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Permalink

https://escholarship.org/uc/item/7n15w550

Journal

Alzheimer's & Dementia, 19(8)

ISSN

1552-5260

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Publication Date

2023-08-01

DOI

10.1002/alz.12989

Peer reviewed

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Author manuscript

Alzheimers Dement. Author manuscript; available in PMC 2023 September 28.

Published in final edited form as:

Alzheimers Dement. 2023 August ; 19(8): 3448–3457. doi:10.1002/alz.12989.

Sex-specific effects of *SNAP-25* genotype on verbal memory and Alzheimer's disease biomarkers in clinically normal older adults

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Abstract

Introduction: We tested sex-dependent associations of variation in the *SNAP-25* gene, which encodes a presynaptic protein involved in hippocampal plasticity and memory, on cognitive and Alzheimer's disease (AD) neuroimaging outcomes in clinically normal adults.

Methods: Participants were genotyped for SNAP-25 rs1051312 (T > C; SNAP-25 expression: C-allele > T/T). In a discovery cohort (N = 311), we tested the sex by *SNAP-25* variant interaction on cognition, Aβ-PET positivity, and temporal lobe volumes. Cognitive models were replicated in an independent cohort (N = 82).

Results: In the discovery cohort, C-allele carriers exhibited better verbal memory and language, lower Aβ-PET positivity rates, and larger temporal volumes than T/T homozygotes among females, but not males. Larger temporal volumes related to better verbal memory only in C-carrier females. The female-specific C-allele verbal memory advantage was evidenced in the replication cohort.

Conclusions: In females, genetic variation in SNAP-25 is associated with resistance to amyloid plaque formation and may support verbal memory through fortification of temporal lobe architecture.

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CONFLICT OF INTEREST STATEMENT

G.D.R. receives research support from Avid Radiopharmaceuticals, GE Healthcare, Genentech, and Life Molecular Imaging. He has received consulting fees from Alector, Eli Lilly, Merck, Genentech, GE Healthcare, and Roche. He is an Associate Editor for *JAMA Neurology*. The remaining authors have no declaration of interest. Author disclosures are available in the supporting information.

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Keywords

Alzheimer's disease; amyloid-beta; cognition; genetics; neuroimaging; neuropsychology; sex differences; SNAP-25; temporal lobe; verbal memory

1 | BACKGROUND

Biological sex and gender (herein referred to as "sex" [female, male]) contributes to cognitive aging across the lifespan and modulates the clinical expression of neurodegenerative diseases, including Alzheimer's disease (AD).[1,2] Converging lines of evidence consistently show that cognitively unimpaired females, on average, exhibit stronger verbal episodic memory abilities than males across the age-span.[3,4] Despite this verbal memory advantage, females are at increased risk for AD dementia and studies consistently report greater amyloid and tau burden in cognitively unimpaired females compared to males.[2,5,6] Furthermore, once they clinically convert, females diagnosed with mild cognitive impairment (MCI) or AD dementia exhibit faster disease progression compared to men, including tau deposition, gray matter atrophy, and cognitive decline.[7,8] Consistent with a model of cognitive resilience,[9] females may maintain a verbal memory advantage while withstanding mounting neuropathologic burden without clinical manifestation, yet once a pathologic threshold is reached, they subsequently experience a steeper and more pronounced cognitive decline than males.[10,11]

Preservation of synaptic integrity is a putative mechanism underlying cognitive resilience,[12] particularly in the presence of proteinopathies that can alter synaptic morphology and function (i.e., synaptopathies).[13] Recent in vivo studies demonstrate that greater synaptic integrity, reflected by lower cerebrospinal fluid (CSF) concentrations of synaptic proteins, attenuates the adverse effect of AD pathology and axonal damage on cognitive decline and brain atrophy.[14–17] Some studies have shown elevated CSF neurogranin in cognitively unimpaired females with a family history of AD or amyloid-beta (Aβ) positivity compared to males,[18,19] concordant with the notion that females are at enhanced risk for AD-related pathological change. However, it remains unclear whether synaptic factors contribute to sex differences in AD-related cognitive and neural outcomes.

Examination of polymorphisms in genes that encode synaptic proteins may facilitate understanding of person-specific factors that influence cognitive and brain aging that cannot be captured with CSF synaptic proteins, which reflect time-variant dynamics of synaptic aging and synaptopathies (e.g., AD). Genetic variation in synaptosomal-associated protein of 25 kDa (SNAP-25), a SNARE complex protein involved in presynaptic vesicle release, has been associated with neuropsychiatric and neurodevelopmental disorders (e.g., attention deficit-hyperactivity disorder [ADHD], schizophrenia), as well as general cognitive abilities in healthy young adults.[20] The relationship between *SNAP-25* single-nucleotide polymorphisms (SNPs) and cognitive aging is poorly characterized, even though numerous studies report abnormal CSF SNAP-25 in preclinical AD.[21,22] Furthermore, data from preclinical studies demonstrate sex hormone-dependent regulation of SNAP-25 and its effects on synaptic plasticity in neurocircuits that support memory (e.g., hippocampus),[23,24]

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leading to hypotheses regarding a role for SNAP-25 genetic variation in neuropsychological sex-differences.[25]

Taken together, SNAP-25 is a synaptic target of AD and may have genetically-driven associations with sex-specific cognitive and neural outcomes. We therefore performed genotyping for the rs1051312 SNP of the *SNAP-25* gene in cognitively unimpaired older adults with cognitive and neuroimaging data. Located in the 3′ untranslated region (UTR), rs1051312 SNP (T>C) regulates post-transcriptional *SNAP-25* expression via microRNA (miRNA) binding,[26,27] with the C-allele relating to greater SNAP-25 protein expression.[28] In a discovery cohort, we systematically examined the interactive effects of sex and *SNAP-25* (T/T homozygotes vs. C-allele carriers) on domain-specific cognitive outcomes and neuroimaging indicators (Aβ –PET positivity and cortical volumes). To test robustness of findings, cognitive analyses were replicated in an independent and demographically-comparable replication cohort with available *SNAP-25* rs1051312 data produced on a different genotyping platform.

2 | METHODS

2.1 | Participants

Our discovery cohort consisted of 311 cognitively unimpaired, community-dwelling, non-Hispanic white adults enrolled in the ongoing Brain Aging Network for Cognitive Health (BRANCH) at the University of California, San Francisco Memory and Aging Center (enrollment year range: 2000–2016). At screening, participants underwent neurological examination, neuropsychological testing, blood draw, and a study partner interview (clinical dementia rating = 0). Participants were classified as cognitively unimpaired per consensus case conference with board-certified neurologists and neuropsychologists. Each participant provided written informed consent to study procedures, which were approved by the UCSF Committee on Human Research. Inclusion in the current study was contingent on availability of DNA genotyped for the *SNAP-25* rs1051312 variant (see Section 2.2).

We also identified an internal replication cohort of 82 cognitively unimpaired participants with more recent entry into the BRANCH study (enrollment year range: 2017–2020). These individuals also had DNA genotyped for *SNAP-25* rs1051312 on a different analytic platform than the discovery cohort (see Section 2.2). These cognitively unimpaired participants underwent the same clinical screening procedures and neuropsychological testing as the discovery cohort, allowing us to test the reliability of the neuropsychological relationships identified in the discovery cohort.

2.2 | Genotyping

Genomic DNA was extracted from peripheral blood using standard protocols (Gentra PureGene Blood Kit, Qiagen). $APOE-\epsilon4$ genotyping (rs429358 and rs7412) was performed with the TaqMan Allelic Discrimination Assay and conducted on an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems) according to the manufacturer's instructions. In the discovery cohort, *SNAP-25* rs1051312 was genotyped using Sequenom iPLEX Technology (Sequenom, San Diego, CA). The SpectroAquire and MassARRAY Typer Software

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packages (Sequenom, San Diego, CA) were used for interpretation and Typer analyzer (v3.4.0.18) was used to review and analyze data. Only genotypes with "Conservative" or "Moderate" quality calls were included in analysis. For the replication cohort, *SNAP-25* rs1051312 was obtained from the Illumina HumanOmni 2.5 array genotyping platform (Illumina Inc., San Diego, CA), processed using manufacturer's instructions.

SNAP-25 rs1051312 genotype distribution across the discovery cohort (Sequenom) participants was 166 (53.4%) T/T, 126 (40.5%) C/T, and 19 (6.1%) C/C, consistent with Hardy-Weinberg equilibrium ($\chi^2 = 0.59$, $p = 0.44$). *SNAP-25* rs1051312 genotype distribution across the replication cohort (OMNI 2.5) participants was 42 (51.2%) T/T, 31 (37.8%) C/T, and 9 (11.0%) C/C, also consistent with Hardy-Weinberg equilibrium ($\chi^2 = 0.78$, $p = 0.38$).

2.3 | Cognitive assessment

Neuropsychological testing included measures of verbal and visual episodic memory, executive functioning, and language, as previously described.[29] All raw test scores were converted to sample-based z-scores. *Verbal episodic memory* was quantified via a composite of three primary metrics from the California Verbal Learning Test, second edition (CVLT-II): total immediate recall, total long (20 min) delay free recall, and recognition discriminability (d'). *Visual episodic memory* was quantified via delayed (10 min) free recall of a complex figure (modified Benson figure). *Executive functions* were quantified via a composite of digit span backwards, modified Trail Making Test, Stroop Inhibition, lexical fluency (number of D-words/60″), and design fluency (DKEFS Condition 1). *Language* was quantified via a composite of the animal fluency task (number of animals/60″) and the 15-item Boston Naming Test.

2.4 | Neuroimaging

A subset of participants ($N = 237$) underwent structural magnetic resonance imaging (MRI) at the UCSF Neuroscience Imaging Center using a Siemens Tim Trio scanner. Magnetization prepared rapid gradient-echo (MPRAGE) sequences were used to obtain whole brain T1-weighted images sagittally using the following parameters: repetition time (TR) = 2300 ms, inversion time (TI) = 900 ms, echo time (TE) = 2.98 ms, flip angle = 9°, field-of-view (FOV) = 240×256 mm with 1×1 mm in-plane resolution and 1 mm slice thickness. As previously described,[17] tissue segmentation was performed using unified segmentation in SPM12[30] and brain volumes of interest were quantified by translating a standard parcellation atlas[31] into International Consortium of Brain Mapping space and summing the gray matter within each region of interest (ROI). For analysis, we computed a previously-validated gray matter volume-based AD meta-ROI comprised of bilateral volumes from temporal lobe regions: hippocampus, entorhinal cortex, amygdala, middle temporal, inferior temporal, and temporal pole.[32] Total intracranial volume was statistically regressed out of the AD meta-ROI prior to analysis.

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2.5 | Amyloid PET imaging

Amyloid status was quantified via Aβ −PET imaging with either ^{18}F-florbetapir (injected dose: ≈ 10 mCi; $n = 90$) or ^{11}C-Pittsburgh compound B (PiB; injected dose: ≈ 15 mCi; $n = 5$). Standard uptake value ratios (SUVR) were calculated for the 50 to 70 min post-injection interval using mean activity in the whole cerebellum (^{18}F-florbetapir) or cerebellar gray matter (PiB) for as the reference region. Frames were co-registered to the corresponding MPRAGE images and global amyloid burden was estimated using a composite of frontal, cingulate, temporal, and parietal areas.[33] Aβ-PET positivity was determined based on processing pipeline- and tracer-specific thresholds: PiB SUVR > 1.21; ^{18}F-florbetapir SUVR > 1.11. A dichotomous Aβ −PET positivity classification was used for analysis given that some participants completed Aβ −PET imaging outside of a 1-year window before or after clinical data collection. Participants with an Aβ-positive status underwent PET imaging within a year or any time *before* the time of clinical data collection (PET ≤ 365 days from clinical data) and participants with an Aβ-negative status underwent PET imaging within a year or any time *after* the time of clinical data collection (PET ≥ −365 days from clinical data).

2.6 | Statistical analysis

All analyses were conducted using JMP Pro version 16.0.0. Sex and *SNAP-25* genotype (C-allele vs. T/T) differences on demographic and clinical characteristics were examined using analysis of variance (ANOVA) and chi-squared statistics with two-tailed tests, as appropriate.

2.6.1 | Discovery cohort analyses—To determine interactive effects of sex and *SNAP-25* on cognition, multivariable linear regression analyses separately modeled cognitive z-scores (verbal memory, visual memory, executive function, language) as a function of sex, *SNAP-25* variant, and their interaction, adjusting for age and education. Given prior evidence of $APOE-\varepsilon4$-dependent sex differences in cognition,[5,10] analyses also included $APOE-\varepsilon4$ genotype and its interaction with sex. Models with significant sex by *SNAP-25* interactions were probed with a priori planned comparisons examining sex differences stratified by *SNAP-25* variant, and *SNAP-25* differences stratified by sex. Multivariable regression analyses also examined the sex by *SNAP-25* interaction on A β −PET positivity and AD meta-ROI volumes, adjusting for age, $APOE-\varepsilon4$, and sex by $APOE-\varepsilon4$. Post-hoc analyses separately examined individual ROIs that composed the AD meta-ROI to determine the regional specificity of sex/*SNAP-25* associations with brain volumes. Based on the pattern of cognitive and biomarker differences identified in primary analyses, post-hoc models also probed relationships between imaging biomarkers and cognitive outcomes by sex/*SNAP-25* group.

2.6.2 | Replication cohort analyses—To determine the robustness of neuropsychological findings, statistically significant ($p < 0.05$) cognitive models from the discovery cohort were re-tested in the replication cohort. Cohen's d statistics are presented for estimates of effect size for pairwise comparisons. Models exhibiting comparable effect

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sizes across the discovery and replication cohorts were interpreted as more generalizable and robust.

3 | RESULTS

3.1 | Participant characteristics

Table 1 presents demographic and clinical characteristics by *SNAP-25* genotype in the discovery and replication cohorts. The discovery cohort was on average 70.2 years old (range: 44–100) with 17.3 years of education, 56% female, and 24%$APOE-\varepsilon4$. The replication cohort was on average 66 years old (range: 45–87) with 17.5 years of education, 52% female, and 25%$APOE-\varepsilon4$. *SNAP-25* groups did not significantly differ with respect to any demographic or clinical characteristics in either cohort. Females and males also exhibited comparable background characteristics within each *SNAP-25* group, with the exception of female C-carriers having slightly less education than male C-carriers in both cohorts (discovery: 17.1 vs. 17.9, $p = 0.023$; replication: 16.4 vs. 18.1, $p = 0.003$) and female T/T homozygotes having a lower prevalence of $APOE-\varepsilon4$ than male T/T homozygotes in the discovery cohort (19% vs. 32%, $p = 0.048$).

3.2 | SNAP-25 and sex differences in cognition

Multiple linear regression analyses in the discovery cohort (Table 2) indicated significant sex by *SNAP-25* interactions for verbal memory ($p = 0.024$) and language ($p = 0.008$), but not for visual memory ($p = 0.112$) or executive functioning ($p = 0.312$). In *SNAP-25*-stratified analyses, females exhibited higher verbal memory z-scores than males in both *SNAP-25* groups, yet this female verbal memory advantage was roughly 2.5 times stronger in C-carriers ($d = 0.89$[0.55, 1.24], $p < 0.001$) than in T/T ($d = 0.36$[0.05, 0.67], $p = 0.022$). Similarly, among C-carriers, females exhibited significantly higher language z-scores compared to males ($d = 0.38$[0.04, 0.73], $p = 0.028$); however, this effect was not evident among T/T homozygotes ($d = -0.22$[−0.09, 0.54], $p = 0.168$). In sex-stratified analyses (Figure 1), C-carriers exhibited significantly higher verbal memory and language z-scores than T/T homozygotes among females (ps < 0.05), but not in males (ps > 0.05). $APOE-\varepsilon4$ genotype did not significantly interact with sex for any cognitive outcomes (p range: 0.194–0.980).

3.3 | SNAP-25 and sex differences in $A\beta$−PET

$A\beta$−PET positivity rates across sex and *SNAP-25* groups (Figure 2) indicated the lowest rates of $A\beta$−PET positivity in female C-carriers ($n = 3/23$, 13.0%), followed by male T/T homozygotes ($n = 7/30$, 23.3%), male C-carriers ($n = 6/19$, 31.6%), and female T/T homozygotes ($n = 8/23$, 34.8%). A significant sex by *SNAP-25* interaction on $A\beta$−PET positivity (OR [95% confidence interval {CI}] = 9.28 [1.04, 82.75], $p = 0.046$) was confirmed in multiple logistic regression analysis. Specifically, female C-carriers were 5 times less likely to be Aβ-positive than female T/T homozygotes (OR [95% CI] = 0.20 [0.03, 1.05], $p = 0.077$), yet male C-carriers were 3.8 times more likely to be Aβ-positive than male T/T homozygotes (OR [95% CI] = 3.81 [0.80, 22.20], $p = 0.094$). $A\beta$−PET positivity

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was not associated with verbal memory or language z-scores (ps > 0.279), nor were these relationships moderated by sex or *SNAP-25* group (ps > 0.304).

3.4 | SNAP-25 and sex differences in AD meta-ROI volumes

The interaction between sex and *SNAP-25* on AD meta-ROI volumes did not reach statistical significance (β[95% CI] = 0.00 [−0.17, 0.16], p = 0.917). After removal of the interaction term, *SNAP-25* exhibited a main effect on AD meta-ROI volumes such that C-carriers had significantly larger cortical volumes than T/T homozygotes (C-carrier vs. T/T: $d = 0.35$[0.10, 0.60], p = 0.006); there was no main effect of sex (females vs. males: $d = -0.22$[−0.11, 0.55], p = 0.192). However, as shown in Figure 3A, the effect of *SNAP-25* on AD meta-ROI volume was driven by females (females: $d = 0.41$[0.07, 0.74], p = 0.018; males: $d = 0.26$[−0.12, 0.64] p = 0.179). Post-hoc regional analyses identified the inferior temporal gyrus ($d = 0.46$[0.12, 0.43], p = 0.008) and hippocampus ($d = 0.38$[0.04, 0.72], p = 0.028) as the two individual components of the AD meta-ROI most strongly associated with *SNAP-25* genotype in females, with the hippocampus showing the largest difference in *SNAP-25* effects between males and females (Figure 3B).

Further, we found that sex significantly moderated the relationship between AD meta-ROI volumes and verbal memory (β[95% CI] = 0.13 [0.00, 0.25], p = 0.045), such that larger AD meta-ROI volumes were associated with higher verbal memory in females (β[95% CI] = 0.25 [0.02, 0.48], p = 0.033), but not males (β[95% CI] = 0.00 [−0.20, 0.19], p = 0.923). *SNAP-25* did not statistically moderate the relationship between AD meta-ROI volumes and verbal memory (p = 0.671); however, when stratified by sex, the largest and only statistically significant relationship between cortical volumes and verbal memory was observed in female C-carriers (Figure 3C). AD meta-ROI volumes were not associated with language z-scores (ps > 0.05), regardless of sex or *SNAP-25* genotype.

3.5 | Replication cohort differences in cognition

The replication cohort exhibited a similar pattern of verbal memory differences that were observed in the discovery cohort (Figure 4), although the sex by *SNAP-25* interaction did not reach statistical significance (p = 0.182). In sex-stratified analyses, C-carriers exhibited significantly higher verbal memory than T/T in females (d = 0.64 [0.01, 1.26], p = 0.046), but not in males (d = 0.03 [−0.61, 0.67], p = 0.924). Neither the sex by *SNAP-25* interaction nor any pairwise comparisons reached statistical significance for language performance in the replication cohort (ps > 0.273).

4 | DISCUSSION

The present study examined sex-dependent associations of *SNAP-25* (rs1051312) with cognition and AD-related biomarkers in cognitively unimpaired adults. Carrying rs1051312 C-allele results in less post-transcriptional inhibition of SNAP-25 and therefore greater SNAP-25 expression. Among females in the discovery cohort, C-allele carriers exhibited stronger verbal memory and language abilities, larger AD-related cortical volumes, and lower A β −PET positivity rates than male or female T/T homozygotes. Moreover, larger AD-related cortical volumes were selectively associated with better verbal memory only

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among female C-allele carriers, who also exhibited superior verbal memory in the independent replication cohort. In contrast, *SNAP-25* rs1051312 did not significantly relate to cognition, brain volumes, or A β −PET among men. Importantly, findings were statistically robust to adjustment for $APOE-\varepsilon 4$, which was a weaker moderator of sex-related brain and cognitive differences than *SNAP-25*.

To date, the few published studies examining *SNAP-25* in the context of cognitive aging have reported some differences in *SNAP-25* variant distributions across the clinical AD continuum.[34,35] Only one study described *SNAP-25* variant associations with categorical fluency and temporo-parietal connectivity in AD patients.[35] Our study is among the first to systematically examine the role of genetic variation impacting SNAP-25 expression on more comprehensive cognitive and neural outcomes and test sex differences in clinically normal adults. As expected, we observed the widely-reported female verbal memory advantage across our discovery and replication cohorts, with amplification of this difference among C-allele carriers. Female C-allele carriers similarly exhibited stronger language abilities (based on semantic retrieval tasks) than female T/T homozygotes in the discovery cohort, although this finding was not reproduced in the replication cohort. Our data in older adults cannot directly address questions regarding the effect of *SNAP-25* variation across the lifespan. Nonetheless, prior studies have demonstrated cognitive effects of additional *SNAP-25* variants in children and young adults,[36] including female-specific differences in verbally-mediated cognition.[37] Given that the female verbal memory advantage is reported to persist across the lifespan in healthy individuals,[4] our cognitive results are consistent with longstanding differences in synaptic function due to gene by sex interactions on early synaptic development.

Although mechanisms underlying cognitive sex differences are likely multifactorial, our neuroimaging data suggest *SNAP-25*-associated differences in brain structure may play a role in the observed pattern of verbal memory differences. The rs1051312 C-allele related to higher AD-related cortical volumes, an effect driven by females. Furthermore, the relationship between AD-related cortical volumes and verbal memory was largest in female C-carriers. Human neuropathological data show that among older adults who were cognitively intact at death, females exhibit higher temporal lobe expression of SNAP-25 protein than males.[38] In animal models, SNAP-25 is densely expressed in the hippocampus, where it regulates presynaptic vesicle release and may also support synaptic maturation and long-term potentiation through postsynaptic actions.[39] Wild-type female mice exhibit higher basal levels than males of the SNAP-25a isoform, which supports hippocampal plasticity in early development.[40] On the other hand, SNAP-25-deficient mice show deficits in hippocampal synaptic plasticity that correlate with impairments in learning.[23] Notably, treatment of female mice with estradiol increases SNAP-25 mRNA expression and enhances hippocampal-dependent memory consolidation,[24,41] further highlighting the importance of the sex-dependent neurohormonal milieu on synaptic development and function. Although speculative, these results coupled with our data raise the possibility that genetically-driven increases in SNAP-25 expression confer *resistance* to verbal memory decline in females through fortification of temporal lobe architecture and function.

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Recent prevalence estimates of Aβ −PET abnormality in cognitively unimpaired adults at age 70 (mean age of our sample) range from 25% to 33% and do not differ by sex.[42,43] In our subsample that under-went Aβ −PET, female C-carriers exhibited a 13% positivity rate, which deviated from other study groups and prevalence estimates. These data raise the intriguing possibility that *SNAP-25* may also contribute to female-specific mechanisms of resistance to amyloid pathology,[44] given that only female C-carriers showed lower-than-expected levels of Aβ −PET positivity. AD-associated alterations in SNAP-25 protein and gene expression are observed post-mortem in brain tissue[45,46] and in vivo in CSF.[22] The synaptic hypothesis posits that soluble Aβ oligomers disrupt synaptic signaling in early AD, which initiates a reciprocal loop of aberrant synaptic firing and Aβ aggregation, leading to plaque and tangle formation with synaptic loss.[47] Thus, one possible explanation for our findings is that higher basal expression of SNAP-25 confers resistance to Aβ oligomer-induced synaptic dysfunction and protein aggregation in a female-specific fashion.

Our study is among the first to leverage *SNAP-25* genetics as a window into sex-specific pathways of cognitive and brain aging; however, we acknowledge several limitations. We conducted a single SNP analysis due to retrospective data availability. Therefore, we cannot definitively localize our results to rs1051312. The rs1051312 C-allele alone is sufficient to alter miRNA-641 binding to the 3′UTR, however, the largest alterations to miRNA-641 binding are observed when accounting for haplotypes of rs1051312 and rs3746544,[28] another 3′UTR SNP that is in linkage disequilibrium with rs1051312. *SNAP-25* is highly polymorphic and SNPs in other regions, including intronic regions with regulatory elements, have also shown associations with cognition and miRNA expression in AD.[26,35] Future work that combines multi-SNP data with cognitive and AD biomarkers would help clarify SNP-specific contributions of *SNAP-25* to sex differences in cognitive and brain aging.

Despite the putative relevance of sex hormones on SNAP-25 activity, our data did not include sex steroid biomarkers or information on menopausal status or hormone replacement therapy. Prospective studies that collect continuous markers of neurohormonal variability would more comprehensively identify the role of sex factors on our findings beyond a dichotomous classification of sex assigned at birth. Similarly, future studies with available tau biomarker data (e.g., tau PET) would help determine whether *SNAP-25* genotype is a stronger contributor to amyloid or tau-related sex differences. Genetic data mitigates some limitations associated with interpretation of directionality of effects in our cross-sectional analysis; however, future longitudinal designs would help tease apart whether the effects of *SNAP-25* truly reflect female-specific resistance to pathological aging orsimply reflect higher baseline function with similar susceptibility to aging. Last, our parent study (BRANCH) has historically enrolled a majority of non-Hispanic White participants and the present analyses lacked genetic ancestry data that would otherwise allow us to account for population substructure. Thus, we restricted analyses to participants who identified as non-Hispanic White (as a proxy for European ancestry) in order to limit potential confounding due to allelic frequency differences in rs1051312 due to population ancestry (www.ncbi.nlm.nih.gov/snp/rs1051312#frequency_tab). Replication of results in diverse cohorts is needed before our findings can be generalized beyond our relatively homogenous cohort.

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Taken together, our data highlight genetic variation in *SNAP-25* as a moderator of the consistently reported female verbal memory advantage in two non-overlapping samples solely comprised of cognitively unimpaired older adults. Moreover, these sexually dimorphic cognitive effects of *SNAP-25* may be related to temporal lobe structure, possibly reflecting the influence of female sex hormones on synaptic development in brain regions where SNAP-25 is densely expressed. Last, we extend the female-specific effects of *SNAP-25* to Aβ-susceptibility, which further implicates synaptic pathways as moderators of early AD pathology. Further investigation into sex-dependent effects of synaptic genes on cognitive and brain aging could inform precision-based medicine approaches that identify AD at early disease stages and tailor interventions based on the interplay between biological sex and synaptic biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This study was supported by NIH-NIA grants K23AG058752 (PI: K.B.C.), R01AG072475 (PI: K.B.C.), R01AG032289 (PI: J.H.K.), R01AG048234 (PI: J.H.K.), UCSF ADRC P30AG062422 (PI: B.L.M), R35AG072362 (PI: G.D.R.), and R01AG062588 (J.S.Y.). Our work was also supported by the Larry L. Hillblom Network Grant (2014-A-004-NET; PI: J.H.K.) and the Alzheimer's Association (AARG-20-683875, PI: K.B.C.).

Funding information

NIH-NIA, Grant/Award Numbers: K23AG058752, R01AG072475, R01AG032289, R01AG048234, P30AG062422, R35AG072362, R01AG062588; Larry L. Hillblom Network, Grant/Award Number: 2014-A-004-NET; Alzheimer's Association, Grant/Award Number: AARG-20-683875

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Highlights

- The *SNAP-25* rs1051312 (T > C) C-allele results in higher basal SNAP-25 expression.
- C-allele carriers had better verbal memory in clinically normal women, but not men.
- Female C-carriers had higher temporal lobe volumes, which predicted verbal memory.
- Female C-carriers also exhibited the lowest rates of amyloid-beta PET positivity.
- The *SNAP-25* gene may influence female-specific resistance to Alzheimer's disease (AD).

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RESEARCH IN CONTEXT

Systematic Review:

The authors used PubMed to identify previous studies examining the effects of sex and SNAP-25, a presynaptic protein, on cognition and Alzheimer's disease (AD) biomarkers. The impact of the *SNAP-25* genetic variant rs1051312 (protein expression: C-allele > T-allele) on sex differences in cognition and AD imaging biomarkers in cognitively unimpaired adults has not been previously studied.

Interpretation

The well-established female verbal memory advantage was magnified in *SNAP-25* C-allele carriers, an effect that was replicated in an independent cohort. Among women only, C-allele carriers exhibited higher temporal lobe volumes, which predicted better verbal memory, and lower amyloid-beta deposition than T/T homozygotes.

Future Directions

Genetically-driven increases in SNAP-25 may contribute to female-specific *resistance* to verbal memory decline through fortification of temporal lobe architecture and reduce susceptibility to amyloid-beta deposition. Future longitudinal designs would help elucidate whether *SNAP-25* truly reflects female-specific resistance to pathological aging or reflects longstanding baseline differences.

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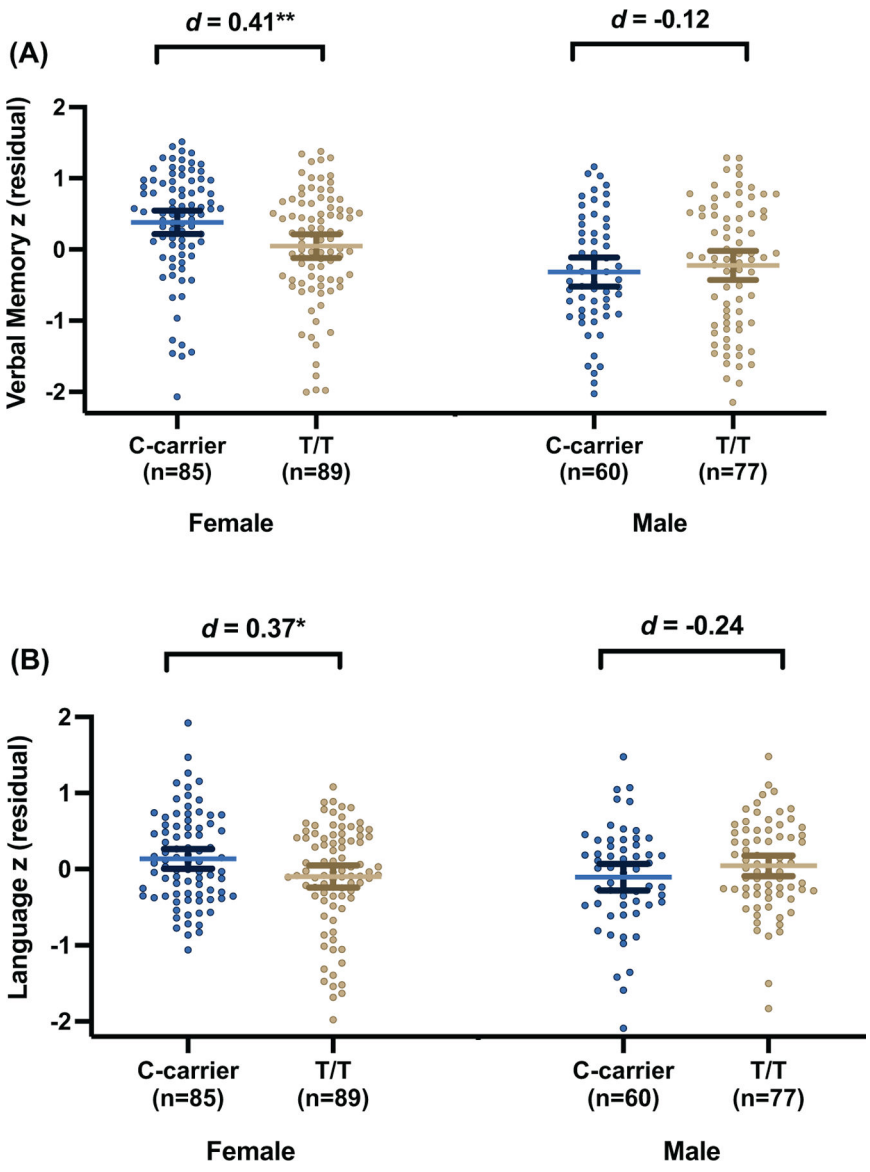

FIGURE 1.

Female-specific association of *SNAP-25* rs1051312 genotype with verbal memory (A) and language (B) in the discovery cohort. Note: For illustrative purposes, verbal memory and language z-scores were regressed against model covariates (age, education, $APOE-\varepsilon 4$) and plotted by *SNAP-25* genotype and sex. Error bars represent 95% confidence intervals. Cohen's *d* estimates reflect C-carrier versus T/T differences. $^{**}p < 0.01$; $^{*}p < 0.05$.

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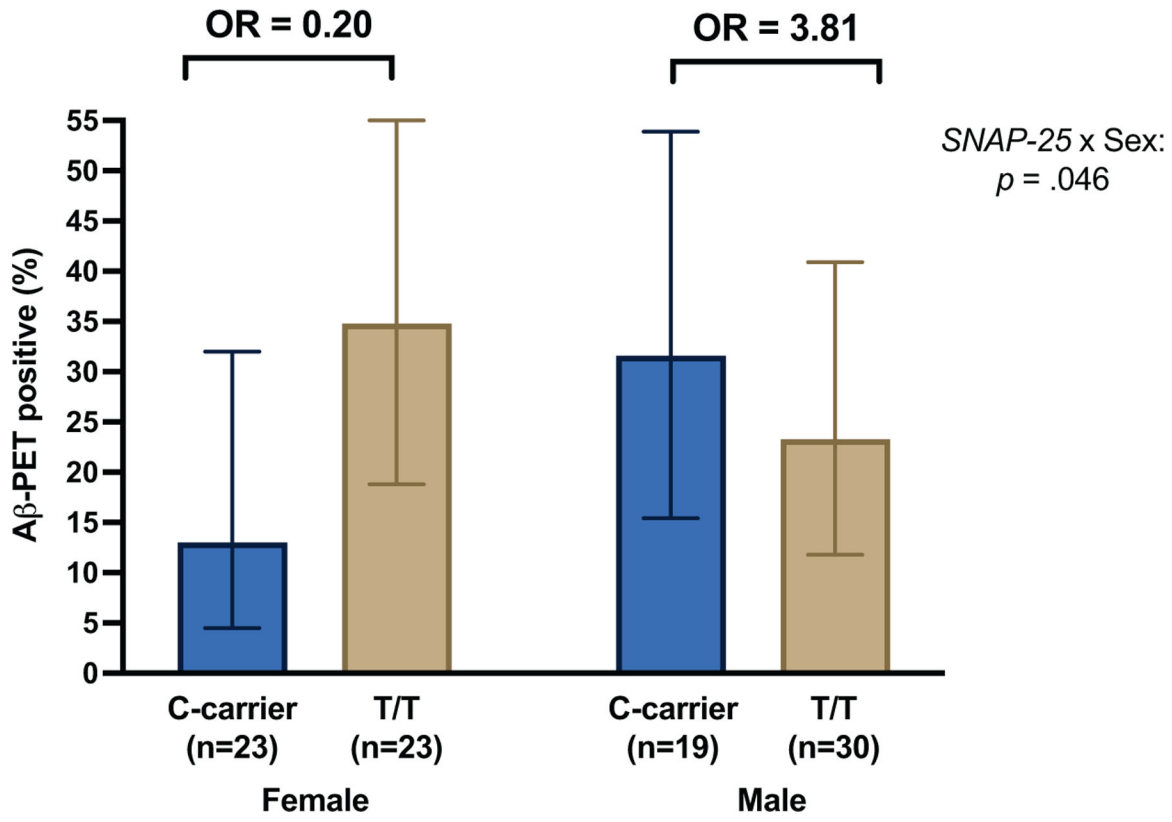

FIGURE 2.

Rates of A β –PET positivity were lowest in female *SNAP-25* rs1051312 C-carriers. Note: Error bars represent 95% confidence intervals. Odds ratios were derived from logistic regression adjusting for age and *APOE* – ε4. Reference group is C-carriers.

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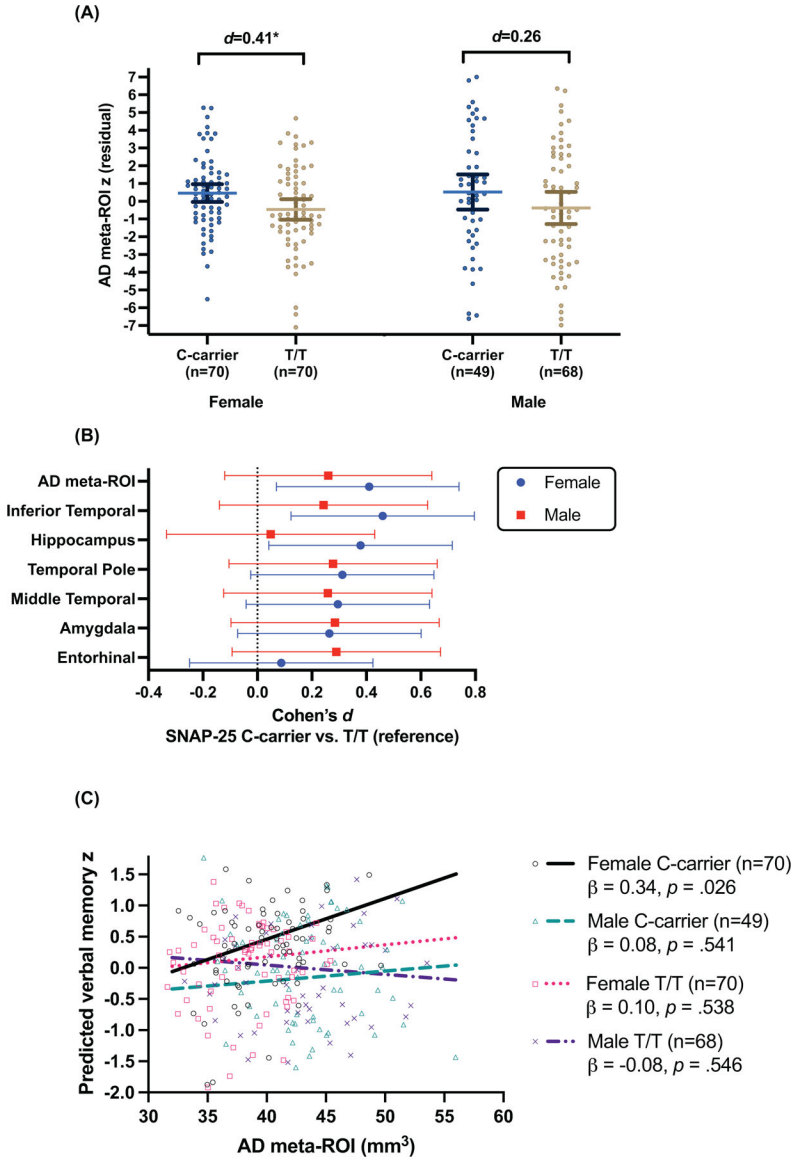

FIGURE 3.

AD meta-ROI volumes were larger and selectively associated with verbal memory performances in female *SNAP-25* rs1051312 C-carriers. Note: (A) For illustrative purposes, AD meta-ROI volumes were regressed against model covariates (age, education, $APOE-\varepsilon4$, intracranial volume) and standardized prior to plotting. Bars represent mean and 95% confidence intervals and Cohen's *d* estimates reflect C-carrier versus T/T differences. $^{*}p < 0.05$. (B) Sex-dependent associations between *SNAP-25* genotype and individual components of the AD meta-ROI. Lines represent Cohen's *d* estimates with 95% confidence intervals. (C) Fitted slopes represent the relationship between AD meta-ROI volumes and predicted verbal memory z-scores, adjusted for model covariates (age, education, $APOE-\varepsilon4$, intracranial volume), across sex and *SNAP-25* group. AD, Alzheimer's disease; ROI, region of interest.

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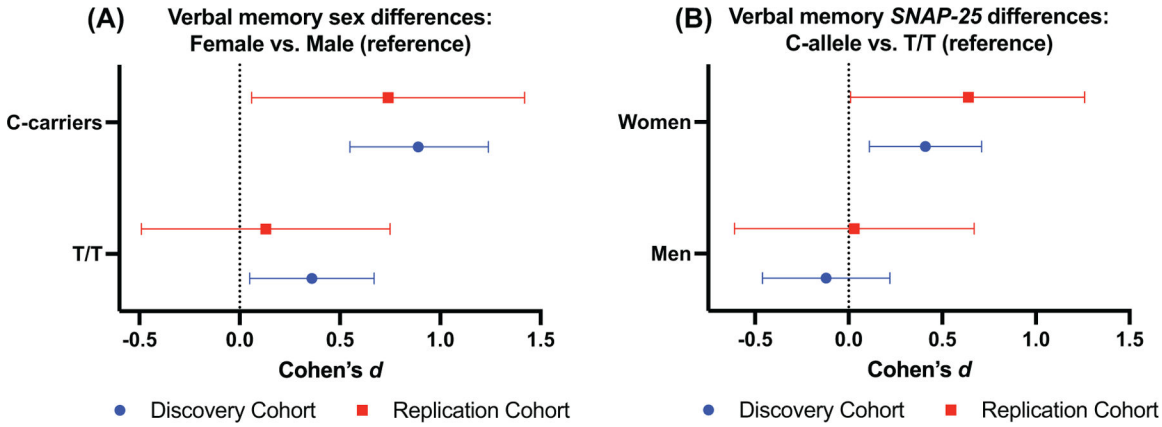

FIGURE 4.

Sex differences stratified by *SNAP-25* (A), and *SNAP-25* differences stratified by sex (B), in verbal memory were replicated across cohorts. Note: Point estimates represent Cohen's *d* effect sizes and error bars represent 95% confidence intervals. Error bars that do not cross Cohen's $d = 0.0$ (dotted line) were statistically significant.

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TABLE 1

Cohort characteristics by *SNAP-25* genotype.

	Discovery cohort			Replication cohort		
Variable	**T/T (*n* = 166)**	**C-carrier (*n* = 145)**	***p***	**T/T (*n* = 42)**	**C-carrier (*n* = 40)**	***p***
Age (years)	70.2 (7.4)	70.2 (8.8)	0.989	66.3 (7.1)	64.9 (9.3)	0.441
Sex (female)	89 (53.6%)	85 (58.6%)	0.375	20 (47.6%)	23 (57.5%)	0.370
Education (years)	17.3 (2.0)	17.4 (2.1)	0.614	17.7 (1.8)	17.1 (1.9)	0.178
MMSE	29.4 (0.8)	29.3 (1.2)	0.546	29.5 (0.7)	29.1 (0.8)	0.051
APOE ε4+	42 (25.3%)	32 (22.1%)	0.504	33 (22.0%)	44 (24.4%)	0.695

Note: Data presented as mean (SD) or *n* (%).

Abbreviations: APOE, apolipoprotein E; MMSE, Mini-Mental State Examination.

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TABLE 2

Interactive effects of sex and *SNAP-25* genotype on cognition.

	Verbal memory		Visual memory		Executive functioning		Language	
Predictor	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
Sex (ref: females)[a]	−0.36 (−0.67, −0.05)	0.023	0.30 (−0.04, 0.63)	0.086	0.24 (−0.08, 0.56)	0.141	0.30 (−0.03, 0.64)	0.073
APOE-ε4 (ref: ε4−)[b]	−0.03 (−0.37, 0.31)	0.868	−0.09 (−0.45, 0.28)	0.643	0.10 (−0.25, 0.44)	0.578	0.27 (−0.08, 0.63)	0.134
SNAP-25 (ref: T/T)[c]	0.37 (0.10, 0.64)	0.007	0.11 (−0.18, 0.41)	0.448	0.20 (−0.08, 0.48)	0.165	0.35 (0.06, 0.64)	0.018
Sex X *APOE-ε4*	0.11 (−0.37, 0.59)	0.642	−0.07 (−0.59, 0.46)	0.804	−0.01 (−0.5, 0.49)	0.980	−0.34 (−0.85, 0.17)	0.194
Sex X *SNAP-25*	−0.48 (−0.89, −0.06)	0.024	−0.36 (−0.81, 0.09)	0.112	−0.22 (−0.64, 0.21)	0.312	−0.60 (−1.04, −0.16)	0.008

[a] Lower-order term representing the effect of sex (male vs. female) in ε4− and T/T individuals.

[b] Lower-order term representing the effect of $APOE-\varepsilon4$ (ε4+ vs. ε4−) in T/T females.

[c] Lower-order term representing the effect of *SNAP-25* (C-carrier vs. T/T) in ε4− females.

Abbreviation: APOE, apolipoprotein E.