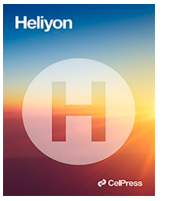
Heliyon 10 (2024) e23963

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Choline kinase alpha genotype is related to hippocampal brain volume and cognition in postmenopausal women

Abigail J. Myers [a], Callum Potts [b], Jenna A. Makarewicz [a], Elizabeth McGee [b], Julie A. Dumas [a,*]

[a] Department of Psychiatry, Larner College of Medicine, University of Vermont, USA
[b] Department of Obstetrics, Gynecology, and Reproductive Sciences, Larner College of Medicine, University of Vermont, USA

ARTICLE INFO

Keywords:
Choline
Choline kinase alpha
Delayed memory
Hippocampal volume
Menopause

ABSTRACT

This study examined how single nucleotide polymorphisms (SNPs) related to choline synthesis and metabolism, processes largely regulated by estrogen, influenced hippocampal volume and neuropsychological function following menopause. We investigated the effect of choline kinase alpha (CHKA) genotype on brain volume and neuropsychological performance in postmenopausal women. The effect alleles of certain CHKA SNPs (*rs6591331* T, *rs10791957* A) are associated with varied responses to choline deficiency and delegation of choline to physiological pathways. The presence of these alleles was hypothesized to correlate with worse cognitive performance in women after menopause. Results from structural MRI scans revealed larger right hippocampal volumes in subjects with a T/T CHKA *rs6591331* genotype compared to A/A subjects. Delayed memory scores from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) were lower in subjects with T/T genotypes compared to those with the A/T genotype and the A/A genotype. Based on these findings, we proposed a CHKA-dependent mechanism present within the brain to compensate for the decreased estrogen and biosynthesized choline associated with menopause.

1. Introduction

Decreased hormonal levels can contribute to menopause-related changes in neuropsychiatric function; however, not all women experience these symptoms with menopause, and there is a high degree of individual variability in the effects of decreased estrogen on cognition in postmenopausal women. Compared to premenopausal women, some studies reported decreased cognitive ability in women during and after menopause, while others found no relationship between cognitive impairment and menopause [1–3]. For individuals who do experience changes to cognition, the exact physiological pathways by which hormone decreases result in cognitive decline are not yet defined, although many studies support an effect of estrogen-related shifts in levels of biosynthesized acetylcholine on structural and cognitive processes in postmenopausal women [4–6].

Choline, an essential micronutrient necessary for overall human health, is a substrate in numerous biochemical reactions that occur in organs such as the liver, kidney, and brain [7]. The compound is crucial to pathways responsible for the biosynthesis of phosphatidylcholine (PC), a highly abundant metabolite that contributes to membrane health and vitality, as well as neuronal plasticity and

* Corresponding author. University of Vermont, 1 South Prospect St Burlington, VT, 05401, USA.
E-mail address: Julie.Dumas@uvm.edu (J.A. Dumas).

https://doi.org/10.1016/j.heliyon.2023.e23963
Received 9 October 2023; Accepted 19 December 2023
Available online 22 December 2023

differentiation [8–10]. PC is synthesized through two major physiological pathways. First, PC synthesis can occur through the phosphorylation of choline via the cytidine diphosphocholine (CDP) choline pathway. Second, choline can act as a methyl donor for triple methylation of phosphatidylethanolamine (PE) to form PC via the phosphatidylethanolamine methyltransferase (PEMT) pathway, which is strongly modulated by estrogen binding [11]. Adequate choline intake is linked to improved neurocognitive performance, and supplementation of the nutrient promotes cell proliferation, enhanced memory and information processing speed, and improved performance on serial learning and recall tasks [12–17].

Estrogen is also a regulator of neuropsychological function, largely via interactions with neurotransmitter systems. In healthy cholinergic neurotransmission, estrogen mediates acetylcholine signaling through effects on cholinergic enzymes and necessary precursors [5,6]. Acetylcholine is synthesized from acetyl coenzyme A (acetyl-CoA) and choline, and it acts in neurons of both the central and peripheral nervous systems to modulate autonomic response, stress response, attention, and memory [18]. Interestingly, the only pathway for endogenous synthesis of choline is catalyzed by an estrogen-mediated enzyme, meaning that acetylcholine availability and resulting neurotransmission are partially dependent on estrogen levels [19]. An examination of specific brain regions responsible for learning and memory further explains the role of estrogen in cognitive functioning. For example, postmenopausal hormone therapy studies showed that estrogen signaling influenced hippocampal functioning, a region highly associated with cholinergic signaling [20]. Estrogen treatments in women led to better performance on hippocampus-mediated tasks, namely verbal learning and memory tasks, as compared to women not receiving estrogen treatment [21].

Similar to the large individual variability in neurocognitive response to decreased estrogen following menopause, there is a large amount of individual variability in exact dietary requirements and physiological responses to choline deficiency, perhaps due to genetics, sex, menopausal status, or a combination of these factors [22]. Several genetic polymorphisms are linked to variations in the biological response to decreased endogenous estrogen production, including certain single nucleotide polymorphisms (SNPs) of genes that encode for enzymes also involved choline metabolism. Because estrogen directly modulates PEMT activity, lower levels of estrogen lead to decreased enzymatic activity in the conversion of PE to PC, ultimately resulting in smaller amounts of endogenously produced choline [23]. In a postmenopausal low estrogen state, levels of PEMT derived PC and choline are effectively diminished. Fischer and colleagues suggested that this difference in endogenous choline production may also explain why men and postmenopausal women have higher dietary choline requirements and are more likely to develop organ dysfunction under a low-choline diet [19].

The estrogen-related genetic variations that alter choline metabolism may also affect the development and mediation of menopausal symptoms. Postmenopausal women with decreased estrogen levels experience decreased activity of the PEMT enzyme, leading to reduced PE methylation and PC synthesis [24]. Thus, these individuals rely more heavily on the CDP-choline pathway for the synthesis of PC. The first enzyme in this pathway, choline kinase α (CHKA), catalyzes the phosphorylation of choline to form phosphocholine [25]. Under physiologically healthy conditions, CHKA provides phosphocholine for PC biosynthesis, ultimately promoting membrane vitality; thus, appropriate CHKA activity is needed to maintain a healthy neural enviornment. Novel findings showed that bi-allelic variants that reduce CHKA activity and significantly impair the first step of the CDP-choline pathway led to neurodevelopmental disorder with epilepsy and microcephaly [26]. Conversely, significantly increased CHKA activity contributes to several disease states, and CHKA is overexpressed in a wide variety of human tumor types [27].

Enzymatic activity and normal physiological function of CHKA may, in part, be modulated by effect alleles that vary by CHKA SNP. These polymorphisms also likely contribute to individual differences in dietary choline requirements for women in various life stages. For example, one study found that carriers of the A allele of CHKA *rs10791957* directed more dietary choline to the CDP-choline pathway compared to the PEMT pathway, an effect that was observed in lactating, pregnant, and non-pregnant young women [28]. Furthermore, da Costa and colleagues examined genetic polymorphisms involved in the alteration of dietary choline requirements and reported that 18 to 70-year-old female carriers of the protective allele for CHKA *rs7928739*, *rs10791957*, and *rs2512612* were at decreased risk for organ dysfunction when fed a low-choline diet [29]. This same study reported that postmenopausal women with a T allele of CHKA *rs6591331* were more susceptible to organ dysfunction when held to a low-choline diet, an effect observed in muscle, the liver, or both [29].

Postmenopausal women endogenously synthesize less choline due to decreased estrogen levels, and women with a CHKA effect alleles experience variations in choline metabolism and response to deficiency. This study aims to examine other pathological markers associated with choline deficiency that may be present in postmenopausal women with one or more CHKA effect alleles. We focused on the effect allele (T) of CHKA *rs6591331* and (A) of CHKA *rs10791957* and examined differences in brain structure and function in postmenopausal women with zero, one, or two effect alleles. We hypothesized an effect allele, dose-dependent decrease in the brain volumes of postmenopausal women due to reduced PC availability for support of membrane health and neuronal plasticity, as well as decreased memory performance resulting from a decline in acetylcholine production and/or excitability of cholinergic neurons in regions of the brain responsible for learning and memory.

2. Material and methods

2.1. Participants

All data were from a cohort of subjects originally recruited for a study that examined the effect of menopause on the dopaminergic system and cognition [30]. In this study, 118 women between 50 and 60 years of age, $M(SD) = 56.18(2.6)$, were recruited through advertisements distributed in the Burlington, VT area. Initial telephone screens were used to identify postmenopausal subjects that had been without menses for at least one year, had not undergone surgically induced menopause, and were not at risk for MRI

complications. Additionally, participants that were on medication affecting the central nervous system and those that had been on postmenopausal hormone treatment in the past year were excluded from this study. Exclusion criteria relating to physical health included a history of breast cancer or cardiovascular disease.

Subjects participated in one 3-h study day at the University of Vermont (UVM) Clinical Research Center (CRC) and the UVM MRI Center for Biomedical Imaging. This study was reviewed and approved by the University of Vermont Institutional Review Board, the Committees on Research in the Medical Sciences. Following the consent process, participants supplied a blood sample for SNP analyses and confirmation of postmenopausal status, as indicated by FSH >20 IU/L. Blood samples for the FSH assay were collected into a 4 ml SST tube by CRC nurses, then sent to the UVM Medical Center laboratory for immediate analysis. Two participants had FSH values that indicated they were not menopausal.

Participants were cognitively evaluated using the Mini Mental State Exam (MMSE [31]; and the Brief Cognitive Rating Scale [32], to establish a Global Deterioration Scale score (GDS) which rated the degree of cognitive impairment [32]. Participants were required to have an MMSE score greater than or equal to 27 and a GDS score of 1 or 2. All participants met these criteria.

General neuropsychological functioning was assessed through the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; [33], which measured immediate memory, visuospatial and constructional ability, language, attention, and delayed memory. Participants received a raw score for each index, which was then scaled to a mean (SD) of 100 (15) for age and education-correction purposes. Episodic memory was also assessed with the Buschke Selective Reminding Test [34]. A full list of cognitive and behavioral assessments not examined in the current study is detailed by Ref. [30].

2.2. Genetic analysis

Samples for genotyping were collected in 10 mL EDTA tubes by CRC nurses, then centrifuged at 3000 rpm for 20 min at 4 °C. A transfer pipette was used to separate the plasma from the buffy coat above the red blood cells, and the buffy coat was collected in a 1.8 ml screw cap vial. Samples were stored at −80 °C until batch analysis was conducted following the conclusion of the study. Genetic analysis of CHKA *rs6591331* and *rs10791957* was performed by the Genome Technologies Core Facility at the University of Vermont Cancer Center.

2.3. MRI procedure

Subjects underwent structural and functional scans on a Philips 3T Achieva d-Stream scanner in the UVM MRI Center for Biomedical Imaging. Relevant to this study are measurements of total and segmented brain volumes, which were acquired from each participant through a sagittal T1-weighted spoiled gradient volumetric MR sequence. The imaging protocol included an orientation perpendicular to the anterior commissure (AC)-posterior commissure (PC) plane, a repetition time (TR) of 9.9 ms, an echo time (TE) of 4.6 ms, a flip angle of 8°, number signal averages (NSA) of 1, a field of view (FOV) of 256 mm, a 256 × 256 matrix, and 160 contiguous slices measuring 1 mm in thickness. A board-certified neuroradiologist reviewed all scans to identify intracranial pathologies, namely age-based abnormal increases in white matter hyperintensities. Incidental findings were detected in eight women. These women were notified of the findings, and their data were removed and were not replaced.

2.4. MRI T1-Weighted scan analysis

The Freesurfer image analysis suite version 7.1.1, which is documented and available for download online (http://surfer.nmr.mgh.harvard.edu/), was used for cortical reconstruction and volumetric segmentation. Processing via the Freesurfer pipeline incorporated multiple corrections, including motion correction and averaging of multiple volumetric T1 weighted images [35], removal of non-brain tissue using a hybrid watershed/surface deformation procedure [36], and segmentation of the subcortical white matter and deep grey matter volumetric structures, including the hippocampus, amygdala, caudate, putamen, and ventricles [37,38]. Structure localization was determined based on Freesurfer's aseg atlas, and volume measurements for structures of interest were obtained using the aseg.stats command [37]. Raw measurements for brain volumes were normalized to account for variations in head size using the previously outlined linear regression-based residual approach [39].

2.5. Statistical analysis

Statistical comparisons were conducted using IBM SPSS Statistics [40]. Subjects were divided into three groups for analysis: those with two effect alleles (*rs*6591331 T/T, rs10791957 A/A), those with one effect allele (*rs*6591331 A/T, *rs*10791957C/A), and those without the effect allele (*rs*6591331 A/A, *rs*10791957C/C). One-way ANOVAs were used to identify the effect of genetic variation on brain volume or cognition, and post-hoc student's t-tests were used to describe specific differences between the three genotype groups. Results were considered significant at $\alpha \leq 0.05$.

3. Results

3.1. CHKA rs6591331 and rs10791957 genetic analysis

Of the original 118 women recruited for the parent study, samples were available from a total of 104 women and were analyzed in

the present study. For the CHKA *rs6591331* genetic analyses, 37 subjects were homozygous T/T, 53 were heterozygous A/T, and 14 were homozygous A/A. Our sample distribution met the Hardy-Weinberg equilibrium ($\chi^2 = 0.53$, df = 1, n.s.). CHKA *rs10791957* genetic analysis revealed 32 subjects were homozygous A/A, 53 were heterozygous A/T, and 19 were homozygous A/A. This sample distribution also met the Hardy-Weinberg equilibrium ($\chi^2 = 0.13$, df = 1, n.s.). Demographic data for these subjects are outlined in Table 1.

Based on previous findings that suggest an effect of the T allele of CHKA *rs6591331* or the A allele of CHKA *rs10791957* on choline metabolism and distribution of choline to the CDP-choline and PEMT pathways, respectively, subjects were placed into one of three groups for analysis: those with no risk alleles (*rs*6591331 A/A; *rs*10791957C/C), those with one risk allele (*rs*6591331 A/T; *rs*10791957C/A), and those with two risk alleles (*rs*6591331 T/T; *rs*10791957 A/A). Brain volume data were available from 92 of the 104 subjects; cognition data were available from 90 of the 104 subjects.

3.2. Hippocampal volume data

We first assessed group differences in segmented brain volumes to identify possible effects of the CHKA SNP on brain volume, with a specific focus on the hippocampus. Fig. 1 shows that those with two CHKA *rs6591331* T alleles had larger right hippocampal volumes ($F(2,89) = 3.148$, $p = .048$; $t(42) = -2.791$, $p = .008$) as compared to homozygous A/A subjects. Although not significant, the same pattern of means was observed between heterozygous A/T individuals and homozygous A/A subjects ($t(57) = -1.953$, $p = .056$). Moreover, no differences were observed in the left hippocampal volumes of these subjects, but the trend was similar to that seen in the right hippocampal volumes ($F(2,89) = 2.467$, $p = .091$; Fig. 1). There was no effect of *rs10791957* genotype on hippocampal volumes (right $F(2,89) = 1.816$, $p = .169$; left $F(2,89) = 1.293$, $p = .297$).

3.3. Neuropsychological test performance

Individual neuropsychological status was assessed through RBANS performance (Table 2), and analysis was conducted based on average group scores. The effect of the CHKA *rs6591331* T allele was observed on delayed memory performance, as displayed in Fig. 2. ANOVA results indicated a difference in delayed memory performance among the homozygous T/T subject group, the heterozygous A/T subject group, and the homozygous A/A subject group ($F(2,87) = 3.508$, $p = .034$). When compared to T/T subjects, results from the post hoc student's t-test analysis showed stronger performance in both A/T individuals ($t(76) = 2.317$, $p = .023$), as well as A/A individuals ($t(42) = 2.086$, $p = .043$). CHKA *rs10791957* did not affect neuropsychological performance (smallest $p > .09$ for RBANS total).

4. Discussion

Individual variability exists in both choline synthesis and the onset and progression of menopausal symptoms, two processes that are mediated by estrogen. To further understand these individual differences, we examined a gene involved in choline synthesis that is potentially affected by the decrease in estrogen at menopause. Our goal was to determine the influence of CHKA genotype on hippocampal volume and memory performance in postmenopausal women. We hypothesized that the presence of the effect alleles in either the *rs6591331* (T) or *rs10791957* (A) would be associated with decreased brain volumes driven by reduced PEMT-mediated phosphatidylcholine (PC) production, as PC is necessary for membrane health and neuronal plasticity. We hypothesized that

Table 1
Demographic Data. The means, standard deviations, and n values of relevant demographic measurements for the participants are outlined below. Subject data were grouped based on the number of effect alleles present in the CHKA *rs6591331* genotype (T) or the CHKA *rs10791957* genotype (A). No significant differences exist in demographic data among the CHKA genotypes of either SNP, or between SNPs ($p \leq .05$) Abbreviations: Follicle Stimulating Hormone (FSH).

A: *rs6591331*			
	Homozygous A/A N = 15	Heterozygous A/T N = 53	Homozygous T/T N = 38
Age (y)	55.3 (2.5)	56.5 (2.2)	55.9 (2.9)
Years since Menopause (y)	7.6 (6.2)	5.3 (3.1)	6.0 (4.4)
Education (y)	16.0 (2.7)	16.0 (2.4)	16.4 (2.1)
FSH (IU/ml)	77.4 (25.7)	84.3 (25.3)	79.7 (23.1)
Estradiol (pg/mL)	10.5 (7.1)	8.6 (6.1)	7.6 (6.0)
B: *rs10791957*			
Rs10791957	Homozygous C/C N = 19	Heterozygous C/A N = 54	Homozygous A/A N = 33
Age (y)	55.8 (2.4)	56.3 (2.4)	56.0 (2.9)
Years since Menopause (y)	7.1 (5.6)	5.2 (3.1)	6.3 (4.5)
Education (y)	16.2 (2.6)	16 (2.4)	16.3 (2.2)
FSH (IU/ml)	76.2 (24.1)	85.0 (25.7)	79.3 (22.6)
Estradiol (pg/mL)	9.5 (6.8)	8.9 (6.1)	7.3 (6.0)

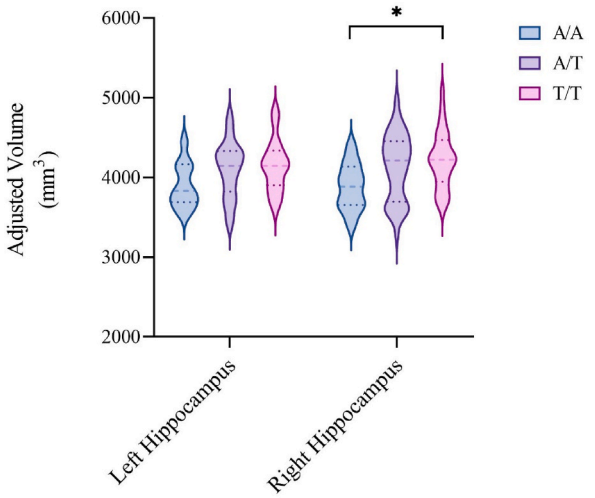


Fig. 1. The effect of CHKA *rs6591331* genotype on hippocampal volume
Results were derived from the FreeSurfer hippocampal segmentation feature. Differences between subjects with a CHKA *rs6591331* genotype of A/A (blue bar), A/T (purple bar), or T/T (pink bar) are illustrated for both the left and right hippocampi. (*p ≤ .05). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) ANOVA Results for *rs6591331*. The average RBANS scores for each of the five domains plus the total average score are presented by group below. Subject data were grouped based on the number of T effect alleles present in the CHKA *rs6591331* genotype. The last column shows the p-value resulting from the ANOVA analysis of groups within each domain.

Cognitive Domain	Average Score ***Homozygous A/A***	Average Score ***Heterozygous A/T***	Average Score ***Homozygous T/T***	One-Way ANOVA p-value
Immediate Memory	114.1	106.9	103.9	.074
Visuo/Construct	116.4	114.9	114.0	.855
Language	112.6	109.2	104.7	.070
Attention	111.7	110.8	114.2	.436
Delayed Memory	109.8	107.0	100.2	.034
Total Average Score	119.8	114.4	110.1	.064

memory performance would be impaired by the presence of the CHKA effect alleles because of the reduced availability of choline for acetylcholine production, as cholinergic signaling is a known regulator of neurological pathways involved in learning and memory.

The results showed larger right hippocampal volumes in women who were homozygous for the CHKA *rs6591331* T allele as compared to those who were homozygous for the A allele. The pattern of means for each genotype group revealed a dose-dependent trend, suggesting a positive relationship between hippocampal volume and the number of CHKA *rs6591331* T alleles. Neuropsychological testing showed that the CHKA *rs6591331* genotype was associated with delayed memory performance. Homozygous T/T women performed worse on delayed memory tasks when compared to heterozygous A/T and homozygous A/A women. These effects were limited to *rs6591331* genotype. The *rs10791957* effect allele did not significantly affect hippocampal volume or neuropsychological functioning. This warrants further examination of *rs10791957*-mediated regulation of choline distribution to the CDP-choline and PEMT pathways specifically in postmenopausal women, as our results imply that these effects may vary from those observed in premenopausal women. Overall, these data revealed the associations of the CHKA *rs6591331* genotype with memory functioning and brain structure in postmenopausal women, a SNP that had only previously been correlated with organ dysfunction in women on a low choline diet after menopause [29].

Although our findings did not support the hypothesized positive association between hippocampal volume and neuropsychological performance, this is not the first study to report better cognition associated with smaller brain volumes. In postmenopausal APOE e4 carriers, hippocampal volume was negatively associated with performance on non-memory cognitive measures [41]. In a study investigating hippocampal atrophy in Alzheimer's disease, delayed verbal recall correlated negatively with hippocampal volume in healthy control subjects [42]. Meta-analyses have established three general perspectives that exist surrounding the relationship between hippocampal volume and memory ability [43]. Support is strong for perspectives that consider both development and the neuropsychological effects of aging and a number of studies contradict hypotheses built on an assumed association between larger brain volumes and stronger cognitive performance [43]. It is likely that a complex combination of the effects of development and aging explains the observed relationships between hippocampal volume and cognitive function in the postmenopausal women included in these previously published studies, as well as in our present results.

Our findings described an observed effect of CHKA genotype on hippocampal structure and function driven by the effect allele

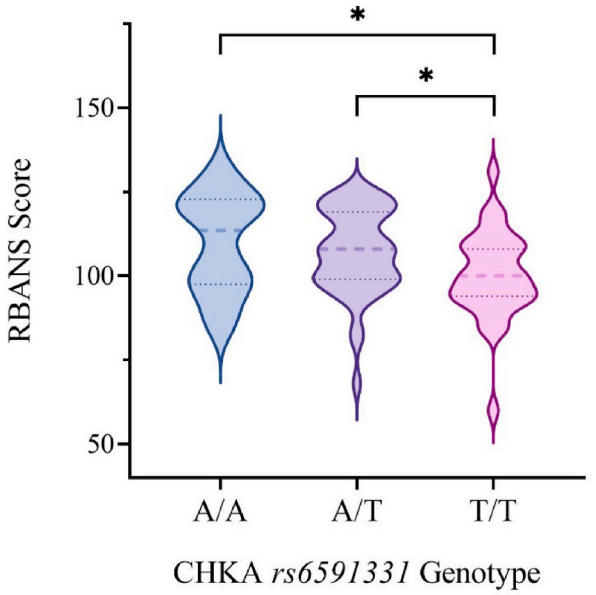


Fig. 2. The effect of CHKA *rs6591331* genotype on delayed memory Results were derived from RBANS scores for delayed memory tasks. Differences in delayed memory performance between subjects who were homozygous A/A, heterozygous A/T, or homozygous T/T for CHKA *rs6591331* are shown. (*p ≤ .05).

rs6591331, but not *rs10791957*, inviting further studies surrounding the mechanisms driving these novel associations. Our results may be explained by a CHKA *rs6591331*-dependent mechanism that compensates for decreased estrogen, and thus decreased bio-synthesized phosphatidylcholine and choline, occurring during menopause. This proposed mechanism aligns with recently reported findings suggestive of an adaptive compensation mechanism in the nervous system of women undergoing the menopausal transition, a conclusion supported by grey matter volume recovery, in vivo brain mitochondria ATP production, and preserved neurocognitive function post-menopause (Mosconi et al., 2021).

Prior studies suggest that low levels of choline may intrinsically heighten CHKA activity; indicating potential enzymatic activity increases in postmenopausal with limited choline synthesis capabilities [44]. The CHKA *rs6591331* SNP is located in the first intron of the gene, a potential regulatory enhancer region [29]. Based on our findings, we anticipate that the T allele may further increase CHKA activity by promoting transcription of the enzyme. Put together, this information suggests that postmenopausal women with a CHKA *rs6591331* T allele may have inherently increased CHKA enzymatic activity, which may promote the use of choline for the CDP-choline pathway in the brains of these individuals. The main product of this pathway is the highly abundant membrane component PC, which contributes to overall cell health and restores neuronal plasticity under inflammatory conditions, such as those observed in normal aging [8]. Typically, this compound is produced largely by the PEMT and CDP-Choline pathways; however, following menopause, PC production occurs largely through the CDP-Choline pathway, as activity of the PEMT pathway is limited by the low levels of estrogen observed in this state. We hypothesize that increased CHKA activity within the CDP-Choline pathway, driven by the CHKA *rs6591331* T allele, leads to accumulation of PC in postmenopausal individuals with a T/T CHKA *rs6591331* genotype. This accumulation promotes neuronal vitality and prevents atrophy in the hippocampus, thus explaining the larger hippocampal volumes observed in these subjects.

In the central nervous system, choline for acetylcholine synthesis is derived from the breakdown of previously released acetylcholine or from PC. Thus, the synthesis of acetylcholine is ultimately limited by choline availability and may be affected by menopausal status or use of PC for other pathways [45,46]. Elevated enzymatic activity of CHKA, as further mediated by the effect allele of the *rs6591331* SNP, may lead to a disproportionate use of PC for the prevention of neuronal atrophy in the hippocampus, leaving less available PC for acetylcholine synthesis. Acetylcholine is essential to processes involved in converting short-term memories for long-term storage, and low levels of the neurotransmitter are associated with impaired cognitive function. Based on these known associations, we anticipate that postmenopausal subjects with the CHKA *rs6591331* effect allele may experience hippocampal PC accumulation combined with limited acetylcholine production, driving the observed increase in right hippocampal volume and decline in delayed memory performance observed in this group.

This study included a relatively small number of subjects and examined the effect of one SNP on brain structure and function. Thus, using larger data sets to further investigate the association between menopausal symptoms and genes involved in choline metabolism may provide more insight into the mechanisms behind the relationship. Furthermore, the findings reported here were assessed from a single measurement time point. Thus, no conclusions can be made on changes in hippocampal volume or cognition throughout pre-peri- and postmenopausal states. Finally, this study examined the effects of one SNP on a singular brain region in well-characterized women. We acknowledge that the effect of CHKA genotype on hippocampal volume is not the single cause of individual differences in

menopause. Rather, the present findings suggest that co-examining choline metabolism and menopause may reveal further information about the variability seen in the onset of menopausal symptoms. These results support further investigation of CHKA genotype, enzymatic activity, PC accumulation, and acetylcholine availability in women throughout their lifetimes to yield additional understanding of the proposed compensation mechanism.

5. Conclusion

Estrogen supports many biological functions, including endogenous choline synthesis and hippocampal neurogenesis [23,47]. However, when estrogen levels drop during the menopausal transition, adaptations must be made to compensate for the decreased hormone levels. We examined how CHKA genotype contributed to variations in this postmenopausal compensation by comparing hippocampal volumes and neuropsychological function among subjects with zero, one, or two effect alleles for CHKA *rs6591331* or *rs10791957*. Individuals with a *rs6591331* T/T genotype had larger right hippocampal volumes but performed worse on delayed memory tasks when compared to individuals with an A allele. We suggest that the CHKA *rs6591331* T allele contributes to a postmenopausal compensation mechanism, possibly by increasing PC production to promote neuronal vitality and act as a protective factor against hippocampal atrophy. The use of PC for this mechanism may result in less available PC for acetylcholine production, explaining the lower delayed memory scores exhibited by *rs6591331* T/T subjects.

CRediT authorship contribution statement

Abigail J. Myers: Conceptualization, Visualization, Writing – original draft, Writing – review & editing, Formal analysis. **Callum Potts:** Conceptualization, Investigation, Resources, Writing – review & editing, Funding acquisition. **Jenna A. Makarewicz:** Investigation, Project administration, Validation. **Elizabeth McGee:** Conceptualization, Supervision, Writing – review & editing. **Julie A. Dumas:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by NIA R21 AG047944>, DoE SC 0001753, and the University of Vermont Mead Fund. The authors would like to thank the volunteer subjects for making this study possible, as well as the University of Vermont Clinical Research Center nursing staff for supporting our subjects. We also wish to thank Jay Gonyea, Scott Hipko, Trevor Andrews, Ph.D., and Richard Watts, D. Phil from the University of Vermont MRI Center for Biomedical Imaging for their continued aid with collection of MRI data. We also thank the staff of the Genome Technologies Core Facility at the University of Vermont Cancer Center.

References

[1] C. Luetters, M.H. Huang, T. Seeman, G. Buckwalter, P.M. Meyer, N.E. Avis, B. Sternfeld, J.M. Johnston, G.A. Greendale, Menopause transition stage and endogenous estradiol and follicle-stimulating hormone levels are not related to cognitive performance: cross-sectional results from the study of women's health across the nation (SWAN), J Womens Health (Larchmt) 16 (3) (2007) 331–344.

[2] M.T. Weber, P.M. Maki, M.P. McDermott, Cognition and mood in perimenopause: a systematic review and meta-analysis, J. Steroid Biochem. Mol. Biol. 142 (2014) 90–98, https://doi.org/10.1016/j.jsbmb.2013.06.001.

[3] N.F. Woods, E.S. Mitchell, Symptoms during the perimenopause: prevalence, severity, trajectory, and significance in women's lives, Am. J. Med. 118 (Suppl 12B) (2005) 14–24, https://doi.org/10.1016/j.amjmed.2005.09.031.

[4] D.M. Conde, R.C. Verdade, A.L.R. Valadares, L.F.B. Mella, A.O. Pedro, L. Costa-Paiva, Menopause and cognitive impairment: a narrative review of current knowledge, World J Psychiatry 11 (8) (2021) 412–428, https://doi.org/10.5498/wjp.v11.i8.412.

[5] B.S. McEwen, Invited review: Estrogens effects on the brain: multiple sites and molecular mechanisms, J. Appl. Physiol. 91 (6) (2001) 2785–2801, https://doi.org/10.1152/jappl.2001.91.6.2785, 1985.

[6] P. Newhouse, J. Dumas, Estrogen-cholinergic interactions: Implications for cognitive aging, Horm. Behav. 74 (2015) 173–185, https://doi.org/10.1016/j.yhbeh.2015.06.022.

[7] D. Wishart, A. Guo, E. Oler, e. al, Choline, 2022.

[8] D. Magaquian, S. Delgado Ocaña, C. Perez, C. Banchio, Phosphatidylcholine restores neuronal plasticity of neural stem cells under inflammatory stress, Sci. Rep. 11 (1) (2021), 22891, https://doi.org/10.1038/s41598-021-02361-5.

[9] L. Paoletti, C. Elena, P. Domizi, C. Banchio, Role of phosphatidylcholine during neuronal differentiation, IUBMB Life 63 (9) (2011) 714–720, https://doi.org/10.1002/iub.521.

[10] S.H. Zeisel, K.A. da Costa, Choline: an essential nutrient for public health, Nutr. Rev. 67 (11) (2009) 615–623, https://doi.org/10.1111/j.1753-4887.2009.00246.x.

[11] C.J. DeLong, Y.J. Shen, M.J. Thomas, Z. Cui, Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway, J. Biol. Chem. 274 (42) (1999) 29683–29688, https://doi.org/10.1074/jbc.274.42.29683.

[12] C.D. Albright, A.Y. Tsai, C.B. Friedrich, M.H. Mar, S.H. Zeisel, Choline availability alters embryonic development of the hippocampus and septum in the rat, Brain Res Dev Brain Res 113 (1–2) (1999) 13–20, https://doi.org/10.1016/s0165-3806(98)00183-7.

[13] M.A. Caudill, B.J. Strupp, L. Muscalu, J.E.H. Nevins, R.L. Canfield, Maternal choline supplementation during the third trimester of pregnancy improves infant information processing speed: a randomized, double-blind, controlled feeding study, Faseb. J. 32 (4) (2018) 2172–2180, https://doi.org/10.1096/fj.201700692RR.

[14] C.N. Craciunescu, C.D. Albright, M.H. Mar, J. Song, S.H. Zeisel, Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus, J. Nutr. 133 (11) (2003) 3614–3618, https://doi.org/10.1093/jn/133.11.3614.

[15] S.L. Ladd, S.A. Sommer, S. LaBerge, W. Toscano, Effect of phosphatidylcholine on explicit memory, Clin. Neuropharmacol. 16 (6) (1993) 540–549.

[16] N. Sitaram, H. Weingartner, E.D. Caine, J.C. Gillin, Choline: Selective enhancement of serial learning and encoding of low imagery words in man, Life Sci. 22 (17) (1978) 1555–1560, https://doi.org/10.1016/0024-3205(78)90011-5.

[17] R. Tönjes, K. Hecht, M. Brautzsch, R. Lucius, G. Dörner, Behavioural changes in adult rats produced by early postnatal maternal deprivation and treatment with choline chloride, Exp. Clin. Endocrinol. 88 (2) (1986) 151–157, https://doi.org/10.1055/s-0029-1210590.

[18] M.R. Picciotto, M.J. Higley, Y.S. Mineur, Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior, Neuron 76 (1) (2012) 116–129, https://doi.org/10.1016/j.neuron.2012.08.036.

[19] L.M. Fischer, K.A. daCosta, L. Kwock, P.W. Stewart, T.S. Lu, S.P. Stabler, R.H. Allen, S.H. Zeisel, Sex and menopausal status influence human dietary requirements for the nutrient choline, Am. J. Clin. Nutr. 85 (5) (2007) 1275–1285, https://doi.org/10.1093/ajcn/85.5.1275.

[20] B.B. Sherwin, Estrogen and cognitive functioning in women, Endocr. Rev. 24 (2) (2003) 133–151, https://doi.org/10.1210/er.2001-0016.

[21] P.M. Maki, A.B. Zonderman, S.M. Resnick, Enhanced verbal memory in nondemented elderly women receiving hormone-replacement therapy, Am. J. Psychiatr. 158 (2) (2001) 227–233, https://doi.org/10.1176/appi.ajp.158.2.227.

[22] A.M. Wiedeman, S.I. Barr, T.J. Green, Z. Xu, S.M. Innis, D.D. Kitts, Dietary choline intake: current state of knowledge across the life cycle, Nutrients 10 (10) (2018), https://doi.org/10.3390/nu10101513.

[23] M.E. Resseguie, K.A. da Costa, J.A. Galanko, M. Patel, I.J. Davis, S.H. Zeisel, Aberrant estrogen regulation of PEMT results in choline deficiency-associated liver dysfunction, J. Biol. Chem. 286 (2) (2011) 1649–1658, https://doi.org/10.1074/jbc.M110.106922.

[24] L.M. Fischer, K.A. da Costa, L. Kwock, J. Galanko, S.H. Zeisel, Dietary choline requirements of women: effects of estrogen and genetic variation, Am. J. Clin. Nutr. 92 (5) (2010) 1113–1119, https://doi.org/10.3945/ajcn.2010.30064.

[25] P. Fagone, S. Jackowski, Phosphatidylcholine and the CDP-choline cycle, Biochim. Biophys. Acta 1831 (3) (2013) 523–532, https://doi.org/10.1016/j.bbalip.2012.09.009.

[26] C. Klöckner, J.P. Fernández-Murray, M. Tavasoli, H. Sticht, G. Stoltenburg-Didinger, L.M. Scholle, S. Bakhtiari, M.C. Kruer, H. Darvish, S.G. Firouzabadi, A. Pagnozzi, A. Shukla, K.M. Girisha, D.L. Narayanan, P. Kaur, R. Maroofian, M.S. Zaki, M.M. Noureldeen, A. Merkenschlager, K. Platzer, Bi-allelic variants in CHKA cause a neurodevelopmental disorder with epilepsy and microcephaly, Brain 145 (6) (2022) 1916–1923, https://doi.org/10.1093/brain/awac074.

[27] J.C. Lacal, T. Zimmerman, J.M. Campos, Choline kinase: an unexpected journey for a precision medicine strategy in human diseases, Pharmaceutics 13 (6) (2021), https://doi.org/10.3390/pharmaceutics13060788.

[28] A.B. Ganz, V.V. Cohen, C.C. Swersky, J. Stover, G.A. Vitiello, J. Lovesky, J.C. Chuang, K. Shields, V.G. Fomin, Y.S. Lopez, S. Mohan, A. Ganti, B. Carrier, O. V. Malysheva, M.A. Caudill, Genetic variation in choline-metabolizing enzymes alters choline metabolism in young women consuming choline intakes meeting current recommendations, Int. J. Mol. Sci. 18 (2) (2017), https://doi.org/10.3390/ijms18020252.

[29] K.A. da Costa, K.D. Corbin, M.D. Niculescu, J.A. Galanko, S.H. Zeisel, Identification of new genetic polymorphisms that alter the dietary requirement for choline and vary in their distribution across ethnic and racial groups, Faseb. J. 28 (7) (2014) 2970–2978, https://doi.org/10.1096/fj.14-249557.

[30] J.A. Dumas, J.A. Makarewicz, J. Bunn, J. Nickerson, E. McGee, Dopamine-dependent cognitive processes after menopause: the relationship between COMT genotype, estradiol, and working memory, Neurobiol. Aging 72 (2018) 53–61, https://doi.org/10.1016/j.neurobiolaging.2018.08.009.

[31] M.F. Folstein, S.E. Folstein, P.R. McHugh, Mini-mental state": a practical method for grading the cognitive state of patients for the clinician, J. Psychiatr. Res. 12 (3) (1975) 189–198.

[32] B. Reisberg, S.H. Ferris, Brief cognitive rating scale (BCRS), Psychopharmacol. Bull. 24 (4) (1988) 629–635.

[33] C. Randolph, M.C. Tierney, E. Mohr, T.N. Chase, The repeatable Battery for the assessment of neuropsychological status (RBANS): preliminary clinical validity, J. Clin. Exp. Neuropsychol. 20 (3) (1998) 310–319, https://doi.org/10.1076/jcen.20.3.310.823.

[34] H. Buschke, Selective reminding for analysis of memory and learning, J. Verb. Learn. Verb. Behav. 12 (1973) 543–550.

[35] M. Reuter, H.D. Rosas, B. Fischl, Highly accurate inverse consistent registration: a robust approach, Neuroimage 53 (4) (2010) 1181–1196, https://doi.org/10.1016/j.neuroimage.2010.07.020.

[36] F. Ségonne, A.M. Dale, E. Busa, M. Glessner, D. Salat, H.K. Hahn, B. Fischl, A hybrid approach to the skull stripping problem in MRI, Neuroimage 22 (3) (2004) 1060–1075, https://doi.org/10.1016/j.neuroimage.2004.03.032.

[37] B. Fischl, D.H. Salat, E. Busa, M. Albert, M. Dieterich, C. Haselgrove, A. van der Kouwe, R. Killiany, D. Kennedy, S. Klaveness, A. Montillo, N. Makris, B. Rosen, A.M. Dale, Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain, Neuron 33 (3) (2002) 341–355, https://doi.org/10.1016/s0896-6273(02)00569-x.

[38] B. Fischl, D.H. Salat, A.J. van der Kouwe, N. Makris, F. Ségonne, B.T. Quinn, A.M. Dale, Sequence-independent segmentation of magnetic resonance images, Neuroimage 23 (Suppl 1) (2004) S69–S84, https://doi.org/10.1016/j.neuroimage.2004.07.016.

[39] O. Voevodskaya, A. Simmons, R. Nordenskjöld, J. Kullberg, H. Ahlström, L. Lind, L.O. Wahlund, E.M. Larsson, E. Westman, The effects of intracranial volume adjustment approaches on multiple regional MRI volumes in healthy aging and Alzheimer's disease, Front. Aging Neurosci. 6 (2014) 264, https://doi.org/10.3389/fnagi.2014.00264.

[40] IMBCORP, IBM SPSS Statistics for Windows, IBM Corp, 2020.

[41] K. Kantarci, M. Dowling, N. Tosakulwong, W. Wharton, C. Gleason, S. Wille, C. Ward, T. Zais, H. Wiste, S. Weigand, C. Jack, G.E. Smith, S. Asthana, V. Miller, P3-298: hippocampal volumes are associated with cognitive performance in recently menopausal APOE ∈4 carriers, Alzheimer's Dementia 7 (4S_Part_17) (2011) S611–S612, https://doi.org/10.1016/j.jalz.2011.05.1740.

[42] S. Köhler, S.E. Black, M. Sinden, C. Szekely, D. Kidron, J.L. Parker, J.K. Foster, M. Moscovitch, G. Winocour, J.P. Szalai, M.J. Bronskill, Memory impairments associated with hippocampal versus parahippocampal-gyrus atrophy: an MR volumetry study in Alzheimer's disease, Neuropsychologia 36 (9) (1998) 901–914, https://doi.org/10.1016/s0028-3932(98)00017-7.

[43] C. Van Petten, Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis, Neuropsychologia 42 (10) (2004) 1394–1413, https://doi.org/10.1016/j.neuropsychologia.2004.04.006.

[44] M. Bussière, R.B. Campenot, D.R. Ure, J.E. Vance, D.E. Vance, Reciprocal regulation of choline acetyltransferase and choline kinase in sympathetic neurons during cholinergic differentiation, Biochim. Biophys. Acta 1259 (2) (1995) 148–154, https://doi.org/10.1016/0005-2760(95)00142-y.

[45] Y.R. Smith, L. Bowen, T.M. Love, A. Berent-Spillson, K.A. Frey, C.C. Persad, N.K. Reame, R.A. Koeppe, J.K. Zubieta, Early initiation of hormone therapy in menopausal women is associated with increased hippocampal and posterior cingulate cholinergic activity, J. Clin. Endocrinol. Metab. 96 (11) (2011) E1761–E1770, https://doi.org/10.1210/jc.2011-0351.

[46] P. Taylor, J.H. Brown, Synthesis, storage and release of acetylcholine, in: G.J. Siegel, B.W. Agranoff, R.W. Albers, e. al (Eds.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects, sixth ed. ed., Lippincott-Raven, 1999.

[47] L. Wan, R.J. Huang, Z.H. Luo, J.E. Gong, A. Pan, J. Manavis, X.X. Yan, B. Xiao, Reproduction-associated hormones and adult hippocampal neurogenesis, Neural Plast. 2021 (2021), 3651735, https://doi.org/10.1155/2021/3651735.