxin was also positively correlated with levels of total tau, p-tau$_{181}$, total tau/Aβ_{1-42}, as well as p-tau$_{181}$/Aβ_{1-42} to a marginal degree. This pattern suggests that CSF autotaxin predicts AD neuropathology in a manner similar to dysmetabolism. Chronic application of insulin *in vitro* in human cortical stem cells appeared to induce insulin resistance and inhibit tau dephosphorylation [46]. Higher IR among cognitively normal, APOE4 late middle-aged participants also predicted higher total tau and phosphorylated tau [47], but not CSF amyloid. Amyloidosis is scant in this late middle-aged cohort, but does show modest regional associations with higher IR [2]. ADNI did not have enough baseline amyloid scans (n=20) to warrant analysis with autotaxin.

This brings to question the underlying mechanism behind the correlation of autotaxin and AD-related outcomes. Individuals with high autotaxin may be more likely to be obese, causing an increase in IR, ischemia, and oxidative stress. Results of our study showed a weak association between systemic glucose and CSF autotaxin. As an alternative or interrelated mechanism, autotaxin stimulates the release of LPA, which may be detrimental [21, 22]. LPA has been shown to prompt neurite withdrawal and tau phosphorylation in neuroblastoma cells [48]. Addition of LPA to neuroblastoma cells led to increased immunoreactivity with antibodies that react with tau phosphorylation [17].

There are several study limitations to address. Regression diagnostic issues were encountered for total tau, p-tau$_{181}$, and Aβ_{1-42}, requiring non-parametric analyses. Autotaxin levels did not differ in MCI conversion or contribute to MCI conversion risk, nor were there significant interactions with MCI conversion, which may be due to small sample size. Instead, baseline values were widely dispersed in the MCI converter group. This mirrors IR MCI interactions [1]. No associations were found with Aβ levels, but there were significant associations with tau and p-tau$_{181}$, as well as tau to amyloid ratios. Given that the mass spectrometry panel was only available at baseline, we were unable to longitudinally assess autotaxin levels and various outcomes. While longitudinal predictions would be useful, it was beyond the scope of this report, which was to systematically assess if autotaxin may be a relevant AD biomarker. While autotaxin was associated with fasting glucose, it was surprising that it was not associated with systemic insulin or HOMA-IR. This may be because insulin was derived from a plasma multiplex, and the values are lower than ELISA. Nonetheless, autotaxin was associated with fasting glucose, risk for pre-diabetes and type 2 diabetes, and was associated with many established metabolic factors in CSF. It also shows a pattern of results comparable to HOMA-IR and either brain atrophy or FDG-PET[7, 27, 28]. The non-significance of MTL ROI CT was unexpected; however, it also provides direction for future research. Glucagon-like peptide-1 (GLP-1) may be a potential element in this phenomenon. GLP-1 stimulates the release of insulin as well as downregulates the release of glucagon and breakdown of amyloid-β protein precursor [49]. GLP-1 receptor production in

the aged mouse medial PFC was decreased compared to young mice, but no similar relationship was seen in the hippocampal regions [49].

In summary, this study demonstrates that CSF-derived autotaxin in elders correlated with greater odds for having MCI or AD, hyperglycemia, AD neuropathology related to total tau, p-tau$_{181}$, and the total tau/Aβ_{1-42} ratio, less gray matter CT in PFC, hypometabolism in PFC and MTL, and worse executive function and memory scores. These results support the theory that autotaxin may be an indicator of central dysmetabolism for a variety of AD outcomes. Further replication is necessary to determine if autotaxin predicts AD and tracks certain aspects of the disease. Future clinical and animal model work should consider examining this biomarker and its applications as a potential target for pharmacologic therapies in the setting of glucose dysregulation and AD. Such work should also examine how autotaxin predicts changes in these outcomes over time.

Acknowledgments

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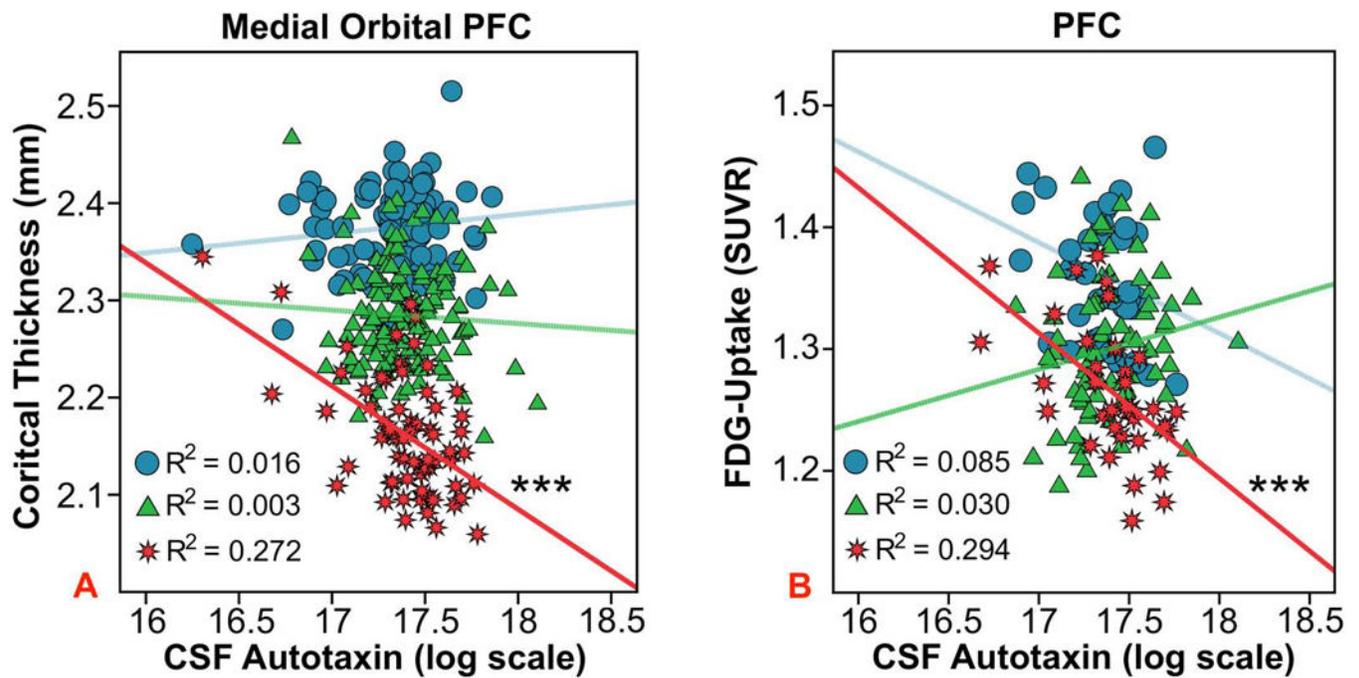
Figure 1.
Autotaxin and PFC region of interest analyses. The association between CSF autotaxin and baseline bilateral medial orbital PFC cortical thickness (A) or bilateral PFC FDG-PET glucose uptake (B), an index of glucose metabolism, among baseline diagnosis groups. The "blue circle", "green triangle", and "red star" symbols correspond to CN, MCI, and AD participants respectively. The R^2 value refers to the proportion of variance in CT or FDG-PET uptake explained by autotaxin for a given group. ***$p < 0.001$.

Table 1

Demographics and sample characteristics

	CN	MCI	AD	MCI-S	MCI-P
Age	75.70 ± 5.54	74.69 ± 7.35	74.98 ± 7.57	74.74 ± 6.96	74.63 ± 7.94
Female	42	44	29	25	19
Male	44	91	37	55	36
Education	15.64 ± 2.97	16.00 ± 2.96	15.11 ± 2.96	16.33 ± 2.91	15.51 ± 3.00
APoE e4-	65	64	19	41	23
APoE e4+	21	71	47	39	52
CDR-sob	0.02 ± 0.11	1.56 ± 0.88	4.34 ± 1.56	1.41 ± 0.79	1.77 ± 0.97
MMSE	29.05 ± 1.02	26.91 ± 1.74	23.52 ± 1.85	27.35 ± 1.62	26.27 ± 1.72
ADAS-cog11	6.05 ± 2.90	11.72 ± 4.33	18.88 ± 6.71	10.70 ± 4.05	13.19 ± 4.32
Memory factor	0.98 ±0.50	−0.15±0.57	−0.91±0.55	0.04 ± 0.57	−0.43 ± 0.45

Numbers represent frequency or unadjusted mean ± SD. MCI-S, Stable MCI at 12 months. MCI-P, Progression to AD from MCI at 12 months.

Table 2

The association of autotaxin with CSF metabolic and inflammatory biomarkers.

	β ± SE	F value	p value
Fructose-bisphosphate aldolase A	0.415±0.097	18.551	<0.001
Cholecystokinin	0.378±0.123	9.369	0.002
Pyruvate Kinase Isozymes M1/M2	0.576±0.108	28.364	<0.001
Neurosecretory protein VGF	0.574±0.162	12.614	<0.001
IGFBP2	0.595±0.056	114.899	<0.001
IL-6 receptor			
CN	0.102±0.011	93.830	<0.001
MCI	0.067±0.010	45.705	<0.001
AD	0.097±0.015	41.301	<0.001

Model estimate beta values and SE of autotaxin for CSF metabolites and inflammation biomarkers.

Table 3

The interaction of clinical diagnosis and autotaxin on prefrontal cortex thickness.

Cortical Thickness	CN		MCI		AD	
	T value	β ± SE	T value	β ± SE	T value	β ± SE
Pars Orbitalis	−1.519	−0.073 ± 0.048	−2.188	−0.073 ± 0.034 *	−4.253	−0.230 ± 0.054 ***
Pars Triangularis	−4.300	−0.062 ± 0.014 ***	−1.914	−0.041 ± 0.021	−4.178	−0.102 ± 0.024 ***
Rostral Middle Frontal	−0.073	−0.001 ± 0.013	−1.592	−0.028 ± 0.018	−4.180	−0.090 ± 0.022 ***
Lateral Orbitofrontal	3.214	0.046 ± 0.014 **	−0.260	−0.005 ± 0.020	−5.306	−0.122 ± 0.023 ***
Medial Orbitofrontal	1.178	0.020 ± 0.017	−0.664	−0.014 ± 0.021	−4.922	−0.127 ± 0.026 ***
Caudal Middle Frontal	−1.208	−0.015 ± 0.013	1.146	0.021 ± 0.019	0.667	0.014 ± 0.021
Superior Frontal	−3.596	−0.062 ± 0.017 **	0.178	0.004 ± 0.025	−1.705	−0.050 ± 0.029
Frontal Pole	1.153	0.023 ± 0.020	1.378	0.034 ± 0.025	−10.925	−0.344 ± 0.031 ***

*
$p < 0.05$,

**
$p < 0.01$,

***
$p < 0.001$

Model estimate beta values and SE of autotaxin for prefrontal CT ROI. T values are for each clinical group. For each ROI, Holm-Bonferroni correction required successive p values of 0.017, 0.025, and 0.050 for a given diagnostic group fit-line to be considered significant.

Table 4

The association of autotaxin with cognitive indices.

Cognitive Parameter	F value	p value	β ± SE
ADAS-cog11	1.251	0.264	1.802 ± 1.611
CDR-sob	0.831	0.363	0.405 ± 0.444
Executive Factor	**4.215**	**0.037**	**−0.464 ± 0.222**
Memory Factor	**4.222**	**0.041**	**−0.333 ± 0.056**
MMSE	0.054	0.816	0.230 ± 0.606

Model estimate beta values and SE of autotaxin for global indices and the executive function and memory factors. Bolded text indicates results with a significant p value.

Table 5

The association of autotaxin and AD CSF markers.

CSF Biomarker	p value	Spearman's R value
$A\beta_{1-42}$	0.738	−0.020
p-tau$_{181}$	**0.028**	**0.130**
p-tau$_{181}$/Aβ_{1-42}	0.070	0.108
Total Tau	**0.002**	**0.185**
Total Tau/Aβ_{1-42}	**0.018**	**0.141**

Due to regression diagnostics, parametric models were not conducted between autotaxin and CSF biomarkers. Bolded text indicates results with a significant p value.