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# Single nucleotide polymorphisms in *aquaporin-4* associate with cognitive impairment status in people with HIV

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## Abstract

Neurocognitive impairments are more frequent in people with HIV (PWH) compared to their uninfected counterparts. HIV-associated neurocognitive disorder (HAND) is a spectrum disorder and up to 50% of PWH are reported to suffer from HAND. Altered waste clearance from the brain, chronic neuroinflammation and impaired metabolic processes may contribute to abnormal aging in PWH and are more common among those who suffer from HAND. Thus, it is important to identify earlier predictors for development of HAND. A key contributor to cognitive impairment in HIV and in Alzheimer's disease (AD) is formation and accumulation of aberrant proteins including hyperphosphorylated Tau (pTau). Previous data from AD and traumatic brain injury studies report that impaired waste clearance from the brain contributes in part to cognitive impairments. Evidence suggests that the *aquaporin 4* (*aqp4*) gene may have an important role in waste clearance from the brain as single nucleotide polymorphisms (SNPs) in *aqp4* have been reported to associate with changes in cognitive decline in AD patients. Given some similarities between HAND and AD, we assessed potential associations of several *aqp4* SNPS with cognitive impairment in PWH. Our data show that homozygous carriers of the minor allele in SNPs rs3875089 and rs3763040 had significantly lower neuropsychological test Z-scores in multiple domains compared to the other genotypes. Interestingly, this decrease in Z-scores was only observed in PWH and not in HIV-control participants. Conversely, homozygosity of the minor allele of rs335929 associated with better executive function in PWH. Based on these data, tracking large cohorts of PWH to determine if the presence of these SNPs associate with cognitive changes during disease progression is of interest. Furthermore, screening PWH for SNPs that may be associated with cognitive impairment risk after diagnosis could be considered in alignment with traditional treatment plans to potentially work on skills in areas shown to have cognitive decline with these SNPs present.

### Keywords

HIV; Brain; Aquaporin 4; Cognitive impairment; Astrocytes

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## Introduction

With the introduction of combination antiretroviral therapy, people with HIV (PWH) now typically live normal life spans (Bandera et al. 2019). However, PWH report to have lower quality of life compared to uninfected individuals, and growing evidence suggests that comorbidities and age-related conditions including cognitive impairment (CI) are more frequent in PWH compared to their uninfected counterparts (Guaraldi et al. 2019; Guo and Buch 2019). The prevalence of PWH over 50 years of age is predicted to account for ~ 70% of the total HIV population by 2030 (Yang et al. 2019). HIV-associated neurocognitive disorder (HAND) is a spectrum disorder and approximately 50% of PWH are reported to suffer from HAND (Ances and Clifford 2008; Cross et al. 2013; Robinson-Papp et al. 2019). Diagnosing HAND clinically is difficult, as CI can fluctuate over time (Ciccarelli 2019; Métral et al. 2020). Accumulation of aberrant proteins, chronic neuroinflammation and impaired metabolic processes contribute to abnormal aging in PWH and are more common among those who suffer from HAND. Moreover, neuroinflammation is linked to impaired waste clearance from the brain (Cassol et al. 2014; Vandenbroucke 2016; Boerwinkle and Ances 2019). For example, accumulation of hyperphosphorylated Tau (pTau) normally associated with Alzheimer's disease (AD), is also observed in the brains of some younger PWH with CI.

Even though links exist among chronic HIV infection of the brain, sustained neuroinflammation, accumulation of aberrant proteins, CNS bioenergetic deficit and age-related brain changes, understanding cell-specific contributions to these factors is limited. Even though CNS bioenergetic deficits and metabolic dysfunction are reported in PWH, potential interactive mechanisms among brain cells and interventions to restore deficits are unknown (Cotto et al. 2019). It was recently reported by Velasquez et al. that circulating levels of ATP are significantly higher in PWH than in uninfected individuals. Also, these higher ATP levels correlate with the degree of CI in samples from participants enrolled in the National NeuroAIDS Tissue Consortium (NNTC) and CNS HIV Antiretroviral Therapy Effects Research (CHARTER) (Velasquez et al. 2020). Importantly, ATP levels in the blood may or may not reflect those in the brain, as astrocyte/neuron metabolic coupling in the brains of PWH is significantly altered (Cotto et al. 2018, 2019; Natarajaseenivasan et al. 2018, Mohseni et al. 2019). Astrocytes are not believed to be the source of significant viral production, but can release several early viral proteins and cytokines that promote chronic inflammation and damage surrounding cells (Ferrell and Giunta 2014). Although neurons are not infected with HIV, damage to neurons is likely due to the release of toxic viral proteins from productively infected cells or inflammatory factors released from infected cells or reactive glia (Ferrell and Giunta 2014). Astrocytes are the primary homeostatic cells of the brain and are the only cell type in the CNS that can undergo rapid volume changes (Thrane et al. 2014; Rasmussen et al. 2018), but little is known about how this can impact neurodegenerative processes. As extracellular volume changes, ion concentration alters neuronal excitability (Dalkara et al. 2011).

Disturbances in water homeostasis in the CNS are typically observed in diseases that have an neuroinflammatory component, such as HIV, AD, and traumatic brain injury (TBI). Cerebrospinal fluid (CSF) and interstitial fluid (ISF) are constantly exchanged in the brain

due to convective influx of CSF along the peri-arterial spaces located in the subarachnoid space (Iliff et al. 2012). Cellular components of the neurovascular unit detect the changes in CNS cells and signal for necessary responses such as vasodilation and/or vasoconstriction (Harder et al. 2002; Koehler et al. 2006). Water channels called aquaporin-4 (AQP4) are located on the perivascular end feet of astrocytes and facilitate the transfer of CSF into the parenchyma (Nakada 2014). Proper functioning of AQP4 is critical for the brain's vascular and waste clearance systems to function effectively. In the parenchyma, CSF influx forces convective ISF exchange within the peri-venous spaces that surround the deep veins within the brain. ISF then collects in the peri-venous space and is transported out of brain toward the cervical lymphatic system (Iliff et al. 2013a, b; Iliff et al. 2013a, b). The fluid flux carries waste products such as aberrant proteins or toxic solutes with it for clearance from the brain.

HIV is thought to negatively impact glymphatic clearance which is proposed to function most efficiently during sleep (St Hillaire et al. 2005; Benveniste et al. 2017). Neuroinflammation in age-associated neurodegenerative diseases such as AD is associated with altered synaptic connectivity and BBB function and neuronal injury. In addition to alterations in activity, post-translational modifications and localization, single nucleotide polymorphisms (SNPs) may contribute to changes in AQP4 protein function during neurodegeneration. Some *aqp4* SNPs are associated with more rapid or slower cognitive decline in some neurodegenerative diseases, but the mechanisms responsible are unknown. Even though these SNPs are common in the general population, the effects are not realized until CNS injury or disease are present. Previous studies demonstrate that *aqp4* SNP rs3763040 is associated with more rapid cognitive decline after AD diagnosis (Burfeind et al. 2017; Rainey-Smith et al. 2018a). Likewise, *aqp4* SNP rs335929 was associated with more rapid decline in the Clinical Dementia Rating score, but with slower decline in the Logical Memory and Digit Symbol test performance (Burfeind et al. 2017). Data also showed association of *aqp4* SNPs rs3875089 and rs9951307 with higher brain Aβ burden and disturbed sleep in AD (Burfeind et al. 2017; Rainey-Smith et al. 2018b). Given the neurocognitive and neuropathological similarities such as chronic inflammation, pTau accumulation and CI between AD and HIV (which increases AQP4 expression), (Orr et al. 2019) (Aoki-Yoshino et al. 2005; Chmelova et al. 2019), we believe that there may be HIV-specific contributions to altered AQP4 in PWH. Thus, we hypothesized that in HIV infection, *aqp4* SNPs influence HIV-mediated signaling alterations and may contribute to changes in CI.

## Methods

### Participant information

Participant DNA and neuropsychological testing information were obtained from the NNTC, an NIH-sponsored research resource that provides tissue and fluid biospecimens from PWH who are a part of a longitudinal cohort and organ donation study. All participants consented to the use of their samples and data for the purpose of HIV research. Refer to http://nntc.org/ for details regarding recruitment.

## Neuropsychological assessment

Neuropsychological battery included 14 tests: Hopkins Verbal Learning Test—Revised—Trials 1–3, Hopkins Verbal Learning Test—Revised—Trials 1–3, Hopkins Verbal Learning Test—Revised—Delayed Recall, Brief Visuospatial Memory Test—Revised—Delayed Recall, Trail Making Test B, WAIS III Letter Number Sequencing, Wisconsin Card Sorting Test, WAIS III Symbol Search, Grooved Pegboard Assessment – dominant hand, Grooved Pegboard Assessment – nondominant hand, Trail Making Test A, Category Fluency Test, and Controlled Oral Word Association Test. Table 1 lists neuropsychological tests and associated cognitive domains that were considered in this study.

We chose to focus on Z-scores for the analyses as it allows for the comparison between two scores that are not in the same normal distribution in their raw score form. A Z-score is the standard score resulting from a transformation of raw neuropsychology testing scores to facilitate interpretation. A Z-score has a mean of zero which indicates average performance, thus a positive Z-score means that the score is above the mean, while a negative Z-score means that the score is below the mean. For each cognitive domain, Z-scores for each of these individual tests are calculated by subtracting the test raw score from the demographically corrected mean score and then divided by standard deviation (Blackstone et al. 2012). All individual Z-scores for that cognitive domain are then averaged to give the Z-score for each domain. Table 2 shows the descriptive statistics for each neuropsychological test within its associated cognitive domain that were considered in this study. Total Z-score was calculated by averaging the Z-scores from the 14 neuropsychological tests.

Impairment was defined by confirmatory factor analysis using the neuropsychiatric Z-scores obtained according to conventional HAND categorization from the Frascati criteria (Blackstone et al. 2012). Z-scores were calculated for each domain. Individuals who had Z-scores ≤ – 1.5 in more than two cognitive domains were considered to have cognitive impairment.

## aqp4 SNP genotyping

The *aqp4* SNPs selected for genotyping were chosen because previous data reports their associations with cognitive status in AD. The frequency of these SNPs and reported associations are listed in Table 3 below. Endpoint genotyping was completed using TaqMan allele specific discrimination assays for SNPs AQP4: C 1303575__20 (rs3875089), C__29992536_20(r s9951307), C__27497828_20 (rs3763040), C__1303566_10 (rs335929), (ThermoFisher Scientific) shown in Table 4. Genotyping was performed using the Roche LightCycler 96 Endpoint Genotyping software program.

## Data analysis

Data analyses were performed using Graphpad Prism v.9. To test the association among genotype and Z-score, we performed one-way ANOVA followed by Tukey post hoc for multiple comparisons. Power analysis was performed using G*Power 3.1.9.4. Post hoc analysis determined that the study's power was 0.8592 and effect size of 0.25 with a total participant sample of 282.

## Results

### PWH have lower neuropsychological test scores compared to uninfected controls

Our study investigated potential associations of *aqp4* SNPs and CI in PWH. Our cohort consisted of 282 participants with 84 belonging to the HIV negative (HIV −) group, 107 in the HIV + no CI group, and 91 in the HIV + CI group (Fig. 1a). To note, HIV + CI is determined by being one or more standard deviations away from norm in two or more domains. Our cohort was predominately Caucasian (61.7%) and male (63.12%) which will not allow us to make any conclusions based on these demographics (Fig. 1a). We first compared the Z-scores for multiple cognitive domains and total Z-score between uninfected (HIV −) individuals and PWH (HIV +) regardless of cognitive impairment status. A Z-score above the mean (0) indicates a better than average score. Thus, a lower Z-score is indicative of worse cognitive function in that domain. Our study focused on five cognitive domains: learning, recall memory, executive function, psychomotor, and language. Total Z is determined by averaging together the Z-scores for the five domains considered. Specific tests used to determine Z-score for specific domains found in Table 1 PWH have significantly lower Z-scores ($p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****) in all domains except for language (Fig. 1b). These results were expected as our HIV + group included both PWH with and without CI, and further validates that our HIV − group can be used as a control for cognitive functioning within our group.

To compare Z-scores for all cognitive domains between our HIV − , HIV + No CI, and HIV + CI groups, we used one-way ANOVA analysis followed by Tukey post hoc for multiple comparisons between groups. We hypothesized that the greatest differences in Z-scores would be observed in the HIV + CI group compared to the HIV − and HIV + No CI groups. We also hypothesized that no differences between the HIV − and HIV + No CI groups would be observed. The HIV + CI group had the lowest Z-scores among groups in learning, recall memory, executive function, psychomotor and total Z-score. Interestingly, the HIV + CI had no significant differences in Z-scores in executive function, psychomotor, and language domains compared to the HIV + No CI group (Fig. 2). As anticipated, there were no significant differences between the Z-scores of the HIV − group and the HIV + No CI group in learning, recall memory, psychomotor, and language domains. However, there was a significant difference between the executive function Z-scores of the HIV − and HIV + No CI group. This finding may be due to our cohort HIV − group having a mean executive function Z-score higher than baseline, while our HIV + No CI group's executive function Z-score was near baseline level Fig. 2. A significant difference in the total Z-score of the HIV − group and the HIV + No CI group was also observed. Next, we assessed if *aqp4* SNPs associated with cognitive functioning in PWH as previously reported in people with AD (Burfeind et al. 2017; Rainey-Smith et al. 2018a).

### Homozygosity of the minor allele of rs3875089 is associated with lower Z-scores in PWH

When comparing genotypes for *aqp4* rs3875089 in all participants regardless of HIV status, our data showed that participants homozygous for the minor allele (C/C) had lower total Z-scores and in learning, recall memory, and executive functioning domains; whereas heterozygous (C/T) carriers had lower total Z-scores and in learning, recall memory, and

psychomotor functioning domains (Fig. 3, left column a, b and d). In the HIV + group, homozygous C/C carriers had lower Z-scores in recall memory and executive functioning than heterozygous carriers (Fig. 3, middle column b and d). Conversely, in the HIV − control group, homozygous carriers for C/C ($n = 3$) had significantly higher Z-scores in learning and in total Z-score (Fig. 3, right column c and f). Overall, our findings are in line with the findings reported by Rainey-Smith et al. that possession of the minor allele (C/C) is linked with worse cognition in PWH. Our data also show that PWH homozygous for the minor allele (C/C) have the lower Z-scores upon cognitive tests considered.

### Homozygosity of the minor allele of rs3763040 is associated with lower Z-scores in PWH

Our data indicate that homozygous carriers of the minor allele (A/A) for rs3763040 associated with lower Z-scores in learning, recall memory, executive function, and overall lower total Z-score among all individuals (HIV − and HIV +) within our cohort (Fig. 4a-c and f). After stratifying groups into HIV − and HIV + , data showed that PWH homozygous for the minor allele (A/A) for rs3763040 had the lowest Z scores for learning, recall memory, and executive function compared to A/G or G/G. No control HIV − participants carried the A/A allele. The minor allele frequency for rs3763040 SNP is 0.15 and A/A is the least commonly occurring. Since an ANOVA could not be conducted due to the small number of A/A participants, we performed a pooled *t*-test to assess for observable differences between heterozygous carriers (A/G) compared to homozygotes for the major allele (G/G) (Fig. 4 right column). Overall, our data also show that PWH in our study that are homozygous for the minor allele (A/A) have the worst cognitive measures.

### Homozygosity of the minor allele of rs335929 is associated with better executive function in PWH

Our data indicate that in all participants in our study (HIV − and HIV +) homozygous carriers of the minor allele (C/C) for rs335929 had higher Z-scores in executive function (Fig. 5c). After stratifying our groups into HIV − and HIV + , we observed that PWH homozygous for the minor allele (C/C) for rs335929 had significantly higher Z-scores for executive function and psychomotor domains compared to those homozygous for the major allele (A/A) for rs335929 (Fig. 5c). No significant differences were observed for other Z score categories or for Total Z-score. These data may suggest that within our cohort, PWH who are homozygous for the minor allele for rs335929 have better executive functioning compared to PWH with C/A or A/A genotypes for this *aqp4* SNP.

### Homozygosity of minor allele of rs9951307 is associated with higher Z-scores in HIV negative individuals

There have been conflicting reports on the role of *aqp4* SNP rs9951307 in cognitive functioning. Our data suggest that carriers of at least one copy of the minor allele (G) for rs9951307 associated with higher Z-scores in recall memory, psychomotor, and overall total Z-score among all participants (HIV − and HIV +) in our cohort (Fig. 6b, d and f). Since Burfeind et al. reported that possession of the minor allele led to decreases in cognitive decline after AD diagnosis, we were interested to investigate the association of this SNP comparing the HIV − and HIV + groups. No significant changes in Z-scores were observed in rs9951307 genotypes in PWH (Fig. 6). Within the control HIV − group, our data

show that those homozygous for the minor allele (G/G) had significantly higher Z-scores in executive function, psychomotor, language, and overall total Z-score (Fig. 6c-f). Our data suggest that overall, rs9951307 genotype does not impact cognition in PWH.

## Discussion

Growing evidence suggests that comorbidities and age-related conditions including functional or neurocognitive impairments are more frequent in PWH compared to their uninfected counterparts (Guaraldi et al. 2019; Guo and Buch 2019). This is a significant health concern because the prevalence of PWH over 50 years of age is increasing and is predicted to be ~ 70% of the total HIV population by 2030 (Yang et al. 2019).

AQP4 is important for cognitive health as it is one of the key regulators of the glymphatic system that promotes the exchange of ISF and CSF through the arterial perivascular spaces into the brain. AQP4 has also been reported to play a role in neuroinflammation, osmotic sensing, cell migration, and Ca2 + signaling (Tice et al. 2020). Changes in expression levels or mislocalization of AQP4 from astrocytic end feet to the soma can alter functions of AQP4. In the case of fluid dynamic changes and waste clearance, decreased ISF flow leads to buildup of extracellular waste products like pTau. pTau accumulation is a neuropathological hallmark in AD, TBI, and HIV (Rasmussen et al. 2018).

There have been conflicting reports on the role of *aqp4* SNP rs3875089 in cognitive functioning. In a 2017 study conducted by Burfeind et al., it was observed that possession of at least one copy of the minor allele was associated with a slower decrease in cognitive decline after AD diagnosis (Burfeind et al. 2017). In 2018, a study by Rainey-Smith et al. reported that carriage of at least one copy of the minor allele of rs3875089 was associated with higher brain Aβ burden as sleep latency increased. These results raised the question of *aqp4* SNP rs3875089's potential impact on cognitive function in PWH, given the abnormal aging trajectory and the increased accumulation of waste products in the brains of some PWH. Our data show that homozygous carriers of the minor allele in rs3875089 (C/C) had significantly lower Z-scores in multiple neuropsychological domains compared to the other genotypes. Interestingly, this drop in Z-scores is only observed in PWH and not in HIV − control participants. This finding is interesting as previous studies in AD report the association of this SNP in individuals after AD diagnosis (Burfeind et al. 2017; Rainey-Smith et al. 2018b).

A previous study reported that homozygous or heterozygous carriers of the minor allele at rs3763040 (A) was associated with significantly faster decline in cognitive scores after AD diagnosis (Burfeind et al. 2017). Our data indicate the homozygous carriers of the minor allele (A/A) for rs3763040 associated with lower Z-scores in learning, recall memory, executive function, and overall lower total Z-score among all individuals (HIV − and HIV +) within our cohort (Fig. 4a-c and f).

Another study reported that homozygous or heterozygous carriers of the minor allele (C) at rs335929 associated with more rapid decline in clinical dementia rating, but slower decline in executive function in patients diagnosed with AD (Burfeind et al. 2017). Our data indicate

that in all participants in our study (HIV − and HIV +), homozygous carriers of the minor allele (C/C) for rs335929 had higher Z-scores in executive function (Fig. 5).

There have been conflicting reports on the role of *aqp4* SNP rs9951307 in cognitive functioning. In a 2017 study conducted by Burfeind et al., it was observed that possession of at least one copy of the minor allele (G) was associated with a slower decrease in cognitive and executive function decline after AD diagnosis (Burfeind et al. 2017). However, a study by Rainey-Smith et al. revealed that at least one copy of the minor allele (G) of rs9951307 was associated with higher brain Aβ burden as sleep latency increased. This finding may be important in HIV given that AQP4 and the glymphatic system is proposed to function most efficiently during sleep (Benveniste et al. 2017, 2019). Our data suggest the carriers of at least one copy of the minor allele (G) for rs9951307 associated with higher Z-scores in recall memory, psychomotor, and overall total Z-score among all participants (HIV − and HIV +) in our cohort (Fig. 6).

As described above very few studies have addressed the potential impact of *aqp4* SNPs and cognitive impairment in disease. Our data in PWH showed associations among minor allele homozygosity and cognitive status, however, several limitations must be recognized. We cannot rule out the possibility that individuals carrying these alleles show undetected impairments prior to disease symptomology. Another limitation is that cognitive impairment is not caused by the presence of *aqp4* SNPs. Rather, numerous factors contribute to CI in PWH. In-depth investigations into downstream signaling, subcellular localization and activity of AQP4 are critical to understand how these SNPs may or may not contribute to changes CI in PWH or other neurodegenerative diseases. In fact, changes in brain ATP levels in PWH and in AQP4 protein expression in PWH are important starting points to explore potential contributions of *aqp4* SNPs in HAND. Our ongoing studies address some of these possible contributing factors. A future study tracking larger cohorts of PWH to determine if the presence of these SNPs associate with cognitive changes during chronic disease is necessary. Furthermore, screening PWH for SNPs that may be associated with cognitive impairment risk after diagnosis could be considered in alignment with traditional treatment plans to potentially work on skills in areas shown to have cognitive decline with SNP present.

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## Data availability

Data will be made available upon request.

## Abbreviations

| | |
|---|---|
| **HIV** | Human immunodeficiency virus |
| **PWH** | People with HIV |
| **CI** | Cognitive impairment |

| | |
|---|---|
| **HAND** | HIV-associated neurocognitive disorder |
| **AD** | Alzheimer’s disease |
| **pTau** | Hyperphosphorylated Tau |
| **NNTC** | National NeuroAIDS Tissue Consortium |
| **CHARTER** | CNS HIV Antiretroviral Therapy Effects Research |
| **CNS** | Central nervous system |
| **CSF** | Cerebral spinal fluid |
| **ISF** | Interstitial fluid |
| **AQP4** | Aquaporin-4 |
| **TBI** | Traumatic brain injury |
| **A2aR** | Adenosine 2a Receptor |
| **SNP** | Single nucleotide polymorphism |

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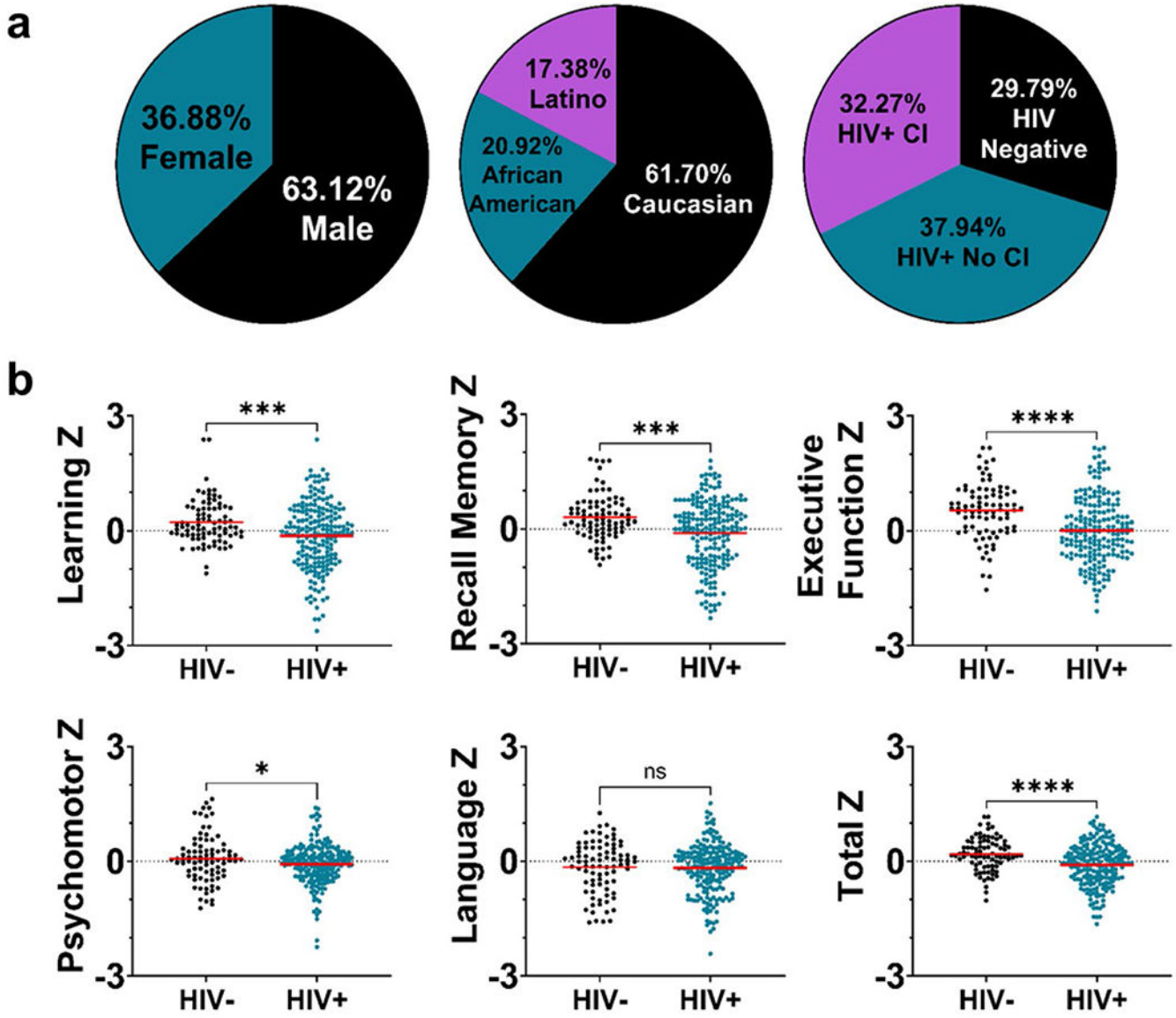


**Fig. 1.**

Demographics and Neuropsychological differences in PWH compared to uninfected individuals **a** Breakdown of 282 NNTC participants by sex, race, and HIV status. **b** Comparison of neuropsychological testing scores between HIV − and HIV + individuals. Red line indicates mean Z-score. Each individual dot represents one participant's Z-score in that domain. The Student *T*-test was used to compute statistical differences between the groups. $p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****

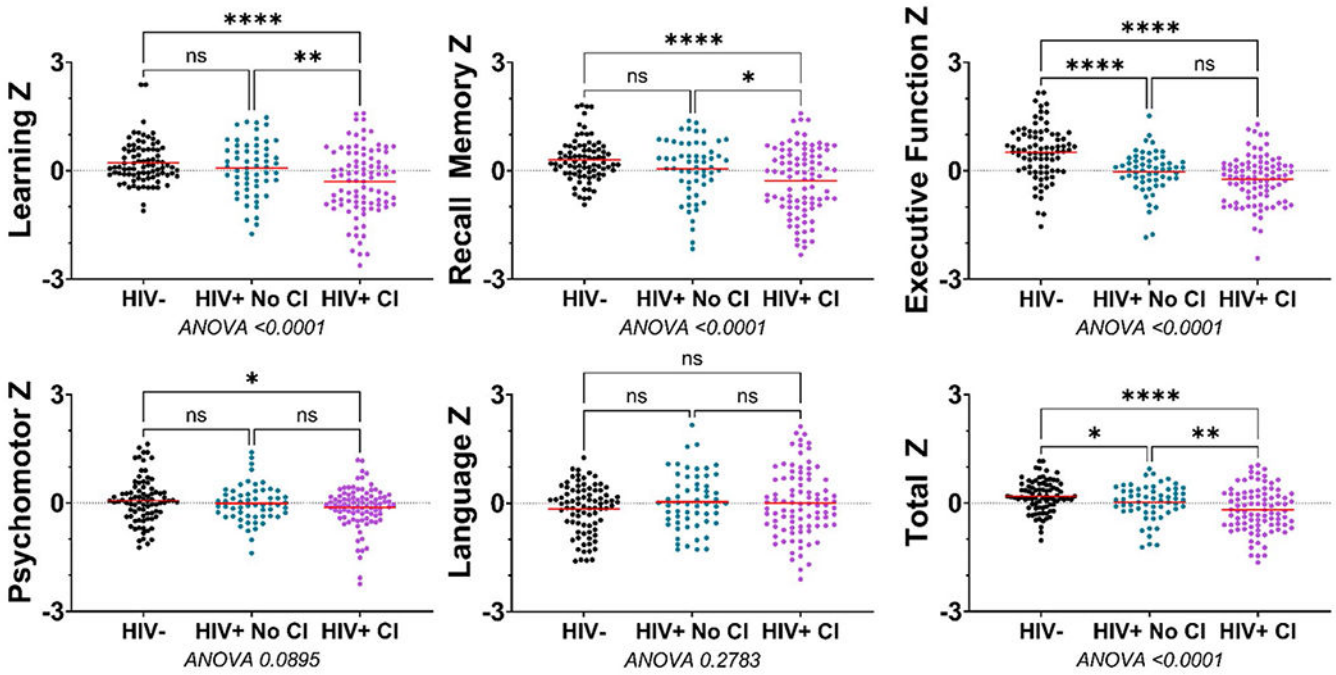


**Fig. 2.**

Z-score differences observed among HIV − , HIV + No CI, and HIV + CI groups

Comparison of neuropsychological testing scores among HIV − , HIV + No CI, and HIV + CI individuals. Red line indicates mean Z-score. Each individual dot represents one participant's Z-score in that domain. One-way ANOVA analysis followed by Turkey post hoc for multiple comparisons between groups. ANOVA *p*-value listed under each graph. CI, cognitive impairment. $p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****

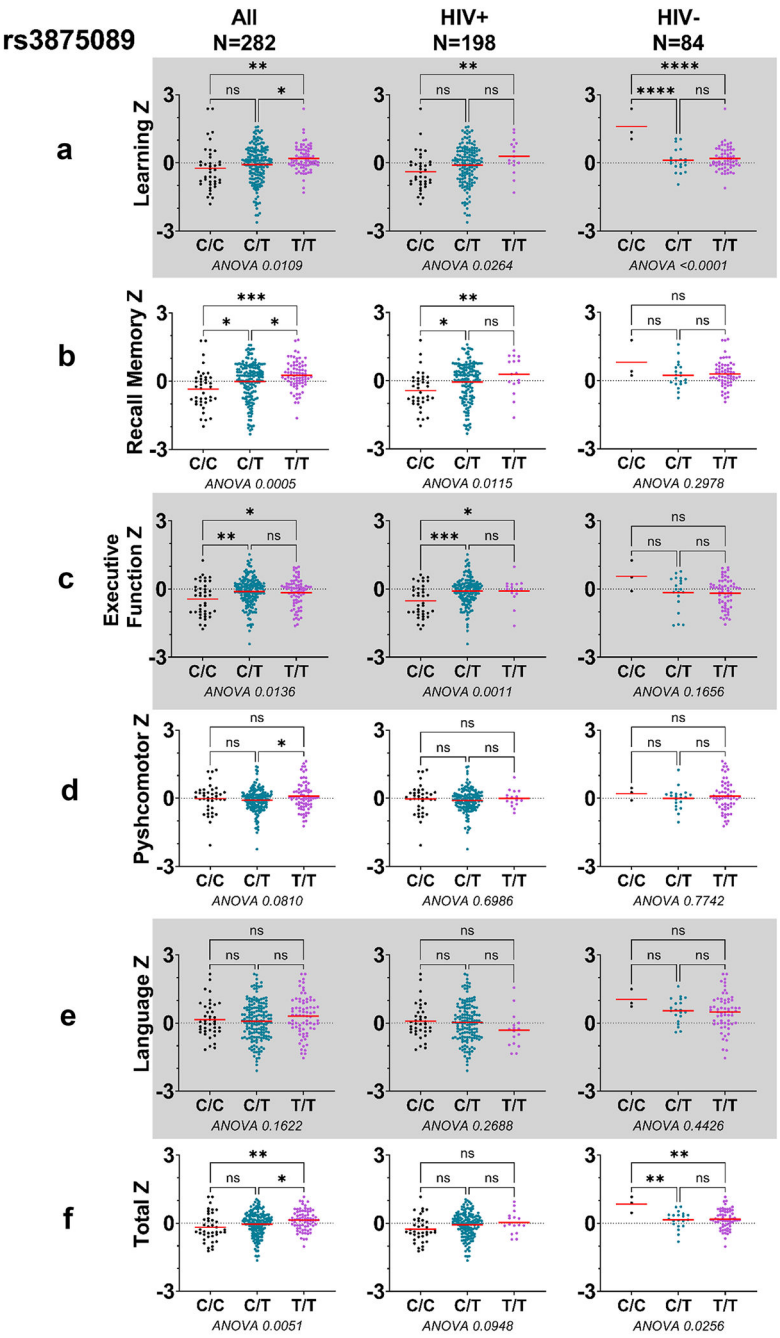


**Fig. 3.**

rs3875089 homozygosity for minor allele is associated with lower Z scores in PWH Comparison of neuropsychological testing Z-scores for rs3875089 (C/C, C/T, and T/T genotypes). Each panel corresponds Z-scores for one of the cognitive domains: **a** Learning Z, **b** Recall Memory Z, **c** Executive Function Z, **d** Psychomotor Z, **e** Language Z, **f** Total Z. Red line indicates the mean Z-score. Each individual dot represents one participant's Z-score in that domain. One-way ANOVA analysis followed by Tukey post hoc for multiple

comparisons between groups was utilized. ANOVA *p*-values are listed under each graph. $p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****

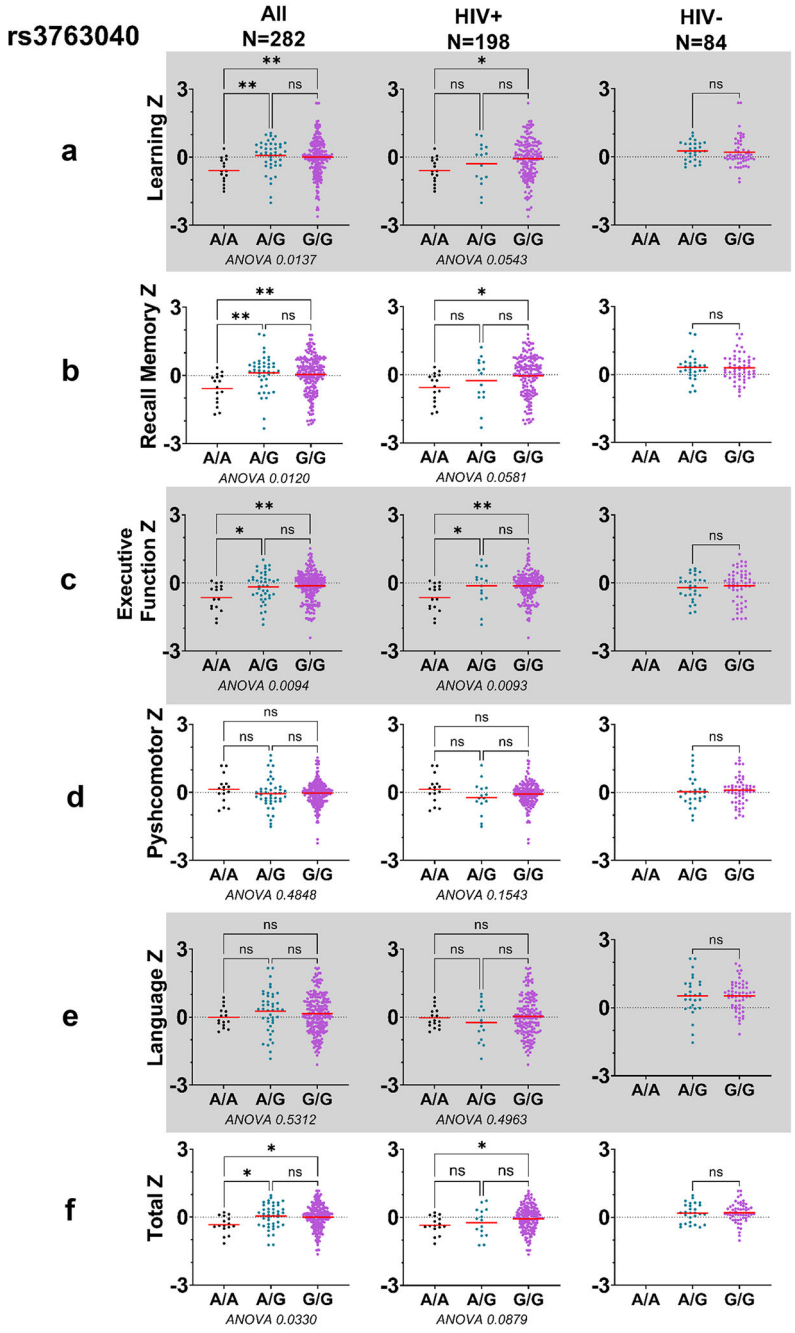


**Fig. 4.**

rs3763040 minor allele homozygosity is associated with lower Z-scores in PWH Comparison of neuropsychological testing Z-scores for rs3763040 (A/A, A/G, and G/G genotypes). Each panel corresponds to Z-scores for one of the cognitive domains: **a** Learning Z, **b** Recall Memory Z, **c** Executive Function Z, **d** Psychomotor Z, **e** Language Z, **f** Total Z. Red line indicates the mean Z-score. Each individual dot represents one participant's Z-score in that domain. One-way ANOVA analysis followed by Turkey post hoc for multiple comparisons between groups was utilized. ANOVA *p*-values are listed

under each graph. For the HIV-graphs, a pooled *t*-test was used to compare groups. $p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****

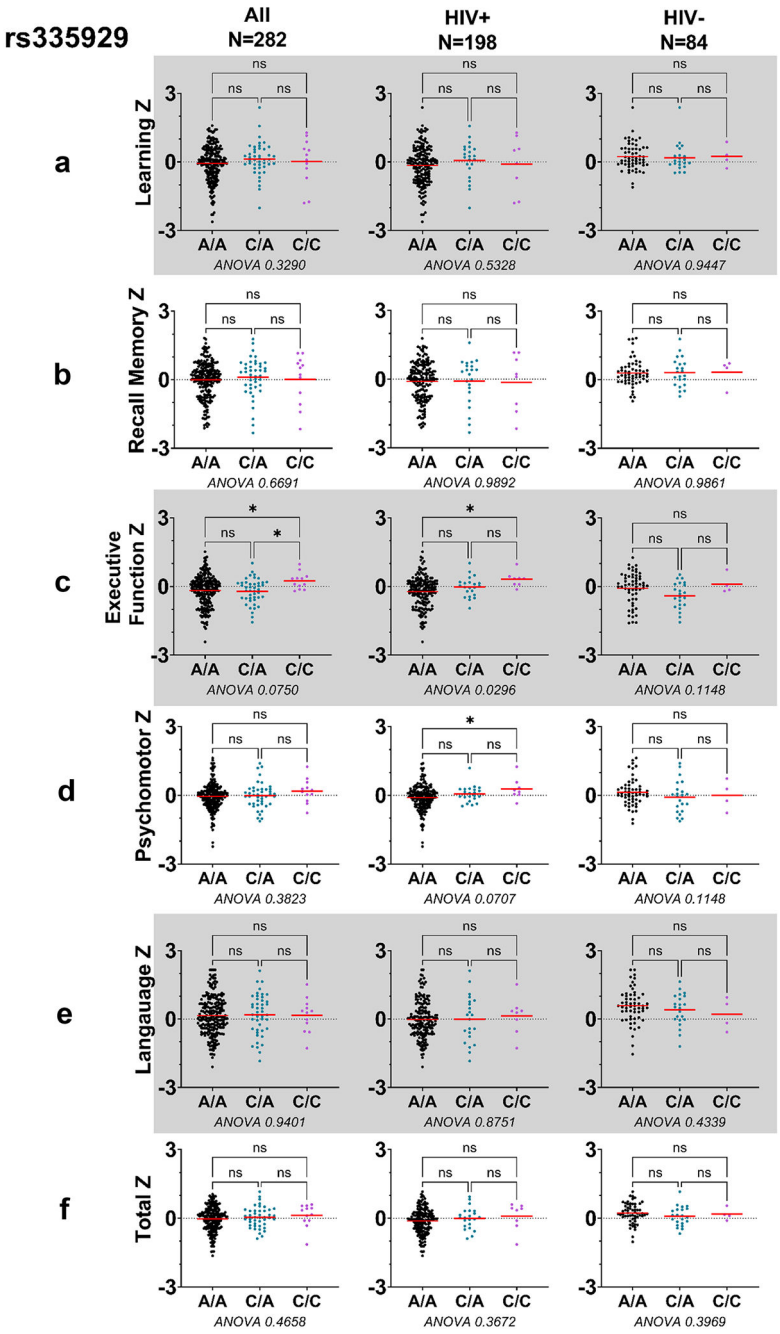


**Fig. 5.**

rs335929 homozygosity for the minor allele is associated with higher Z-scores in PWH Comparison of neuropsychological testing Z-scores for rs335929 (A/A, A/C, and C/C genotypes). Each panel corresponds Z-scores for one of the cognitive domains: **a** Learning Z, **b** Recall Memory Z, **c** Executive Function Z, **d** Psychomotor Z, **e** Language Z, **f** Total Z. Red line indicates the mean Z-score. Each individual dot represents one participant's Z-score in that domain. One-way ANOVA analysis followed by Tukey post hoc for multiple

comparisons between groups was utilized. ANOVA *p*-values are listed under each graph. $p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****

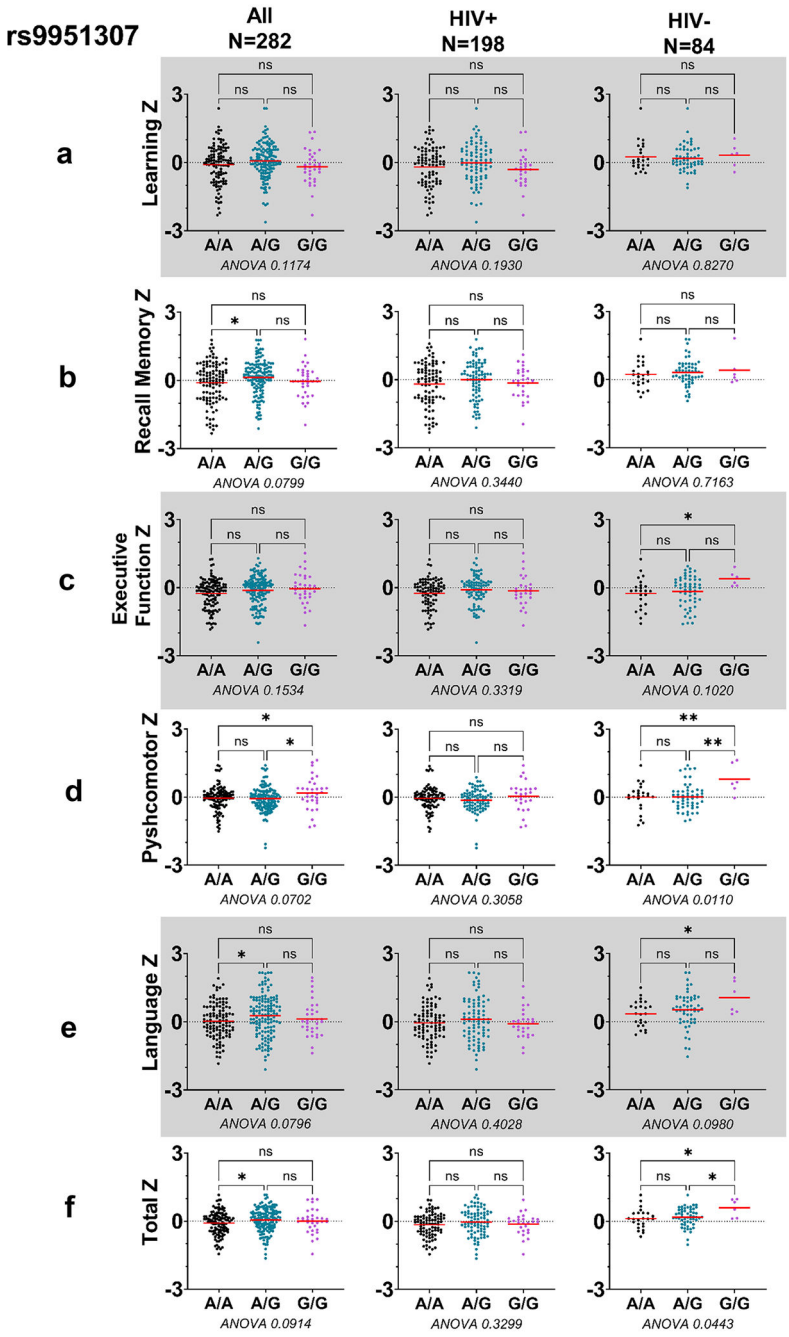


**Fig. 6.**

rs9951307 minor allele homozygosity is associated with higher Z-scores in HIV negative individuals Comparison of neuropsychological testing scores A/A, A/G, and G/G genotypes. Each panel corresponds Z-scores for one of the cognitive domains: **a** Learning Z, **b** Recall Memory Z, **c** Executive Function Z, **d** Psychomotor Z, **e** Language Z, **f** Total Z. Red line indicates mean Z-score. Each individual dot represents one participant's Z-score in that domain. One-way ANOVA analysis followed by Tukey post hoc for multiple comparisons

between groups. ANOVA $p$-value listed under each graph. $p < 0.05$* $p < 0.01$** $p < 0.001$***, and $p < 0.0001$****

**Table 1**

Domain-specific neuropsychological tests

| Cognitive Domain | Neuropsychological Test |
|---|---|
| **Learning** | Hopkins Verbal Learning Test - Revised - Trials 1-3 |
| | Brief Visuospatial Memory Test - Revised - Trials 1-3 |
| **Recall Memory** | Hopkins Verbal Learning Test - Revised - Delayed Recall |
| | Brief Visuospatial Memory Test - Revised - Delayed Recall |
| **Executive Function** | Trail Making Test B |
| | WAIS III Letter Number Sequencing |
| | Wisconsin Card Sorting Test |
| **Psychomotor** | WAIS III Symbol Search |
| | WAIS III Digit Symbol |
| | Grooved Pegboard Assessment - Dominant hand |
| | Grooved Pegboard Assessment - Nondominant hand |
| | Trail Making Test A |
| **Language** | Category Fluency Test |
| | Controlled Oral Word Association Test |

**Table 2**

Descriptive statistics of Z-Scores for neuropsychological testing performed by NNTC

| Cognitive Domain | Neuropsychological Test | Min | 25% Percentile | Median | 75% Percentile | Max | Mean | Std. Dev |
|---|---|---|---|---|---|---|---|---|
| **Learning** | Hopkins Visual Learning Z1 | −2.58 | −0.67 | −0.14 | 0.71 | 2.52 | 0.00270 | 1.005 |
| | Hopkins Visual Learning Z2 | −2.95 | −0.47 | 0.14 | 0.76 | 2.00 | 0.00028 | 1.000 |
| | Hopkins Visual Learning Z3 | −3.24 | −0.40 | 0.11 | 0.68 | 1.65 | 0.00450 | 0.993 |
| | Brief Visuospatial Memory Z1 | −2.16 | −0.77 | −0.21 | 0.61 | 2.92 | −0.02543 | 0.984 |
| | Brief Visuospatial Memory Z2 | −2.69 | −0.71 | −0.01 | 0.88 | 1.78 | −0.01514 | 0.988 |
| | Brief Visuospatial Memory Z3 | −3.50 | −0.59 | 0.33 | 0.79 | 1.35 | −0.00840 | 0.996 |
| **Recall Memory** | Hopkins Visual Learning Z | −2.97 | −0.55 | −0.03 | 0.64 | 2.04 | 0.00234 | 0.999 |
| | Brief Visuospatial Memory Z | −4.01 | −0.70 | 0.16 | 0.69 | 1.47 | −0.01443 | 0.985 |
| **Executive Function** | Trail Making B Z | −1.39 | −0.64 | −0.18 | 0.33 | 5.29 | 0.01869 | 1.014 |
| | Letter Number Seguencing Z | −2.30 | −0.66 | −0.08 | 0.56 | 3.42 | −0.00415 | 1.013 |
| | Wisconsin Card Sorting Z | −3.01 | −0.58 | 0.18 | 0.64 | 1.70 | −0.02418 | 1.003 |
| **Psychomotor** | Symbol Search Z | −2.18 | −0.75 | −0.03 | 0.69 | 2.38 | −0.01287 | 1.006 |
| | Digit Symbol Z | −2.70 | −0.66 | 0.02 | 0.77 | 2.55 | −0.00642 | 1.012 |
| | Pegboard (dominant hand) Z | −1.32 | −0.71 | −0.17 | 0.37 | 4.56 | 0.03188 | 1.009 |
| | Pegboard (nondominant hand) Z | −1.30 | −0.59 | −0.22 | 0.39 | 4.31 | 0.02826 | 1.011 |
| | Trail Making A Z | −1.71 | −0.75 | −0.14 | 0.47 | 3.27 | 0.02121 | 1.002 |
| **Language** | Correct Words (Animals) Z | −2.61 | −0.62 | −0.13 | 0.49 | 2.55 | −0.00394 | 1.001 |
| | Controlled Oral Word Z | −2.10 | −0.73 | −0.05 | 0.66 | 2.73 | 0.00174 | 0.992 |

**Table 3**

Minor allele frequencies of chosen AQP4 SNPs and effect in Alzheimer's disease

| SNP | Minor Allele | Minor Allele Frequency | SNP Reported Effect in Alzheimer's Disease |
|---|---|---|---|
| **rs3875089** | C | 0.15 | Minor allele carriers at rs3875089 have higher brain Aβ burden as sleep latency increased (Burfeind, Murchison et al. 2017, Rainey-Smith, Mazzucchelli et al. 2018). |
| **rs3763040** | A | 0.18 | Minor allele carriers of rs3763040 were associated with significantly faster decline in clinical dementia rating (Burfeind, Murchison et al. 2017, Rainey-Smith, Mazzucchelli et al. 2018) |
| **rs335929** | C | 0.18 | Possession of the minor allele at rs335929 was associated with more rapid decline in clinical dementia rating, but slower decline in Logical Memory and Digit Symbol tests performance (Burfeind, Murchison et al. 2017, Rainey-Smith, Mazzucchelli et al. 2018) |
| **rs9951307** | G | 0.34 | Carriage of homozygosity of the MAJOR allele rs9951307 was associated with higher brain Aβ burden as sleep latency increased (Burfeind, Murchison et al. 2017, Rainey-Smith, Mazzucchelli et al. 2018). |

**Table 4**

Context sequence for AQP4 TaqMan assays used to genotype participants

| SNP | Context Sequence |
|---|---|
| rs3875089 | [VIC/FAM] ATATGGAGGATTTGGCTAAAAAGCA**[C/T]**CCCTTTTCTTCTACCTTCTCTATGC |
| rs3763040 | [VIC/FAM] ATAAAGGAAGAGCTGGCTCCACAGG**[G/A]**GGGTGGCCAGCCACATCCCACGCAT |
| rs335929 | [VIC/FAM] GGAAGAAATACCATGAAATGAAGTT**[A/C]**GAATTGAAACTTGGGTATTTGTGAT |
| rs9951307 | [VIC/FAM] GCTTTCATAATTCTATAACTCTTCT**[A/G]**TGAAAAGCAGTCCTTGAAAAGATAG |