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A lipid storage–like disorder contributes to cognitive decline in HIV-infected subjects

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ABSTRACT

Objective: In this multicenter cohort study, we sought to identify prognostic and associative metabolic indicators for HIV-associated neurocognitive disorders (HAND).

Methods: A quantitative lipidomic analysis was conducted on 524 longitudinal CSF samples collected from 7 different performance sites across the mainland United States, Hawaii, and Puerto Rico. Subjects included HIV-infected individuals with longitudinal clinical and cognitive testing data and cognitively normal HIV-negative healthy controls.

Results: At baseline, HIV+ subjects could be differentiated from HIV− controls by reductions in a single ceramide species and increases in multiple forms of cholesterol. Perturbations in cholesterol metabolism and ceramide were influenced by combined antiretroviral therapy (cART) use. There were no cross-sectional baseline differences in any lipid metabolite when HIV+ subjects were grouped according to cognitive status. However, a single sphingolipid metabolite and reduced levels of esterified cholesterols were prognostic indicators of incident cognitive decline. Longitudinal patterns of these disturbances in sphingolipid and sterol metabolism suggest that a progressive disorder of lipid metabolism that is similar to disorders of lipid storage may contribute to the pathogenesis of HAND.

Conclusions: These findings suggest that HIV infection and cART are independently associated with a CNS metabolic disturbance, identify surrogate markers that are prognostic for cognitive decline, and implicate a lipid storage–like disorder in the progression of HAND. *Neurology*® 2013;81:1–8

GLOSSARY

ARV = antiretroviral drug; **cART** = combined antiretroviral therapy; **CI** = confidence interval; **HAND** = HIV-associated neurocognitive disorders; **MSK** = Memorial Sloan-Kettering; **NNRTI** = nonnucleoside reverse transcriptase inhibitor; **OR** = odds ratio; **SM** = sphingolipid metabolite.

The widespread use of combined antiretroviral therapy (cART) in developed countries has dramatically decreased the death rates due to AIDS and decreased the incidence of dementia.[1,2] However, cART has not reduced the prevalence rates for milder forms of HIV-associated neurocognitive disorders (HAND) that are associated with increased mortality.[3–6] HAND frequently manifests in the domains of memory and executive functions,[7] and is associated with decreased adherence to pharmacotherapy, lower cognitive reserve, and increased incidence of psychiatric comorbidities.[8,9] Despite cART, there is evidence for ongoing neurologic damage that includes persistent astrocyte infection, brain volume loss, inflammation, synaptodendritic damage, and disruptions in white matter integrity.[5,10–12] The mechanisms underlying these residual neuropathologic impairments remain obscure.

Several studies have suggested that dysregulations in CNS lipid metabolism may be involved in the pathogenesis of HAND.[13–15] In particular, roles for ceramides, sphingomyelins, and cholesterol have been described, but the interrelationships of these bioactive lipids to the temporal progression

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Supplemental data at www.neurology.org

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of HAND have not been determined. In this study, we sought to elucidate the interactions between CSF lipidoses and temporal shifts in the cognitive status of a demographically diverse multicenter collection of HIV-infected subjects. Our findings provide evidence that the onset and progression of HAND involves a progressive disturbance in sphingolipid and cholesterol metabolism that is reminiscent of the biochemical manifestations associated with a lysosomal storage disorder.

METHODS

Subjects. CSF samples for this study were collected from 7 performance sites: The Johns Hopkins University, School of Medicine; the Mount Sinai School of Medicine; the University of California in San Diego; the University of Texas Medical Branch; the University of Washington; the University of Hawaii; and the University of Puerto Rico. HIV-seronegative CSF was available from 30 healthy subjects (table e-1 and appendix e-1 on the *Neurology*® Web site at www.neurology.org).

Standard protocol approvals, registrations, and patient consents. The collection and use of human samples was approved by the institutional review board at each of the study sites.

Table 1 Comparison of baseline CSF sphingolipid levels in HIV+ individuals and HIV− cognitively normal controls

Log sphingolipids[a]	HIV+, mean (SE)	HIV−, mean (SE)	*p* Value
Ceramide			
C16:0	10.69 (0.21)	10.53 (0.21)	0.59
C18:0	9.51 (0.24)	8.80 (0.24)	0.04
C20:0	10.48 (0.21)	10.31 (0.21)	0.56
C24:1	9.67 (0.22)	9.08 (0.22)	0.06
C24:0	10.27 (0.24)	9.69 (0.24)	0.08
Sphingomyelin			
C16:0	14.98 (0.23)	14.85 (0.23)	0.70
C18:1	11.82 (0.25)	11.27 (0.25)	0.12
C18:0	15.56 (0.24)	15.37 (0.24)	0.57
C20:1	10.63 (0.24)	10.15 (0.24)	0.16
C22:1	12.64 (0.25)	12.03 (0.24)	0.08
C22:0	13.68 (0.24)	13.18 (0.24)	0.14
C24:1	13.61 (0.26)	13.16 (0.26)	0.22
C24:0	11.11 (0.30)	10.79 (0.30)	0.46
Sphingomyelin/ceramide ratio			
C16:0	1.41 (0.03)	1.42 (0.03)	0.81
C18:0	1.65 (0.03)	1.78 (0.03)	<0.001
C24:1	1.42 (0.03)	1.46 (0.03)	0.34
C24:0	1.09 (0.03)	1.11 (0.03)	0.68
Total ceramide	12.01 (0.22)	11.60 (0.22)	0.19
Total sphingomyelin	16.34 (0.24)	16.03 (0.23)	0.36
Total sphingomyelin/ceramide ratio	1.36 (0.02)	1.39 (0.02)	0.37

[a] Means are adjusted for site.

Cognitive outcome. The Memorial Sloan-Kettering (MSK) dementia severity scale was used to categorize the level of cognitive impairment[16] as this measure was used at all study sites. A score of 0 indicates no cognitive impairment; a score of 0.5 indicates mild cognitive impairment; 1 indicates mild dementia; 2 indicates moderate dementia; 3 or more indicates severe dementia. For the purposes of this study, a decline in cognition was defined by an increase in MSK between baseline and follow-up, while cognitive improvement was defined by a decrease in MSK.

Lipid extraction and mass spectrometry. CSF samples were centrifuged to remove any cells, aliquoted, and immediately frozen at −80°C. A crude lipid extraction of CSF was conducted using a modified Bligh and Dyer procedure as described.[13] Analyses of sphingolipids and sterols were performed independently on 2 separate high-performance liquid chromatography coupled electrospray ionization tandem mass spectrometers (API3000s, Sciex Inc., Thornhill, Canada) (see e-Methods for further details).[13,14]

Statistical analyses. Student *t* tests for continuous variables and χ^2 tests for categorical variables were used to examine baseline group differences. Analyses of variance were used to examine whether baseline lipids varied by severity of cognitive impairment. As there were no baseline differences in lipid levels by MSK status, all HIV-positive individuals were combined into one group and logistic regression determined whether baseline mean CSF lipids were altered among groups. Several covariates were examined as potential confounders of the association between the CSF lipids and cognitive change including demographics (age, sex, race, education), HIV medication use by antiretroviral drug (ARV) class (nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors [NNRTIs], protease inhibitors, entry/fusion inhibitors, and integrase inhibitors), indicators of HIV status (CD4 cell count, nadir CD4 count, plasma and CSF viral loads), drug/alcohol use, and time between visits. Covariates were retained in the models if they were associated with baseline lipids and MSK status and significantly ($p < 0.05$) contributed to the model based on the likelihood ratio test. Final models for analyses controlled for time between visits and site, sex, and NNRTI use. Analyses were conducted using STATA version 12 (StataCorp, College Station, TX).

Prognostic and associative analyses. For the prognostic analysis, we examined baseline (visit 1) CSF lipid levels, and odds of subsequent cognitive decline or improvement. For the associative analyses, changes in CSF lipid levels between the 2 visits were examined in association with change in cognitive status. Both prognostic and associative analyses used multinomial logistic regression to separately examine metabolites among those who experienced cognitive decline or improvement compared to those with no cognitive change over the 2 visits (see appendix e-1 for additional information).

RESULTS

Participant characteristics. There were 321 subjects with CSF samples: 291 HIV-positive and 30 HIV-seronegative (cognitively normal healthy participants). Subject demographics are shown in table e-1. Of 291 HIV+ participants, 203 (69.8%) had an additional visit with lumbar puncture and CSF. Compared to subjects with a follow-up visit, those lost to follow-up were more cognitively impaired ($p = 0.008$), and were more likely to be women (37.9% women vs 25.1% men, χ^2 [1] = 5.41, $p = 0.020$). There were no other demographic or disease-related differences between

those with or without a follow-up visit. Slightly more than half the HIV+ subjects, 128 (63.1%), were cognitively stable between visits, 29 (14.3%) exhibited a decline in cognitive stats, and 46 (22.7%) showed improvement in cognitive status. The average number of days between visits was 278.7 ± 191.4 (SD).

Relationship between CSF sphingolipid and sterol levels and HIV status. There were no baseline differences in any of the lipid levels when HIV+ subjects were grouped based on cognitive status. Thus, for cross-sectional comparisons, HIV+ individuals were combined into a single group and compared with HIV− controls. Total ceramide and sphingolipid metabolite (SM) concentrations in CSF were greater in HIV− control subjects compared with HIV+ subjects (table 1). Compared to HIV− subjects, HIV+ subjects had decreased CSF levels of a single ceramide species (C18:0) that resulted in an elevated SM/ceramide C18:0 ratio (table 1; we used SM/ceramide ratios as an index of metabolism. An increase in this ratio suggests a preference for SM production, and decreases suggest a preference for ceramide production). Thus, in this demographically diverse population, HIV+ subjects can be differentiated from HIV− controls by elevations in a single ceramide species.

Total sterol concentrations in CSF were greater in HIV+ compared with HIV− subjects (table 2). Compared with HIV− subjects, HIV+ individuals showed higher levels of all but one sterol species measured including cholesterol, multiple esterified forms of cholesterol, and triglyceride (table 2).

Table 2 Comparison of baseline CSF cholesterol levels in HIV+ individuals and HIV− cognitively normal controls

Log cholesterol[a]	HIV+, mean (SE)	HIV−, mean (SE)	*p* Value
Cholesterol esters			
20:6	9.38 (0.19)	11.33 (0.19)	<0.001
22:6	9.88 (0.18)	12.05 (0.19)	<0.001
20:5	8.86 (0.18)	10.74 (0.18)	<0.001
22:5	10.70 (0.24)	13.22 (0.24)	<0.001
18:4	6.94 (0.18)	7.53 (0.19)	0.02
20:4	9.56 (0.19)	10.39 (0.19)	0.002
18:3	11.63 (0.18)	12.75 (0.18)	<0.001
20:3	8.61 (0.16)	9.91 (0.16)	<0.001
16:2	8.98 (0.15)	10.26 (0.15)	<0.001
18:2	12.79 (0.17)	13.69 (0.17)	0.001
16:1	9.81 (0.16)	11.52 (0.16)	<0.001
18:1	11.39 (0.16)	12.60 (0.17)	<0.001
16:0	9.37 (0.16)	11.34 (0.16)	<0.001
Total cholesterol	13.62 (0.15)	14.95 (0.15)	<0.001
Triglyceride	11.23 (0.22)	13.06 (0.38)	<0.001

[a] Means are adjusted for site.

The influence of cART and statin use on CSF lipid metabolites and HIV status. Since cART use is associated with a metabolic disturbance manifest by increased circulating cholesterol and triglycerides,[17] we next determined if cART may have contributed to these perturbations in brain sphingolipid and sterol metabolism. Information on cART use was available for 95.6% of the subjects, and 85.3% reported taking a cART regimen. Mean baseline CSF levels of SMs, ceramides, and triglycerides did not differ by cART use ($p > 0.05$). However, cART use was associated with higher baseline levels of cholesterol and nearly all cholesterol esters ($p < 0.05$ or greater). When we separated cART by drug class, we found that nucleoside reverse transcriptase inhibitors and NNRTIs were associated with higher mean levels of multiple forms of cholesterol ($p < 0.05$; table e-2). Protease, fusion, or integrase inhibitors were not associated with CSF levels of SM, ceramides, or sterols. Since most individuals taking an NRTI were also taking an NNRTI, we included NNRTI use as a covariate in subsequent analyses of cholesterol esters. Associations for individual ARVs with SM, ceramide, and sterols species are shown in tables e-3 and e-4. We also examined the relationships between CNS penetration effectiveness score and all lipid species, but did not find any significant associations.

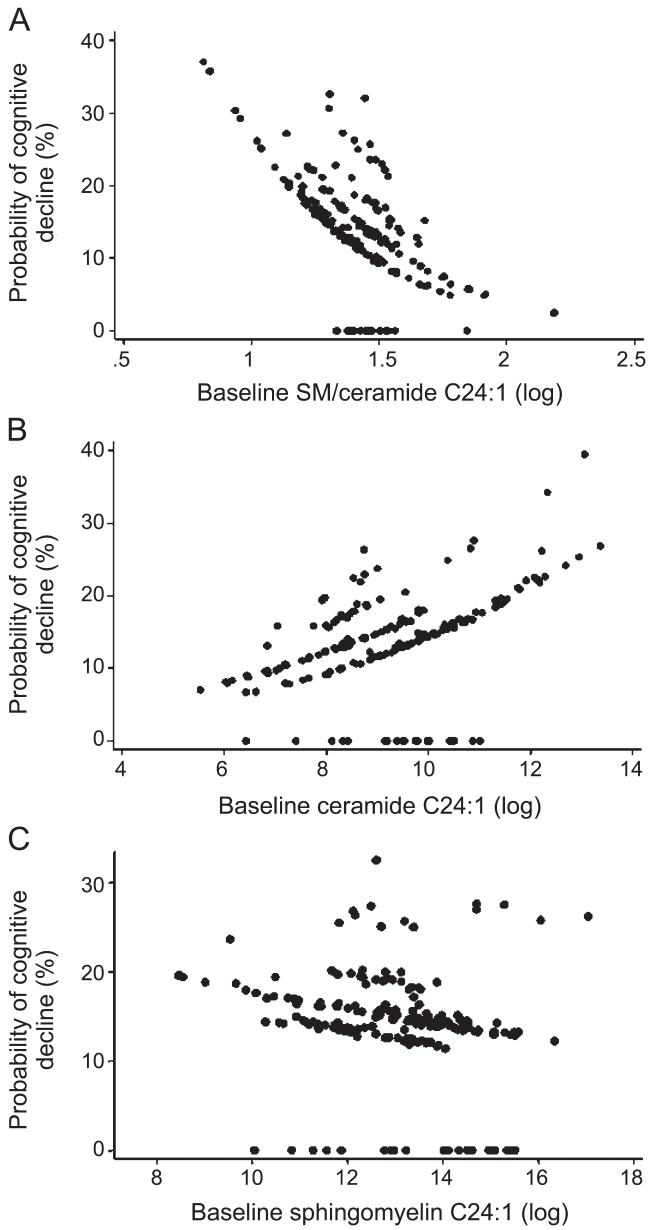
There were no correlations between CSF levels of SM, ceramide, or sterols and HIV disease markers including CD4 cell count, CD4 nadir, and plasma or CSF viral load ($p > 0.1$). Nadir CD4 was associated with cognition at some study sites when examined independently, but was not significant when all sites were combined.

There were 19 subjects taking a statin at baseline. Statin use itself was not a prognostic or associative indicator of worsening or improvement in cognitive status. At baseline, there was no association between statin use and cholesterol or cholesterol esters. However, statin users did have significantly higher levels of ceramide C16:0 ($p = 0.038$), C20:0 ($p = 0.009$), C24:1 ($p = 0.005$), and sphingomyelin C22:0 ($p = 0.020$) at baseline. When included as a covariate in the regression models, statin use had no effect on the odds ratios (ORs).

CSF sphingolipids and esterified cholesterols are prognostic indicators of cognitive decline. Among HIV+ participants, there were no apparent cross-sectional differences at baseline in CSF levels of any sphingolipid or sterol product when grouped according to cognitive status. This included when cognitive status was examined as a categorical variable or dichotomized as cognitively impaired (MSK 0.5–2.0) vs cognitively normal (MSK = 0). Therefore, we determined if clinical or lipid measures at baseline could be used as prognostic

indicators of cognitive function. We did not find any prognostic relationships between clinical markers of HIV disease status (e.g., CD4 count, nadir CD4, ARV use, plasma or CSF viral load) or demographics (e.g., age, sex, education) and odds of improvement or decline in cognitive status (data not shown). However, a higher SM/ceramide ratio for C24:1 at baseline was associated with a reduced odds of cognitive decline at the next visit (OR 0.06, 95% confidence interval [CI] 0.01–0.96, $p = 0.047$, controlling for time between visits and site) (table e-2, figure 1A). Thus, a lower SM/ceramide C24:1 ratio was a prognostic indicator for cognitive decline. Analyzing each of these metabolites separately revealed that higher levels of ceramide C24:1 at baseline were associated with greater odds of cognitive decline (OR 1.31, 95% CI 0.91–1.88; table e-5 and figure 1B), while SM C24:1 was not associated with cognitive decline (OR 0.94, 95% CI 0.70–1.26). Restricting these analyses to only include individuals with less than 1 year of follow-up did not alter the results (data not shown).

Figure 1 **Baseline sphingolipids that are prognostic indicators for cognitive decline**



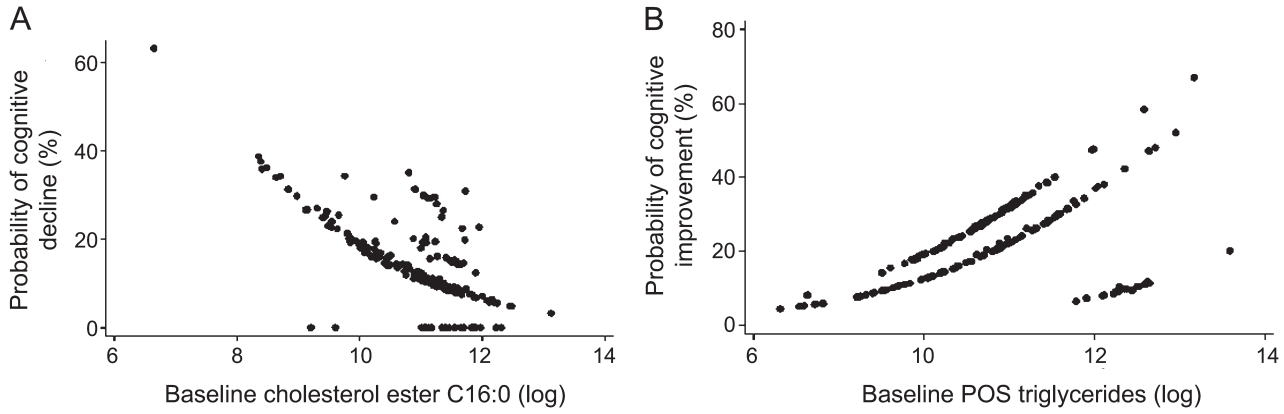
(A) Lower ratios of CSF sphingomyelin/ceramide C24:1 at baseline was a prognostic indicator for cognitive decline (odds ratio [OR] 0.06, 95% confidence interval [CI] 0.01–0.96, $p = 0.047$). (B) Elevated baseline levels of CSF ceramide C24:1 were associated with a greater probability of cognitive decline at the next visit (OR 1.31, 95% CI 0.91–1.88, $p = 0.252$). (C) Baseline levels of sphingomyelin did not predict cognitive decline (OR 0.94, 95% CI 0.70–1.26, $p = 0.679$).

Elevations of baseline CSF cholesterol esters but not triglycerides were associated with reduced odds for cognitive decline at the next visit (table e-6, figure 2A). Thus, lower CSF levels of multiple cholesterol esters at baseline were prognostic indicators for cognitive decline. When we restricted the data to those subjects with a follow-up visit within 1 year, these associations became stronger, and decreases in all but one of the cholesterol esters (12 of the 13 identified) were prognostic indicators for cognitive decline (table e-6). As cART can result in a metabolic syndrome that is characterized by lipodystrophy and increased circulating levels of cholesterol, it was possible that the association between decreased cholesterol ester levels in CSF and cognitive decline were the result of cART failure or poor drug compliance. Thus, we used plasma viral loads and CD4 levels as surrogate markers for cART efficacy, and examined these levels by change in cognitive status. There were no differences in mean plasma CD4 count or viral load between HIV+ subjects who remained unimpaired at both visits and those with either worsened or improved cognitive status ($p > 0.10$; data not shown), suggesting that the prognostic value of low cholesterol esters for declines in cognitive status was not due to cART failure or poor compliance.

CSF triglycerides are prognostic indicators of cognitive improvement. We next determined whether any of the lipid metabolites measured were prognostic indicators for cognitive improvement. None of the SM, ceramide, or cholesterol metabolites were prognostic indicators for improvements in cognitive status (data not shown). However, elevated baseline levels of triglycerides were associated with increased odds for cognitive improvement (OR 1.93, 95% CI 1.21–3.10, $p = 0.006$) (table e-6; scatterplot for triglyceride is shown in figure 2B). This prognostic relationship between elevated triglycerides and improved cognitive status remained strong when we restricted our analysis to subjects who had a follow-up within 1 year (OR 2.07, 95% CI 1.24–3.47, $p = 0.006$). Thus, elevated levels of triglycerides were prognostic indicators for cognitive improvement.

Progressive dysregulations in sphingolipid and sterol metabolism are associated with cognitive decline and metabolic normalization is associated with cognitive improvement. To determine whether there were

Figure 2 Specific forms of sterols and triglycerides that are prognostic indicators for change in cognitive status



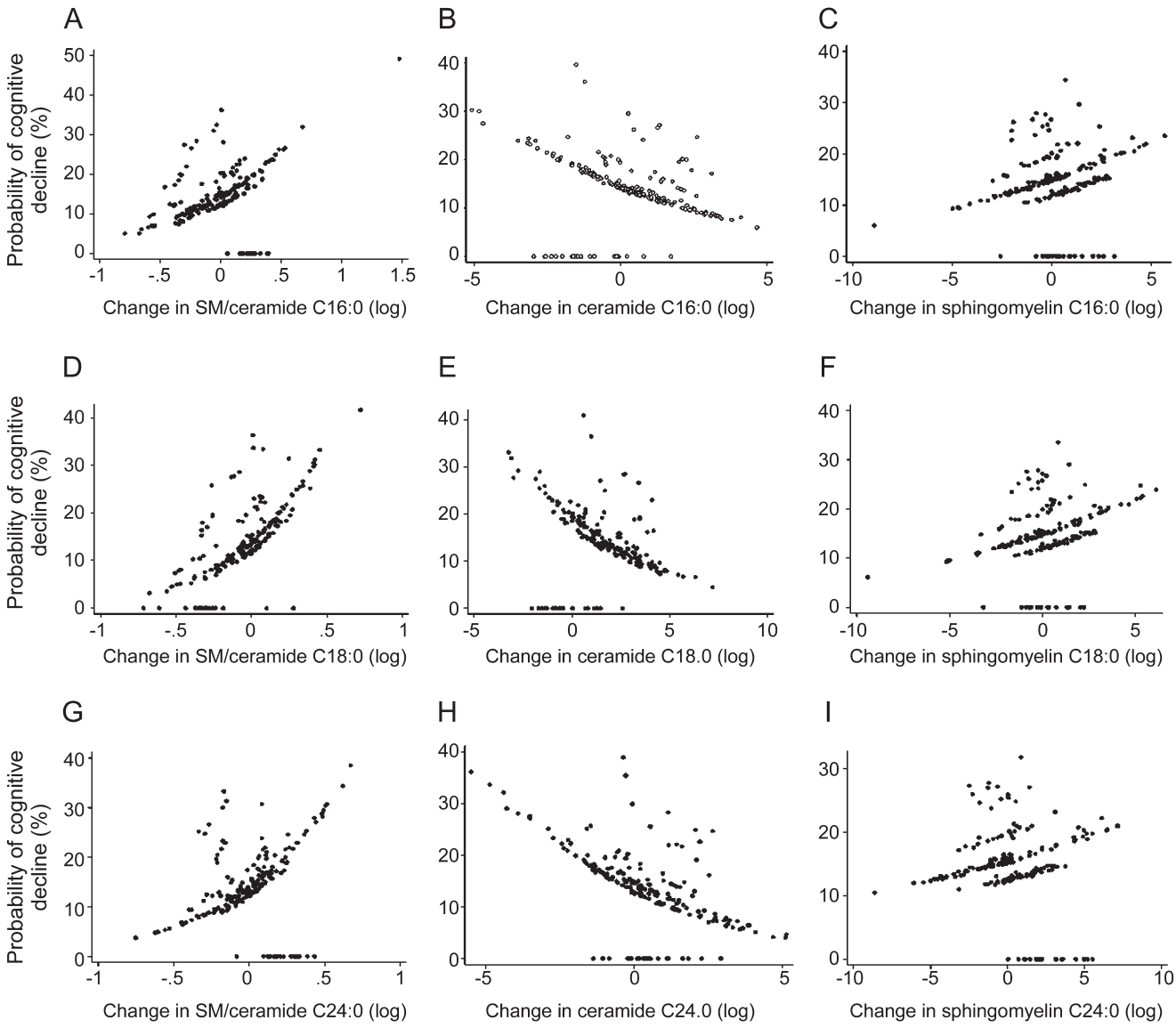
Decreased CSF levels in multiple forms of cholesterol esters at baseline were prognostic indicators for cognitive decline. (A) Scatterplot shows the relationship between baseline levels of the cholesterol ester C16:0 and the probability of cognitive decline at the next visit (odds ratio [OR] 0.51, 95% confidence interval [CI] 0.31–0.84, $p = 0.008$). This plot is representative of the 16 cholesterol esters with similar relationships to cognitive decline (see table 1 for a complete list). (B) Higher levels of triglycerides were associated with a greater probability of cognitive improvement at the next visit. Data show the relationship of triglyceride to the probability of cognitive decline at the next visit (OR 1.93, 95% CI 1.21–3.10, $p = 0.006$).

progressive disruptions in ceramide, SM, or sterol metabolism that paralleled changes in cognitive status, we examined the longitudinal association between each CSF lipid and sterol metabolite and the odds of either cognitive decline or improvement. We found that increasing SM/ceramide ratios from the baseline to follow-up visit for C16:0 (OR 5.07, 95% CI 1.06–24.15, $p = 0.042$), C18:0 (OR 11.86, 95% CI 1.39–100.90, $p = 0.024$), and C24:0 (OR 7.98, 95% CI 1.18–54.21, $p = 0.034$) were associated with increasing odds of cognitive decline (table e-7). These progressive changes in SM/ceramide ratios were driven by decreases in ceramide and increases in SM (table e-7; figure 3, A–I). Levels of cholesterol esters did not further change over time as cognitive status declined. In contrast to these subjects with progressively worsening cognitive impairment, cognitively impaired subjects who improved over time showed a progressive normalization of all SM, ceramide, and sterol species examined (data not shown).

DISCUSSION Our data suggest that disturbances in CSF lipid metabolites are a common feature of HAND. The CNS normally has the highest cholesterol content in humans, with daily turnover rates estimated to be approximately 20% of the total brain cholesterol content.[18] While this high turnover rate permits rapid plasma membrane turnover and remodeling, excess cholesterol can be sequestered into endosomal/lysosomal compartments. For example, genetic mutations in Niemann-Pick disease, type C1 leads to the sequestration of unesterified cholesterols in endosomal/lysosomal compartments, culminating in the development of pulmonary, hepatic, and neurodegenerative disease. Although cART has been associated with metabolic disturbances that include elevated blood cholesterol and triglycerides,[19–21] effects on CSF lipids were unknown. We found HIV infection increased CSF cholesterol ester content, and this was further increased by cART. Moreover, and perhaps surprisingly en face, we found that decreased cholesterol esters were associated with increased risk for cognitive decline. Since increased cholesterol production is balanced by esterification, we interpret these data to suggest that HIV and cART are independently associated with brain cholesterol accumulation. Consistent with this concept of sterol clearance, we found that increased CSF triglycerides were prognostic indicators for cognitive improvement, suggesting that removal of sterols from brain in the form of triglycerides improves cognition.

Further evidence that a lysosomal storage-like disorder may play a role in HAND was suggested by alterations in sphingolipid levels. Sphingolipids regulate a wide variety of neurobiologic processes, including neurotropic signaling,[22] neural cell adhesion and migration,[23,24] synaptic transmission/axonal guidance,[23,25] and neuron–glial interactions.[26] Sphingolipids regulate these (and other) biological functions by affecting the biophysical properties of membranes, and through lipid–protein interactions that control protein location, scaffolding, and posttranslational modifications. In a previous cross-sectional study, we found that both ceramide and sphingomyelin species (C18 and C24) were elevated in CSF of HIV-positive subjects with cognitive impairment.[13] In a longitudinal study on a single cohort of subjects, we found that an inactive dementia was associated with elevated CSF sphingomyelin, while an active (progressing) dementia was associated with elevations in

Figure 3 A progressive disturbance in sphingolipid metabolism is associated with cognitive decline



Increasing ratios for 3 sphingomyelin/ceramide species over time were associated with declining cognitive status. Scatterplots show the continuous relationships of sphingomyelin and ceramide to cognitive decline. Data are expressed as a ratio of SM/ceramide (A, D, G), individual ceramide (B, E, H), and SM (C, F, I) species to the probability of cognitive decline for the indicated species. (A–C) C16:0 (odds ratio [OR] 5.07, 95% confidence interval [CI] 1.06–24.15, $p = 0.042$), (D–F) C18:0 (OR 11.86, 95% CI 1.39–100.90, $p = 0.024$), and (G–I) C24:0 (OR 7.98, 95% CI 1.18–54.21, $p = 0.034$).

multiple ceramide species and reductions in 25OH cholesterol.[14] These results combined with the current multicenter study suggest that elevations of CSF ceramide C18 are associated with HIV infection, and are not related to cART or cognitive status. Some of the earliest biochemical changes in CSF that predict cognitive impairment appear to involve reductions in brain efflux of sterols, with accumulations of very long-chain ceramides (notably C24). The onset of asymptomatic or mild neurocognitive impairment coincides with accumulations of sphingomyelins. Rapid declines in cognitive function are associated with conversions of sphingomyelin to ceramide. These data begin to define temporal perturbations in sphingolipid biochemistry that are similar to those seen in lysosomal storage disorders. Indeed, inherited disorders of sphingolipid metabolism are frequently associated with nervous system dysfunction (reviewed in reference 27). For example, the infantile type A variant of Niemann-Pick disease results in the accumulation of sphingomyelin within lysosomes. Other examples include glycolipids in Fabry disease and gangliosides in Tay-Sachs disease.[27] There are striking similarities between these genetic disorders and sphingolipid accumulations in HIV-infected subjects that suggest that the underlying mechanism of HAND includes early and specific disturbances in ceramide and sphingomyelin metabolism that become progressively more involved and directed

toward the overproduction of sphingomyelin. These metabolites were not associated with cognitive status in cross-sectional comparisons, suggesting that the progression of HAND involves temporal patterns of metabolic disturbance. This abnormal pattern of lipid metabolism in HIV-infected subjects is similar to metabolic disturbances associated with lysosomal storage diseases. Indeed, pathologic observations in white matter from subjects with HAND found evidence for expansions in the lysosomal apparatus that are consistent with our biochemical findings of perturbed sphingolipid balance.[28] Future studies in which direct comparisons are made with samples obtained from subjects with defined lysosomal storage disorders are required to confirm these findings.

These findings suggest that therapeutics targeting sphingolipid or sterol metabolism may preserve lysosomal function in HIV. For example, pretreatment of neurons with HMG-coenzyme A reductase inhibitors (the rate-limiting enzyme for cholesterol synthesis) has been shown to protect neurons from HIV by reducing the induction of stress-associated pathways including Hsp70, isoprostanes, and total nitrate levels.[29] A second intriguing possibility is that dietary manipulations could modify brain sphingolipid and sterol content. Age-associated accumulations of ceramides and long-chain glycosphingolipids can be reduced by caloric restriction, or by a diet rich in n-3 polyunsaturated fatty acids.[30,31] In contrast, diets high in fat, cholesterol, and sugar elevated galactosyl ceramide and sphingomyelin levels in hippocampus, and were associated with increased oxidative stress.[32] Thus, both dietary and pharmacologic interventions could potentially restore brain sphingolipid and sterol balance in HIV-infected individuals, and may preserve the function of endolysosomal systems.

AUTHOR CONTRIBUTIONS

Veera Venkata Ratnam Bandaru, PhD: developed mass spectrometry methods, extracted and conducted analyses of samples, contributed to writing the manuscript. Michelle M. Mielke, PhD: conducted statistical analysis, prepared figures and tables, and contributed to writing of the manuscript. Ned Sacktor, MD: provided samples and clinical data from the Johns Hopkins HIV cohort, contributed to the interpretation of data and editing of manuscript. Justin C. McArthur, MBBS: provided samples from the Johns Hopkins HIV cohort, contributed to interpretation of finings and clinical aspects of the study, contributed to editing of the manuscript. Igor Grant, MD: provided samples from the CHARTER cohort, contributed to interpretation of data and editing of the manuscript. Scott Letendre, MD: provided samples from the CHARTER cohort, contributed to interpretation of data and editing of the manuscript. Linda Chang, MD: provided samples from the Hawaii cohort, assisted with data interpretation and editing of the manuscript. Valerie Wojna, MD: provided samples from the Puerto Rico cohort, assisted with data interpretation and editing of the manuscript. Carlos Pardo, MD: provided samples from patients with MS and contributed to interpretation of the data. Peter Calabresi, MD: provided samples from patients with MS and contributed to data interpretation. Sody M. Munsaka, PhD: contributed to sample handling and data collection at the Hawaii site. Norman J. Haughey, PhD: oversaw all aspects of experimental design, analyses, data interpretation, and manuscript preparation.

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DISCLOSURE

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