Ph.D. Dissertation of Medicine

Association of L-alpha glycerylphosphorylcholine with subsequent stroke risk

among individuals without the history of the anti-dementia drugs

치매 치료제 복용력이 없는 성인에서
L-alpha glycerylphosphorylcholine과
뇌졸중 발생 관련성에 관한 연구

August 2022

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Association of L-alpha glycerylphosphorylcholine with subsequent stroke risk among individuals without the history of the anti-dementia drugs

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Submitting a Ph.D. Dissertation of
Clinical Medical Science

April 2022

Graduate School of Clinical Medical Science
Seoul National University

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Abstract

Introduction: L-alpha glycerylphosphorylcholine (alpha-GPC, choline alfoscerate) is used globally by elderly populations based on its potential function as a precursor of acetylcholine. However, choline has previously been linked to high risk of cardiovascular disease via trimethylamine-N-oxide, a metabolite of choline produced by microbiota. We, therefore, investigated the association between alpha-GPC use and subsequent 10-year stroke risk.

Methods: This population-based, retrospective cohort study was performed using the National Health Insurance Service of Korea. Men and women aged ≥50 years without history of anti-dementia drug use or underlying stroke or transient ischemic attack (n = 12,008,977) were participants. All participants were divided based on whether they were prescribed alpha-GPC during 2006–2008. Alpha-GPC users were matched with non-users for all covariates to create a matched cohort. Alpha-GPC users were further divided into <2, 2–6, 6–12, and >12 months of alpha-GPC prescription use. The adjusted hazard ratios and 95% confidence intervals for total stroke, ischemic stroke, and hemorrhagic stroke during 2009–2018 were calculated by multivariate Cox proportional hazards regression.

Results: A total of 12,008,977 men (46.7%) and women (53.3%) aged ≥50 years were included in the study. Alpha-GPC users (n = 108,877) had higher risk for total stroke (adjusted hazard ratio [95% confidence interval]: 1.46 [1.43–1.48]), ischemic stroke (1.36 [1.33–1.39]), and hemorrhagic stroke

(1.36 [1.28–1.44]) than alpha-GPC non-users (n = 11,900,100). After matching for all covariates, alpha-GPC users had higher risk for total stroke (1.43 [1.41–1.46]), ischemic stroke (1.34 [1.31–1.37]), and hemorrhagic stroke (1.37 [1.29–1.46]). Increasing intake of alpha-GPC was associated with higher risk for total stroke in a dose-responsive manner ($p < 0.001$).

Conclusions: Alpha-GPC use was associated with higher 10-year incident stroke risk in a dose-responsive manner after adjusting for traditional cerebrovascular risk factors. Future studies are needed to determine the possible mechanisms behind the potential cerebrovascular risk-elevating effects of alpha-GPC.

Keywords: L-alpha glcerylphosphorylcholine; Choline alfoscerate; Stroke; Dementia; Pharmacoepidemiology; Big data science
Student Number: 2017-36173

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Chapter 1. Introduction

1.1. Study Background

Dementia is an acquired loss of cognition in multiple cognitive domains sufficiently severe to affect social or occupational function (Livingston et al., 2017). The prevalence of dementia among the elderly approximately doubles every 5 years (Hugo & Ganguli, 2014), with 131 million adults expected to be diagnosed with dementia by 2050 (Prince et al., 2016).

Alzheimer's disease (AD), the most common cause of dementia, is a progressive, unremitting, neurodegenerative disorder that affects wide areas of the cerebral cortex and hippocampus. AD is associated with the accumulation of insoluble amyloid-β plaques in extracellular spaces, as well as in the walls of blood vessels, and aggregation of the microtubule protein tau in neurofibrillary tangles in neurons (Arvanitakis et al., 2019). Additionally, abnormalities in neurotransmitters have been reported, affecting cholinergic, monoaminergic, and glutamatergic systems (Arvanitakis et al., 2019).

Early animal studies highlighted the importance of cholinergic function in learning and memory (Deutsch, 1971). Cholinergic therapies introduced in clinical practice included cholinergic precursors and inhibitors of the acetylcholine catabolic enzymes acetylcholinesterase (AChE) and

cholinesterase (Jones, 2003).

For treatment of AD, most regulatory authorities have approved AChE inhibitors as drugs, while approving cholinergic precursors as dietary supplements. L-alpha glycerylphosphorylcholine (alpha-GPC, choline alfoscerate, or choline alphoscerate) is one of the cholinergic precursors and globally used as a drug or a dietary supplement, depending on the relevant regulatory authority. Only a few countries, including Italy, Russia, and South Korea, approved alpha-GPC as a drug, unlike most developed countries, including the US, EU, and Japan.

This discrepancy in the approval of alpha-GPC as a prescription drug suggests that there may be a lack of sufficient evidence on its efficacy, safety, or both. A few randomized controlled trial (RCT) on alpha-GPC reported that active treatment using AChE inhibitors, donepezil and alpha-GPC, might slow progressive decline compared with donepezil treatment alone among 113 participants with AD with cerebrovascular injury, after a 12-month and 24-month observation period (ASCOMALVA trial) (Amenta et al., 2012; Amenta et al., 2014). In addition, most previous clinical trials on alpha-GPC were enrolled acute phase of disease or different route of administration like per intramuscular injection (Parnetti et al., 2001).

Alpha-GPC is largely considered to be safe due to its structure-function feature. Choline, a metabolite of alpha-GPC (Traini et al., 2013), is an essential nutrient naturally present in some foods and supplements (National Institutes of Health Offiice of Dietary Supplements, 2020), with

potential adverse effects such as fishy body odour, vomiting, excessive sweating and salivation, hypotension, and liver disease (Erdman JW et al., 2012; National Institutes of Health Offiice of Dietary Supplements, 2020).

However, a growing body of evidence suggests that high plasma choline level is associated with a high risk of cardiovascular diseases via trimethylamine-N-oxide (TMAO) produced by gut microbiota from choline (Koeth et al., 2013; Tang et al., 2013; Wang, Klipfell, Bennett, Koeth, Levison, DuGar, Feldstein, Britt, Fu, & Chung, 2011; Zheng et al., 2016). Recent studies suggest that TMAO is associated with stroke as well as cardiovascular disease (Nam, 2019; Nie et al., 2018; Wu et al., 2018).

1.2. Previous literature reviews

1.2.1 Cholinergic hypothesis of memory dysfunction

For decades, neurological studies in those with aging and AD have consistently reported damage or abnormalities in these pathways (particularly basal forebrain projections) that appeared to correlate well with the level of cognitive decline. As a result, the so-called “cholinergic hypothesis” was developed, which essentially states that a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age and AD (Bartus et al., 1982).

Alterations in high-affinity choline uptake, impaired acetylcholine release, deficits in the expression of nicotinic and muscarinic receptors, dysfunctional neurotrophic support, and deficits in axonal transport are manifested in early AD neurons (Terry & Buccafusco, 2003).

1.2.2 History of cholinergic precursor therapy

The efficacy of AChE inhibitors in the treatment of adult-onset dementia depends on the existence of an adequate central cholinergic tone (Amenta et al., 2001). Acetylcholine is a neurotransmitter derived from choline and acetyl coenzyme A (Stanciu et al., 2019).

Nervous tissue cannot synthesize choline, which is ultimately derived from the diet and transmitted to neurons via the bloodstream (Amenta et al., 2001). Cholinergic precursor loading therapy was the first attempted approach to relieve cognitive impairment in AD, using lecithin, phosphatidylserine, cytidine 5'-diphosphocholine (CDP-choline or citicoline), and alpha-GPC.

First, based on the assumption that lecithin is a major dietary source of choline and, in some circumstances, may be transformed into acetylcholine (Blusztajn et al., 1987; Chung et al., 1995; Jope, 1982), lecithin was used for the treatment of cognitive impairment due to adult-onset dementia in clinical studies. However, the results of 12 RCTs failed to show relevant effects induced by choline or the choline-containing phospholipid phosphatidylcholine (lecithin) (Amenta et al., 2001; Higgins & Flicker, 2000).

Second, phosphatidylserines, an endogenous phospholipid and dietary nutrient, also failed to show significant effects on AD in RCTs (Engel et al., 1992), conferring only a short-term benefit and its limited effect on AD (Heiss et al., 1994).

Third, CDP-choline is an essential intermediate in the biosynthetic pathway of the structural phospholipids of cell membranes, especially in that of phosphatidylcholine. CDP-choline resulted in improved scores on cognitive evaluation scales and probably slowed the evolution of AD (Secades & Frontera, 1995). However, studies comparing the effects of CDP-choline and alpha-GPC in patients with vascular dementia showed more favorable psychometric scores from alpha-GPC (Muratorio et al., 1992; Perri et al., 1991).

1.2.3 L-alpha glycerylphosphorylcholine

Alpha-GPC (Figure 1) is hydrolyzed to choline which is the precursor of the neurotransmitter acetylcholine. The alpha-GPC is completely absorbed following oral or intramuscular administration so that the active major metabolite, choline, appears in the plasma (Kang et al., 2010).

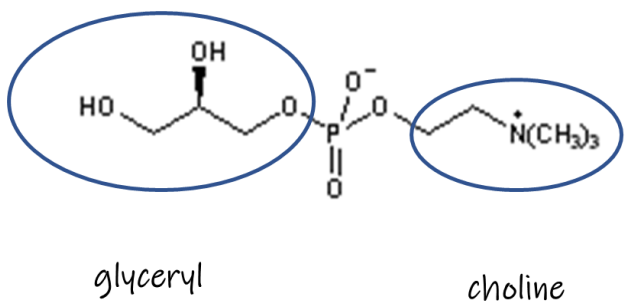

Figure 1. The structure of L-alpha glycerylphosphorylcholine (alpha-GPC or choline alfoscerate)

Alpha-GPC, per oral administration, is commercially available as a soft capsule and administered at a dose of 400 mg three times a day. Alpha-GPC is hydrolyzed by phosphodiesterases in the gut mucosa to form free choline (Min et al., 2019). The enzyme transforms alpha-GPC into a molecule of choline and glycerol-3-phosphate (Amenta & Tayebati, 2008).

The kinetics and metabolism of alpha-GPC were investigated in male and female rats after i.v. injection (10mg/kg) or oral doses (100–300 mg/kg) of the compound labeled with L-α-(3-[^{14}C])-glycerylphosphorylcholine ([^{14}G]-GPC, i.e., glycerol part) and L-α-glycerylphosphoryl(1,2-[^{14}C])choline

([^{14}C]-GPC, i.e., choline part) (Abbiati et al., 1993). After oral administration, the main circulating metabolite was choline; intact alpha-GPC was not present after either labelled compound was administered, indicating that alpha-GPC was all hydrolyzed in the rat intestinal mucosa by a specific enzyme system degrading alpha-GPC to choline and glycerol-3-phosphate (Abbiati et al., 1993).

Peak concentration (t_{max}) occurred 24 h after oral administration of radioactive alpha-GPC. Tissues with radioactivity levels (in descending order) higher than that of the blood were the liver (highest at 7–10 times the blood levels, corresponding to 5-11% of the administered dose), kidneys (highest at 5–6 times the blood levels, corresponding to 1-1.5% of the administered dose), gut, spleen, and lung. The brain radioactivity was approximately half the radioactivity than in blood, corresponding to <1% of the administered dose (Abbiati et al., 1993). Other three types of [^{14}C]-GPC metabolites were also detected in blood after oral administration of alpha-GPC. On the other hand, only one [^{14}C]-GPC metabolite was detected in blood after i.v. injection detection.

1.3. Adverse effects of choline

1.3.1 Choline

Choline, a metabolite of alpha-GPC, is an essential nutrient that is naturally present in some foods and supplements (National Institutes of Health Offiice of Dietary Supplements, 2020) and important for normal membrane function and acetylcholine synthesis and methyl group metabolism (Zeisel & Blusztajn, 1994). The recommended amount of choline depends on age and sex. Average daily recommended amounts are summarized in Table 1, based on the National Institutes of Health Office of Dietary Supplements.

Table 1. The recommended dosage of choline according to life stages.

Life Stage	Recommended Amount
Birth to 6 months	125 mg
Infants 7–12 months	150 mg
Children 1–3 years	200 mg
Children 4–8 years	250 mg
Children 9–13 years	375 mg
Teen boys 14–18 years	550 mg
Teen girls 14–18 years	400 mg
Men 19+ years	550 mg
Women 19+ years	425 mg
Pregnant teens and women	450 mg
Breastfeeding teens and women	550 mg

Potential adverse side effects include fishy body odor, vomiting, excessive sweating and salivation, hypotension, and liver disease (National Institutes of Health Offiice of Dietary Supplements, 2020). The daily upper limits for choline, summarized in Table 2, are based on the National Institutes of Health Office of Dietary Supplements.

Table 2. Upper limit of dietary choline intake according to life stages.

Life Stage	Upper Limit
Birth to 12 months	Not established
Children 1–3 years	1,000 mg
Children 4–8 years	1,000 mg
Children 9–13 years	2,000 mg
Teens 14–18 years	3,000 mg
Adults	3,500 mg

1.3.2 Gut microbiota and choline

A growing body of evidence suggests that a high choline level in the plasma is associated with a high risk of cardiovascular diseases due to TMAO produced by the gut microbiota from choline (Koeth et al., 2013; Tang et al., 2013; Wang, Klipfell, Bennett, Koeth, Levison, DuGar, Feldstein, Britt, Fu, Chung, et al., 2011; Zheng et al., 2016). Recent studies suggest that TMAO is associated with stroke as well as cardiovascular diseases (Nam, 2019; Nie et al., 2018; Wu et al., 2018).

Considering that alpha-GPC is used as a drug or a dietary supplement owing to its potential beneficial effects on memory and cognitive function, the possible risk of stroke associated with high choline levels gives rise to the apprehension surrounding the safety and efficacy of alpha-GPC. Alpha-GPC was frequently prescribed for the prevention of cognitive decline in the elderly, even without dementia, in South Korea. It might be because of its broad indications through the insurance coverage of alpha-GPC in South Korea, include dementia or secondary symptoms caused by cerebrovascular defects, changes in mood and behaviors, and senile depression.

1.4. Purpose of research

The author aimed to investigate the association between alpha-GPC intake and the risk of incident stroke across a 10-year period, including ischemic and hemorrhagic stroke, using the Korean national claim database.

Chapter 2. Methods

2.1. Systematic review of clinical trials on oral alpha-GPC

2.1.1 Search strategy and selection criteria

The author searched the Medline and Embase databases, Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov registry for articles published through January 1, 2020. Table 3 lists the PICOS search strategy on Medline, similar to other databases as follows: P (population): human; I (intervention): alpha-GPC and its relevant expressions; C (comparison): placebo or non-exposed groups; S (study design): RCT or cohorts. When searching the databases, the author did not use the outcome keywords to identify any adverse events. Additionally, the author reviewed all registered clinical trials including unpublished reports about alpha-GPC. Additional publications were retrieved from the bibliographies of relevant manuscripts when they were considered potentially pertinent. All citations were eligible for inclusion regardless of publication year or language.

Table 3. Search strategy on Medline

Number	Search queries
#1	MeSH descriptor: [Glycerylphosphorylcholine] explode all trees
#2	(choline alfoscerate OR choline alphoscerate OR choline alfocerato OR alpha-GFC OR alpha-GPC OR alpha-glyceryl-phosphorylcholine OR acetylcholine precusor):ti,ab,kw
#3	#1 OR #2
#4	Search randomized controlled trial [Publication Type]
#5	Search cohort [Publication Type]
#6	#4 OR #5
#7	#3 AND #6 Filters: Adults

The same keywords were used in other databases, including Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), and the ClinicalTrials.gov registry.

After removing duplicate citations, the author screened the titles and abstracts to identify potentially relevant citations. Full texts were then reviewed to establish whether all pre-specified inclusion criteria were met; participants were adults with chronic dementia or stroke (i.e., with no acute treatment period); group allocation was based on oral administration (i.e., not i.v. injection); the study reported at least two groups; and participants were followed up for at least 12 weeks.

2.1.2 Data extraction and risk of bias assessment

Although the author planned to use a standardized form to extract data from each included study, there were only two studies to summarize. The Cochrane Collaboration's tool for assessing the risk of bias was used to examine the quality of eligible RCTs (Corbett et al., 2014).

2.2. Retrospective cohort study on the association between alpha-GPC use and 10-year incident stroke risk

2.2.1 Database overview

The retrospective cohort study was derived from the Korean National Health Insurance Service (NHIS). In Korea, all citizens are required to enrol in the NHIS for health insurance, which covers nearly all forms of health services (Bahk et al., 2017). The NHIS collects and maintains information on all insured health services for health claim purposes and provides part of the database for research purposes. The health services include information on all inpatient and outpatient department visits, laboratory examinations, diagnostic and surgical procedures, and pharmaceutical prescriptions. Moreover, all citizens aged 40 years, or more are eligible for a biannual health screening examination, which includes the results from a self-reported questionnaire on health behaviours and laboratory blood examinations. The NHIS database has previously been used in several epidemiological studies, and its validity has been described in detail elsewhere (Cheol Seong et al., 2017; Son et al., 2018).

2.2.2. Study population

1) Total study population

Among 13,533,281 men and women aged 50 years or more in 2008, we excluded 54,266 subjects who were prescribed alpha-GPC during 2002–2005. Then, 118,514 subjects with a history of anti-dementia drug use were excluded. Those who died (n = 196,600) or were diagnosed with stroke (n = 549,558) were also excluded. Finally, 605,366 subjects who had transient ischemic attack (TIA) were also excluded, resulting in a final study population of 12,008,977 participants (total cohort) (Figure 2).

Main ingredient codes for the anti-dementia drugs are as follows: 148601ATB and 148602ATB, as donepezil, 385205ACR, 385203ACR, 385204ACR, 385201ATB, and 385202ATB, as galantamine, 224501ACH, 224503ACH, 224504ACH, 224505ACH, 224507CPC, 224508CPC, 224506CPC, and 224502ALQ, as rivastigmine, 190001ATB, 190001BIJ, 190031ALQ, 190001ALQ, 190030ASY, and 190002ASY, as memantine. History or TIA (G45) and stroke (I60-I69) before index date were applied all diagnosis code, including all minor as well as main diagnosis code.

Additionally, for sensitivity analysis, 5,425,467 participants (45% of total population) who underwent health screening examinations with additional information on health behaviours and health status were extracted.

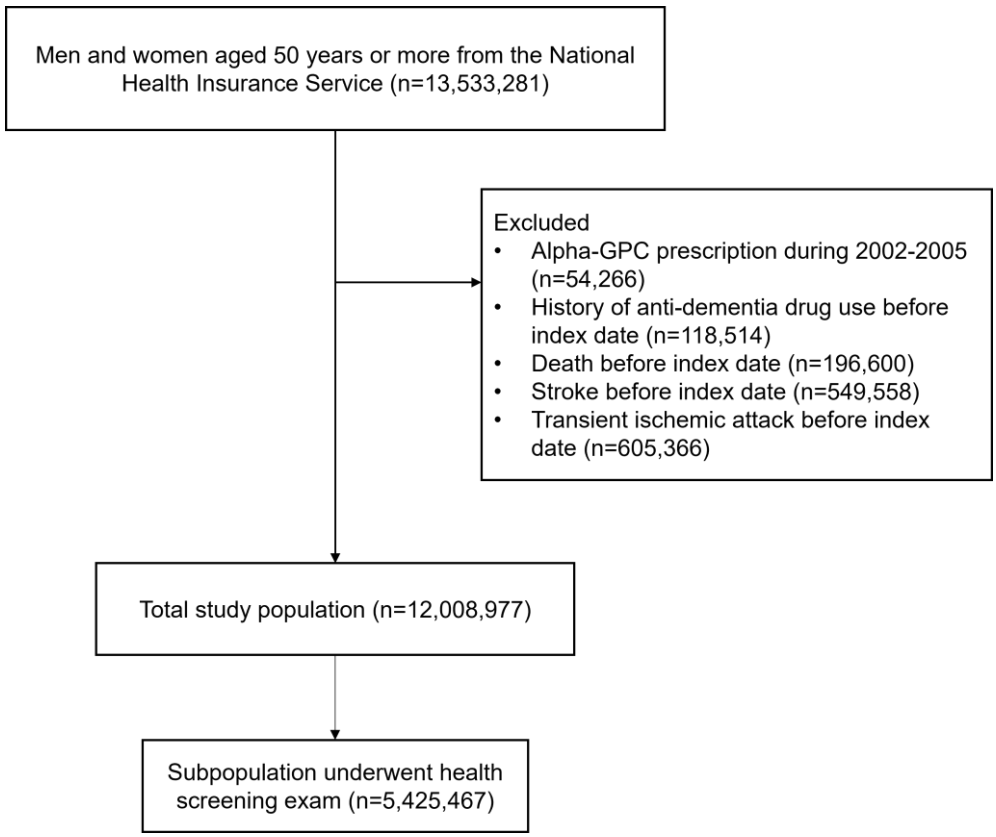

Figure 2. Total study population

Abbreviation: GPC, glycerylphosphorylcholine.

2) Matching population

To minimize the possible confounding effects of covariates, a separate cohort (matched cohort) was created after matching each alpha-GPC user with 10 non-alpha-GPC users according to age, sex, household income, and Charlson comorbidity index (CCI) as comorbidities using the greedy matching method (1:10 age, sex, income, and comorbidity exact matching). After an alpha-GPC non-user (control) was matched with a user (case), that non-user was not reused to match with another user. For sensitivity analysis, 1:1 matching with age, sex, income, and comorbidities was also conducted.

Among 108,877 alpha-GPC new users during 2006–2008 without previous history of anti-dementia drug use, stroke, or TIA, 661 users who were not matched by age, sex, income, and CCI with alpha-GPC non-users were excluded. Then, 108,216 alpha-GPC users were 1:10 matched with 1,082,160 alpha-GPC non-users, also without a history of anti-dementia drug use, stroke, or TIA, resulting in a matched cohort of 1,190,376 subjects (Figure 3).

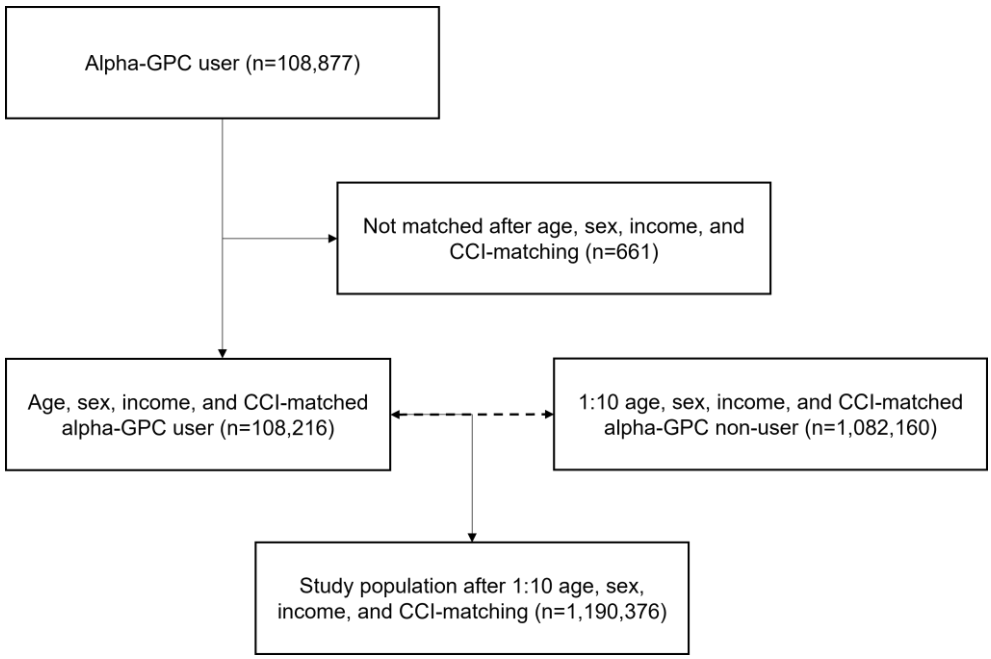

Figure 3. Matching cohort generation

Abbreviation: GPC, glycerylphosphorylcholine; CCI, Charlson comorbidity index

2.2.3. Study design

All participants were divided according to alpha-GPC use within 3 years prior to the index date (2006–2008) and then followed up from January 1, 2009, until the date of stroke event, death, or January 31, 2018, whichever was the earliest date (Figure 4). This study adhered to the guidelines set by the STROBE statement for reporting observational studies in epidemiology (Von Elm et al., 2014).

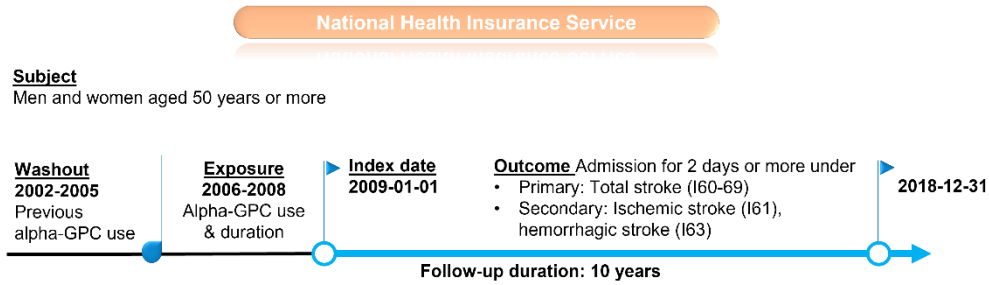

Figure 4. Study design

2.2.4. Study variables

1) Exposures

Alpha-GPC use was determined during 2006–2008, within 3 years prior to the index date. Records of pharmaceutical prescriptions were used to determine alpha-GPC use, and alpha-GPC users were defined as those who were prescribed alpha-GPC for at least one day. Alpha-GPC users were further divided into those who had <2, 2–6, 6–12, and ≥12 months of alpha-GPC intake during 2006–2008. For alpha-GPC use that extended beyond 2008, only prescription days up to December 31, 2008, were counted.

2) Outcomes

Stroke was defined as hospitalization for two or more days, with stroke being the main diagnosis according to the International Classification of Diseases, Tenth Edition (ICD-10) codes. The ICD-10 codes for stroke (I60-I69) were in accordance with the guidelines of the American Heart Association (Writing Group et al., 2016). Along with the risks of stroke, the risks of ischemic stroke (I61) and hemorrhagic stroke (I63) were also determined. The ICD-10 codes for ischemic and hemorrhagic stroke were adopted from a previous study, which also used the NHIS database to define stroke outcomes (Song & Cho, 2008).

3) Covariates

Covariates included age (continuous, years), sex (categorical, men and women), household income (categorical, 1st, 2nd, 3rd, and 4th quartiles), and CCI for the total cohort.

The additional covariates were from health screening examinations, which included smoking (categorical, never, past, and current smokers), alcohol consumption (categorical, 0, 1–2, 3–4, and ≥5 times per week), physical activity (categorical, 0, 1-2, 3-4, and ≥5 times per week), body mass index (BMI, continuous, kg/m^2), presence of hypertension, diabetes mellitus, and dyslipidemia (categorical, yes or no).

Hypertension defined as being prescribed anti-hypertensive medication for hypertension (ICD-10 codes: I10) or having blood pressure levels of ≥140/90 mmHg. Diabetes defined as being prescribed anti-diabetic medication for diabetes (ICD-10 codes: E11-E14) or having fasting serum glucose levels of ≥126 mg/dL. Dyslipidemia defined as being prescribed statin medication for dyslipidemia (ICD-10 codes: E78) or having total cholesterol levels of ≥240 mg/dL.

2.2.5. Statistical analysis

1) General analysis of total and matching cohort

Upon comparing the descriptive characteristics of the study population according to alpha-GPC use, the t-test was used for continuous variables and the Chi-squared test for categorical variables. The adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs) for stroke according to alpha-GPC use were determined by multivariate Cox proportional hazards regression after adjustments for age, sex, household income, and CCI for the total cohort.

The risk for stroke was determined by HRs with 95% CI without adjustments for the matched cohort, as all covariates were matched between alpha-GPC users and non-users. Among alpha-GPC users, the risk of stroke according to alpha-GPC prescription duration was determined.

2) Sensitivity and stratified analysis of total or matching cohort

Competing risk analysis using the cause-specific hazard model regression was conducted for sensitivity analysis. Using death, ischemic stroke (for hemorrhagic stroke assessment), and hemorrhagic stroke (for ischemic stroke assessment) as competing events, the association of alpha-GPC use with stroke risk and number of days of alpha-GPC use with stroke was determined.

For alpha-GPC users, HR values for stroke per one interquartile

range (IQR) increase in alpha-GPC prescription days were determined.

A stratified analysis on the association of alpha-GPC uses with stroke according to subgroups of age, household income and CCI was conducted.

3) Sensitivity analysis of subpopulation underwent health screening test

Hazard ratios for all factors in the prediction model for total stroke were analysed among those who underwent health screening examinations. Sensitivity analyses on the association of alpha-GPC use with the risk of stroke among participants who underwent health screening examinations or after exclusion of participants with stroke events within the first 1 to 4 years of follow-up were conducted. The additional covariates considered upon determination of the association of alpha-GPC use with the risk of stroke among those who underwent health screening examinations, included smoking, alcohol consumption, physical activity, BMI, and presence of hypertension, diabetes mellitus, and dyslipidemia.

Statistical significance was determined in a two-sided manner with a p-value < 0.05. All data collection and statistical analyses were conducted using SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA).

2.2.6. Ethical approval

This study was conducted according to the guidelines in the Declaration of Helsinki and approved by the Seoul National University Hospital Institutional Review Board (IRB number: E-1812-041-933). All participants were informed of the objective of the survey, and they provided their consent. The Korean NHIS database was anonymized according to strict confidentiality guidelines prior to distribution by the NHIS.

Chapter 3. Results

3.1. Systematic review of clinical trials on oral alpha-GPC

3.1.1. Study characteristics of reviewed trials

Figure 5 shows the flow diagram of this study according to the PRISMA statement. The literature search identified 466 reports. After screening the titles and abstracts, 363 reports did not meet the predetermined selection criteria, including study design (study protocol, review, animal experiment study, or case report), study population (epilepsy, tumor, ocular disease, hepatic encephalopathy, bipolar or schizophrenia), and other drugs. The full texts of the remaining 39 reports were reviewed, and 37 reports were excluded (Table 4).

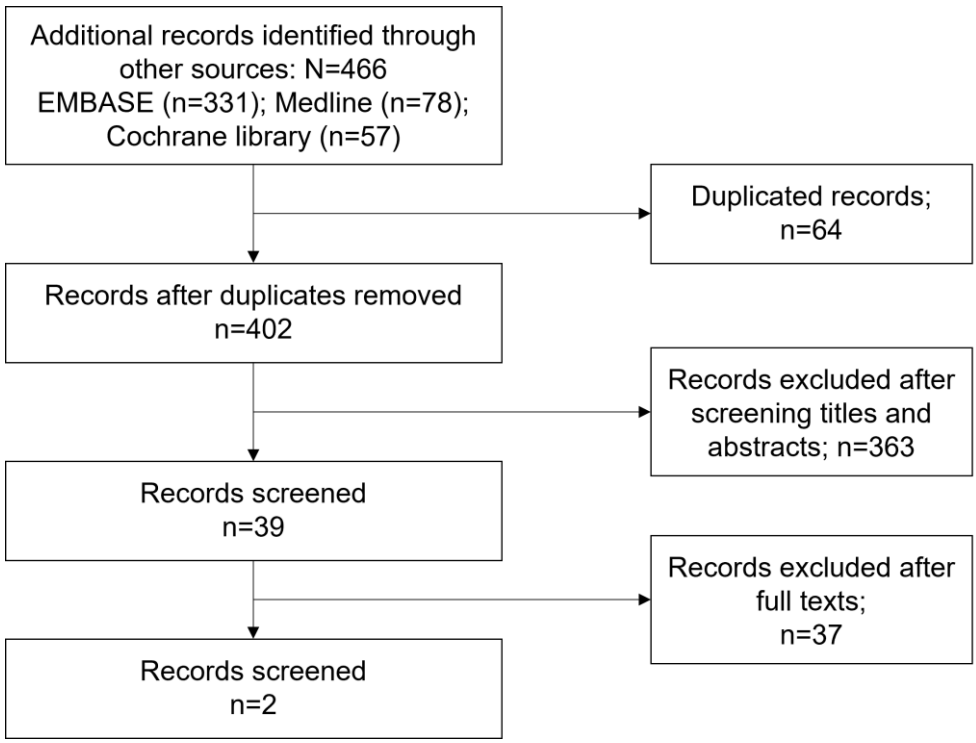

Figure 5. PRISMA diagram

Table 4. The information of excluded reports and exclusion reason

#	Title	Author, year	Journal	Exclusion reason
1	Nootropic therapy of cerebral aging	Abbati et. al., 1991	Advances in therapy	•CA IM vs. oxiracetam IM •not available full text
2	The ASCOMALVA trial: association between the cholinesterase inhibitor donepezil and the cholinergic precursor choline alphoscerate in Alzheimer's disease with cerebrovascular injury: interim results	Amenta et. al., 2012	Journal of the neurological sciences	•ASCOMALVA interim results
3	Diagnosis and treatment of neurogenic dysphagia after acute ischemic stroke	Balashova et. al., 2018	Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova	•10 days acute intervention after stroke
4	alpha-Glycerophosphocholine in the mental recovery of cerebral ischemic attacks. An Italian multicenter clinical trial	Barbagallo Sangiorgi et. al., 1994	Annals of the New York Academy of Sciences	•single arm •28 days (IM)+5 months (PO) •after acute stroke or TIA
5	The use of cereton in the rehabilitation of patients with hemorrhagic stroke	Builova et al, 2009	Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova	•15 days acute intervention after stroke •cereton
6	The Effect of the Association between Donepezil and Choline Alphoscerate on Behavioral Disturbances in Alzheimer's Disease: interim Results of the ASCOMALVA Trial	Carotenuto et al, 2017	Journal of Alzheimer's disease	•ASCOMALVA interim results •Behavioral Disturbances outcomes
7	Effect of choline alphoscerate on cognition in patients with mild-to-moderate Alzheimer's disease treated with a cholinesterase inhibitor therapy	Cumbo et al, 2013	Functional neurology	•Cochrane Conference Abstract •not available data

8	A multicentre trial to evaluate the efficacy and tolerability of alpha-glycerylphosphorylcholine versus cytosine diphosphocholine in patients with vascular dementia	Di Perri et al, 1991	Journal of international medical research	•CA IM vs. cytosine diphosphocholine IM •open trial
9	Multicenter clinical comparison of the effects of choline alfoscerate and cytidine diphosphocholine in the treatment of multi-infarct dementia	Frattola et al, 1998	Current therapeutic research - clinical and experimental	•CA IM vs. cytosine diphosphocholine IM •open trial
10	A comparative study of free plasma choline levels following intramuscular administration of L-alpha-glycerylphosphorylcholine and citicoline in normal volunteers	Gatt et al, 1992	International journal of clinical pharmacology, therapy, and toxicology	•pharmacodynamic study following intramuscular administration
11	[Clinical efficacy and safety of choline alfoscerate in the treatment of late-onset cognitive impairment]	Gavrilova et al, 2018	Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova	•single arm •not available full text
12	P300 latency changes in patients with mild cognitive impairment after taking choline alphoscerate; A preliminary study	Han et al, 2018	eNeurologicalSci	•single arm • a preliminary study
13	Efficacy of cereton in the acute period of ischemic stroke	Ismagilov et al, 2009	Zhurnal Nevrologii i Psihiatrii imeni S.S. Korsakova	•21 days acute intervention after stroke
14	Efficacy of cereton in acute ischemic stroke: results of the trial SOLNTSE	Kamchatnov et al, 2012	Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova	•21 days acute intervention after stroke
15	[Analysis of choline alfoscerate effectiveness in chronic ocular ischemic syndrome]	Kamilov et al, 2016	Vestnik Oftalmologii	•Russian language •patients with chronic ocular ischemic syndrome

16	Effect of choline alfoscerate on cognitive dysfunction after mild traumatic brain injury, prospective case-control study	Kang et al, 2018	Journal of Neurotrauma	•conference abstract •not available full text
17	[The use of cerepro (choline alfoscerate) in the treatment of outpatients with chronic progressive cerebrovascular disease]	Kostenko et al, 2012	Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova	•Russian language •after strke
18	A comparative study of addition therapy of choline alfoscerate and omega 3 fatty acid in older depressed patients with or without executive dysfunction	Kwak et. al., 2013	International Psychogeriatrics	•conference abstract •not available full text
19	Efficacy and tolerability of choline alphoscerate (cereton) in patients with Parkinson's disease with cognitive disorders	Levin et al., 2009	Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova	•an open 10-day study of treatment effect
20	Efficacy and safety of choline alphoscerate (cereton) in patients with parkinson's disease with cognitive impairments	Levin et al., 2011	Neuroscience and behavioral physiology	•an open 10-day study of treatment effect
21	The Optimization of Cortical Function in Patients with Severe Head Injury During Gliatiline (Alpha-GPC) Treatment	Madorsky, 1995	8th european college of neuropsychopharmacology congress.	•conference abstract •not available full text
22	Evaluation of the effects of two doses of alpha glycerylphosphorylcholine on physical and psychomotor performance	Marcus et al., 2017	Journal of the international society of sports nutrition	•college aged males
23	[The use of cereton in ischemic stroke]	Maslova et al., 2008	Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova	•Russian language •not available full text

24	A neurotropic approach to the treatment of multi-infarct dementia using L-α-glycerylphosphorylchlorine	Muratorio A et. al., 1992	Current Therapeutic Research - Clinical and Experimental	•IM L-choline alfoscerate vs. IM GDP-choline
25	The Efficacy of Gliatiline® on Post-stroke Patients With Vascular Cognitive Impairment no Dementia	NCT01363648	Not available	•No results although ending the study in March, 2013 •Clinicaltrials.gov data
26	A Clinical Trial for an Evaluation of Choline Alfoscerate and Donepezil for Cognitive Improvements of Patients With Cerebrovascular Injury in Alzheimer Patients	NCT02648906	Not available	•No results although ending the study in November, 2018 •Clinicaltrials.gov data
27	Effect of Choline Alphoscerate on Cognitive Function in Alzheimer's Dementia	NCT03441516	Not available	•ongoing study •Clinicaltrials.gov data •open trial
28	Multicentre study of l-alpha-glyceryl-phosphorylcholine vs ST200 among patients with probable senile dementia of Alzheimer's type	Parnetti et al., 1993	Drugs aging	•choline alfoscerate vs. acetyl-l-carmitine •not available full text
29	The use of cereton in patients with chronic brain ischemia and moderate cognitive impairment	Pizova et al., 2014	Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova	•Russian language •not available full text •single arm
30	Clinical efficacy and safety of choline alfoscerate in the treatment of predementia cognitive impairment in late life	Ponomareva et al., 2019	European Journal of Neurology	•Russian language •not available full text •single arm
31	Apathy Treatment in Alzheimer's Disease: interim Results of the ASCOMALVA Trial	Raffaele et al., 2015	Journal of Alzheimer's disease	•ASCOMALVA interim results

32	The domestic drug cereton in the treatment of patients in the acute period of ischemic stroke	Shmyrev et al., 2008	Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova	•Russian language •not available full text •acute intervention period
33	Choline alfoscerate: Short-term effect on sleep in healthy subjects	Strambi et al, 1991	Current Therapeutic Research - Clinical and Experimental	•short-term effect on sleep
34	Choline alphoscerate (ceretone) in the treatment of patients with chronic cerebral ischemia	Stulin et al., 2009	Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova	•Russian language •not available full text
35	Influence of neuroprotectors with choline-positive action on the level of brain-injury markers during acute ischemic stroke	Vaizova et al., 2012	Eksperiment al'naia i klinicheskaia farmakologiia	•pharmacokinetic study
36	The use of choline alfoscerate (gliatiline) in patients with ischemic stroke	Vinogradov et al., 2013	Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova	•Russian language •not available full text
37	Improvement of cognitive function in early stage of vascular cognitive impairment (VCI) after treatment with the acetylcholine precursor choline alfoscerate	Yang et al., 2017	Cerebrovascular diseases (basel, switzerland)	•conference abstract •not available full text

Two RCTs were selected on the effect of oral alpha-GPC on AD (Amenta et al., 2014; De Jesus Moreno Moreno, 2003), and Tables 5 and 6 summarize the characteristics. Moreno (2003) reported the dramatic effect of alpha-GPC on cognitive dysfunction. The mean change in the Mini-Mental State Examination (MMSE) score in the placebo group was -0.5 (from 17.62 at the baseline to 17.12 on Day 180) and that in the alpha-GPC intervention group was +6.33 (from 18.19 at the baseline to 24.52 on Day 180).

Table 5. Detailed information on the selected randomized controlled trial I (De Jesus Moreno Moreno, 2003).

Title	Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial
Author, year	Moreno, 2003
Journal	Clinical therapeutics
Country	Mexico
Study design	multicenter, double-blind, randomized, placebo-controlled
Diseases of participants	Outpatients with a history of mild-to-moderate Alzheimer's disease
Intervention	CA 400mg capsule orally 3 times a day for 6 months
Placebo	reference treatment including ChE inhibotors
Population	Total N=261; CA (n=132) vs. PLB (n=129)
Baseline characteristics	

Groups	CA intervention	PLB
Gender	T132; F105; M27	T129; F94; M35
Age, years	72.2 (7.5) range: 60-80	71.4 (7.4) range 60-80
MMSE at baseline	18.19 (3.38)	17.62 (3.43)

Acronyms/abbreviations: MMSE; Mini-Mental State Examination; CA: choline alfoscerate; PLB: placebo; ChE: cholinesterase; T: total; F: female; M: male

Another double-blind multicenter trial was conducted in Italy (Amenta et al., 2012; Amenta et al., 2014). Active treatment using the AChE inhibitors, donepezil and alpha-GPC, may delay progressive decline compared with treatment using donepezil alone in 113 participants with AD and cerebrovascular injury after a 12-month and 24-month observation period (Amenta et al., 2012; Amenta et al., 2014).

Table 6. Detailed information on the selected randomized controlled trial II. ASCOMALVA trial

Title	The ASCOMALVA (Association between the Cholinesterase Inhibitor Donepezil and the Cholinergic Precursor Choline Alphoscerate in Alzheimer's Disease) Trial: interim results after two years of treatment
Author, year	Amenta et. al., 2014
Journal	Journal of Alzheimer's disease
Country	Italy
Study design	multicenter, randomized, placebo-controlled, double-blind
Diseases of participants	Alzheimer's disease with concomitant cerebrovascular damage
Intervention	Donepezil+choline alfoscerate
Placebo	Donepezil+placebo
Population	Total N=113; CA+donepezil (n=57) vs. PLB+donepezil (n=56)
Baseline characteristics	

Groups	CA+donepezil	PLB+donepezil
Gender	T57; F33; M24	T56; F34; M22
Age, years	76 (8)	78 (5)
Education, years	7 (3)	8 (5)
MMSE at baseline	20.3 (2.9)	19.9 (3.1)
24-21	42.9 %	37.5 %
20-18	39.3 %	25.0 %
<17	17.9 %	37.5%

Acronyms/abbreviations: CA: choline alfoscerate; PLB: placebo; T: total; F: female; M: male; MMSE; Mini-Mental State Examination

3.1.2. Risk of bias in two selected trials

Tables 7 and 8 show risk of bias examined using the Cochrane Collaboration's tool. Although two trials reported the study design as a double-blind RCT, there are possibilities of some bias.

Table 7. Risk of bias of the selected randomized controlled trial I (De Jesus Moreno Moreno, 2003).

Bias domain	Source of bias and judgement	Its reason
Selection bias	Random sequence generation: unclear	- Expression “blocks of 4 patients” is unclear because there were any information on the other patients rnadomization. - Quote: “Patients were randomized to receive CA or placebo. Randomization was done in blocks of 4 patients, and allocation to the active-treatment or the placebo group was done according to tables of random numbers.”
	Allocation concealment: unclear	- Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned.
Performance bias	Blinding of participants and personnel: Low (good)	- Quote: “Double-Blind Clinical Trial …”
Detection bias	Blinding of outcome assessment: unclear	- Not stated
Attrition bias	Incomplete outcome data: Low (good)	- Quote: “All 261 (100.0%) enrolled patients were considered for intent-to-treat (ITT) analysis,~”
Reporting bias	Selective reporting: Low (good)	All prespecified outcome reported.

Table 8. Risk of bias of the selected randomized controlled trial II. ASCOMALVA trial

Bias domain	Source of bias and judgement	Its reason
Selection bias	Random sequence generation: unclear or high (bad)	- Quote: “Randomization lists were prepared in groups of 20 subjects per centre. Hence, each centre could work independently with the advantage of an easy possible combination of the results obtained. This WEB-based procedure was followed also for economic reasons, being ASCOMALVA a clinical study of spontaneous generation.”
	Allocation concealment: unclear	- Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned.
Performance bias	Blinding of participants and personnel: Low (good)	- Quote: “Double-Blind Clinical Trial …”
Detection bias	Blinding of outcome assessment: unclear	- Not stated
Attrition bias	Incomplete outcome data: unclear or high (bad)	- Missing data have not been imputed using appropriate methods; there was no intent-to-treat (ITT) analysis.
Reporting bias	Selective reporting: Low (good)	All prespecified outcome reported.

3.2. The association between alpha-GPC use and 10-year incident stroke risk of total and matching cohort

3.2.1 Baseline characteristics

Table 1 shows the descriptive characteristics of the study population. The numbers of alpha-GPC non-users and users were 11,900,100 and 108,877, respectively. The mean (standard deviation, SD) ages for non-users and users were 61.6 (9.4) and 68.3 (10.0) years, respectively. Alpha-GPC users tended to be older (mean 68.3 vs. 61.6 years) and had lower household income (lowest quartile 33.7% vs. 24.1%) and more comorbidities (CCI ≥ 2, 65.7% vs. 29.5%) than non-users in the total cohort (all $p < 0.001$). Among the matched cohort, there was no difference in distribution for the matched variables, including age, sex, income, and CCI (all $p = 1.000$).

Table 9. Descriptive characteristics of the study population

	Alpha-GPC Non-user	Alpha-GPC User	*p* value
Total cohort			
Number of participants	11,900,100	108,877	
Age, years, mean (SD)	61.6 (9.4)	68.3 (10.0)	<0.001
Sex, N (%)			
Men	5,568,179 (46.8)	38,833 (35.7)	<0.001
Women	6,331,921 (53.2)	70,044 (64.3)	
Household income, quartiles, N (%)			
1st (highest)	4,124,629 (34.7)	36,273 (33.3)	<0.001
2nd	2,778,519 (23.4)	21,319 (19.6)	
3rd	2,131,128 (17.9)	14,561 (13.4)	
4th (lowest)	2,865,824 (24.1)	36,724 (33.7)	
Charlson comorbidity index			
0	5,496,687 (46.2)	13,812 (12.7)	<0.001
1	2,898,511 (24.4)	23,486 (21.6)	
≥2	3,504,902 (29.5)	71,579 (65.7)	
Matched cohort			
Number of participants	1,082,160	108,216	
Age, years, mean (SD)	68.2 (9.9)	68.2 (9.9)	1.000
Sex, N (%)			
Men	386,170 (35.7)	38,617 (35.7)	1.000
Women	695,990 (64.3)	69,599 (64.3)	
Household income, quartiles, N (%)			
1st (highest)	361,900 (33.4)	36,190 (33.4)	1.000
2nd	212,300 (19.6)	21,230 (19.6)	
3rd	144,870 (13.4)	14,487 (13.4)	
4th (lowest)	363,090 (33.6)	36,309 (33.6)	
Charlson comorbidity index			
0	138,120 (12.8)	13,812 (12.8)	1.000

1	234,810 (21.7)	23,481 (21.7)
≥2	709,230 (65.5)	70,923 (65.5)

p value calculated by the t-test for continuous variables and the Chi-squared test for categorical variables.
Acronyms: alpha-GPC, L-alpha glycerylphosphorylcholine; SD, standard deviation; N, number of participants.

3.2.2 The association between alpha-GPC and risk of stroke among total and matching cohort

The results of the association of alpha-GPC use with the risk of stroke within the total and matched cohorts are summarized in Table 10. Those who were prescribed alpha-GPC had a higher risk for total stroke (aHR 1.46, 95% CI 1.43–1.48), ischemic stroke (aHR 1.36, 95% CI 1.33-1.39), and hemorrhagic stroke (aHR 1.36, 95% CI 1.28-1.44) than alpha-GPC non-users. Alpha-GPC users had a higher risk for total stroke than non-users among men (aHR 1.56, 95% CI 1.52-1.60) and women (aHR 1.39, 95% CI 1.36-1.42).

Within the matched cohort, alpha-GPC use was associated with higher risk for total stroke (aHR 1.43, 95% CI 1.41-1.46), ischemic stroke (aHR 1.34, 95% CI 1.31-1.37), and hemorrhagic stroke (aHR 1.37, 95% CI 1.29-1.46).

Similar associations were observed upon competing risk analysis via cause-specific hazard model regression (Table 11).

Table 10. Hazard ratios for stroke according to alpha-GPC use among total and matching cohort

	Total		Men		Women	
	Non-user	User	Non-user	User	Non-user	User
Total cohort						
Total stroke						
Events	745,589	14,138	362,154	5,426	383,435	8,712
Person-years	107,830,473	870,174	49,775,056	295,203	58,055,417	574,970
aHR (95% CI)	1.00 (reference)	1.46 (1.43-1.48)	1.00 (reference)	1.56 (1.52-1.60)	1.00 (reference)	1.39 (1.36-1.42)
Ischemic stroke						
Events	446,469	8,342	233,444	3,311	213,025	5,031
Person-years	107,830,473	870,174	49,775,056	295,203	58,055,417	574,970
aHR (95% CI)	1.00 (reference)	1.36 (1.33-1.39)	1.00 (reference)	1.44 (1.39-1.49)	1.00 (reference)	1.30 (1.27-1.34)
Hemorrhagic stroke						
Events	69,376	1,089	35,767	413	33,609	676
Person-years	107,830,473	870,174	49,775,056	295,203	58,055,417	574,970
aHR (95% CI)	1.00 (reference)	1.36 (1.28-1.44)	1.00 (reference)	1.39 (1.26-1.53)	1.00 (reference)	1.34 (1.24-1.44)
Matched cohort						
Total stroke						
Events	101,067	14,056	36,623	5,407	64,444	8,649
Person-years	8,939,584	867,451	3,072,198	294,503	5,867,386	572,948
aHR (95% CI)	1.00 (reference)	1.43 (1.41-1.46)	1.00 (reference)	1.54 (1.50-1.58)	1.00 (reference)	1.38 (1.34-1.41)
Ischemic stroke						
Events	63,895	8,295	24,185	3,299	39,710	4,996
Person-years	8,939,584	867,451	3,072,198	294,503	5,867,386	572,948
aHR (95% CI)	1.00 (reference)	1.34 (1.31-1.37)	1.00 (reference)	1.42 (1.37-1.48)	1.00 (reference)	1.29 (1.25-1.33)
Hemorrhagic stroke						
Events	8,139	1,083	3,102	412	5,037	671
Person-years	8,939,584	867,451	3,072,198	294,503	5,867,386	572,948
aHR (95% CI)	1.00 (reference)	1.37 (1.29-1.46)	1.00 (reference)	1.38 (1.25-1.53)	1.00 (reference)	1.37 (1.26-1.48)

Acronyms: alpha-GPC, L-alpha glycerylphosphorylcholine; SD, standard deviation; N, number of participants.

Table 11. Hazard ratios for stroke according to alpha-GPC use with competing risk analysis via cause-specific hazard model regression

	Total		Men		Women	
	Non-user	User	Non-user	User	Non-user	User
Total cohort						
Total stroke						
Events	745,589	14,138	362,154	5,426	383,435	8,712
Person-years	107,830,473	870,174	49,775,056	295,203	58,055,417	574,970
aHR (95% CI)	1.00 (reference)	1.46 (1.43-1.48)	1.00 (reference)	1.56 (1.52-1.60)	1.00 (reference)	1.39 (1.36-1.42)
Ischemic stroke						
Events	446,469	8,342	233,444	3,311	213,025	5,031
Person-years	107,830,473	870,174	49,775,056	295,203	58,055,417	574,970
aHR (95% CI)	1.00 (reference)	1.36 (1.33-1.39)	1.00 (reference)	1.44 (1.39-1.49)	1.00 (reference)	1.30 (1.27-1.34)
Hemorrhagic stroke						
Events	69,376	1,089	35,767	413	33,609	676
Person-years	107,830,473	870,174	49,775,056	295,203	58,055,417	574,970
aHR (95% CI)	1.00 (reference)	1.36 (1.28-1.44)	1.00 (reference)	1.38 (1.25-1.52)	1.00 (reference)	1.34 (1.24-1.44)
Matched cohort						
Total stroke						
Events	101,067	14,056	36,623	5,407	64,444	8,649
Person-years	8,939,584	867,451	3,072,198	294,503	5,867,386	572,948
aHR (95% CI)	1.00 (reference)	1.44 (1.42-1.47)	1.00 (reference)	1.56 (1.51-1.60)	1.00 (reference)	1.38 (1.35-1.41)
Ischemic stroke						
Events	63,895	8,295	24,185	3,299	39,710	4,996
Person-years	8,939,584	867,451	3,072,198	294,503	5,867,386	572,948
aHR (95% CI)	1.00 (reference)	1.35 (1.32-1.38)	1.00 (reference)	1.45 (1.40-1.50)	1.00 (reference)	1.29 (1.25-1.33)
Hemorrhagic stroke						
Events	8,139	1,083	3,102	412	5,037	671
Person-years	8,939,584	867,451	3,072,198	294,503	5,867,386	572,948
aHR (95% CI)	1.00 (reference)	1.41 (1.32-1.50)	1.00 (reference)	1.43 (1.29-1.58)	1.00 (reference)	1.39 (1.29-1.51)

Adjusted hazard ratios calculated by cause-specific hazard model regression after adjustments for age, sex, household income, and Charlson comorbidity index.
Competing events for total stroke include death.
Competing events for ischemic stroke include death and hemorrhagic stroke.
Competing events for hemorrhagic stroke include death and ischemic stroke.
Acronyms: aHR, adjusted hazard ratio; CI, confidence interval.

3.2.3 The association between alpha-GPC prescription duration and risk of stroke

Figure 5 depicts the risk of stroke with alpha-GPC prescription duration among alpha-GPC users. Those with 2-6 (aHR 1.13, 95% CI 1.09-1.18), 6-12 (aHR 1.18, 95% CI 1.12-1.24), and >12 (aHR 1.36, 95% CI 1.29-1.43) months of alpha-GPC use had a higher risk for stroke in a dose-responsive manner than those who were prescribed alpha-GPC for less than two months (p for trend < 0.001).

Similarly, participants with 2-6 (aHR 1.10, 95% CI 1.05-1.17), 6-12 (aHR 1.15, 95% CI 1.08-1.23), and >12 (aHR 1.37, 95% CI 1.28-1.46) months of alpha-GPC use had a higher risk for ischemic stroke than those with <2 months of use (p for trend < 0.001).

Finally, those with 2-6 (aHR 1.23, 95% CI 1.06-1.44), 6-12 (aHR 1.07, 95% CI 0.89-1.29), and >12 (aHR 1.34, 95% CI 1.11-1.61) months of alpha-GPC prescription had a higher risk of hemorrhagic stroke than the users with <2 months of use (p for trend 0.003).

Similar associations were observed upon competing risk analysis via cause-specific hazard model regression (Figure 6).

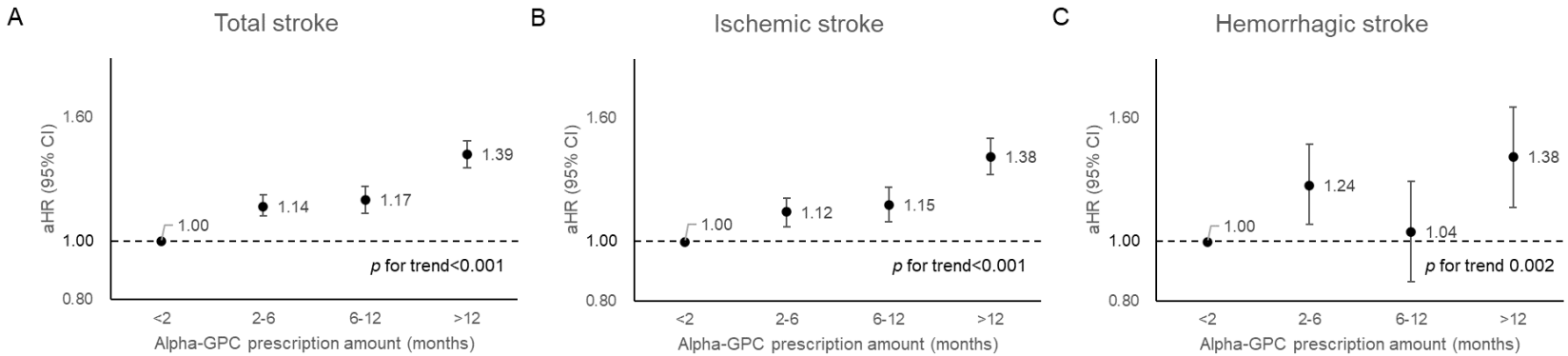

Figure 6. Hazard ratios for stroke according to alpha-GPC prescription duration among those who were prescribed alpha-GPC.

Adjusted hazard ratios calculated by Cox proportional hazards regression after adjustments for age, sex, household income, and Charlson comorbidity index.
Acronyms: alpha-GPC, L-alpha glycerylphosphorylcholine; aHR, adjusted hazard ratio; CI, confidence interval.
This result was generated with S. Choi.

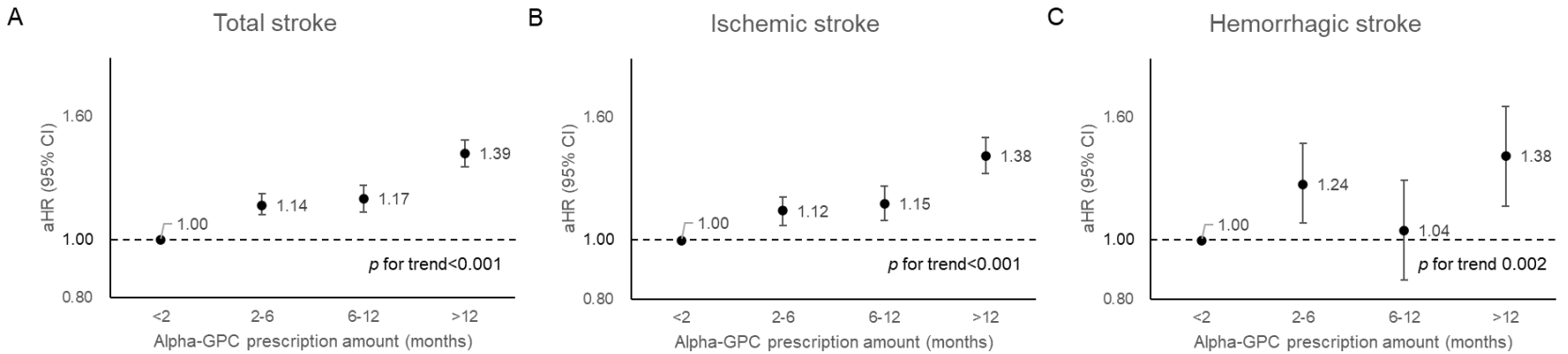

Figure 7. Hazard ratios for stroke according to alpha-GPC prescription duration among those who were prescribed alpha-GPC with competing risk analysis via cause-specific hazard model regression.

Adjusted hazard ratios calculated by cause-specific hazard model regression after adjustments for age, sex, household income, and Charlson comorbidity index.
Acronyms: alpha-GPC, L-alpha glycerylphosphorylcholine; aHR, adjusted hazard ratio; CI, confidence interval.
This result was generated with S. Choi.

3.2.4 The stratified analysis on the association between alpha-GPC and the risk of stroke among total cohort

The results of the stratified analysis on the association of alpha-GPC use with the risk of total stroke according to subgroups of household income and CCI within the total cohort are summarized in Table 12. Users within the upper (aHR 1.44, 95% CI 1.41-1.48) or lower (aHR 1.49, 95% CI 1.46-1.53) half of household income had a higher risk for total stroke. Alpha-GPC users with CCI ≤ 1 (aHR 1.52, 95% CI 1.47-1.56) or ≥2 (aHR 1.49, 95% CI 1.46-1.52) both had a higher risk for total stroke than non-users. Similar results were observed for men and women.

Table 12. Stratified analysis on the association of alpha-GPC uses with total stroke risk among subgroups of age, household income, and Charlson comorbidity index within the total cohort.

	Total		Men		Women	
	Non-user	User	Non-user	User	Non-user	User
Age, years						
<65	1.00 (reference)	1.79 (1.73-1.86)	1.00 (reference)	1.85 (1.76-1.95)	1.00 (reference)	1.73 (1.65-1.82)
≥65	1.00 (reference)	1.52 (1.49-1.55)	1.00 (reference)	1.66 (1.61-1.72)	1.00 (reference)	1.45 (1.41-1.48)
Household income						
Upper half	1.00 (reference)	1.44 (1.41-1.48)	1.00 (reference)	1.51 (1.46-1.57)	1.00 (reference)	1.39 (1.35-1.44)
Lower half	1.00 (reference)	1.49 (1.46-1.53)	1.00 (reference)	1.64 (1.58-1.71)	1.00 (reference)	1.41 (1.36-1.45)
CCI						
≤1	1.00 (reference)	1.52 (1.47-1.56)	1.00 (reference)	1.59 (1.51-1.67)	1.00 (reference)	1.47 (1.41-1.54)
≥2	1.00 (reference)	1.49 (1.46-1.52)	1.00 (reference)	1.62 (1.57-1.67)	1.00 (reference)	1.43 (1.39-1.46)

Adjusted hazard ratios calculated by Cox proportional hazards regression after adjustments for age, sex, household income, and Charlson comorbidity index.

Acronyms: alpha-GPC, L-alpha glycerylphosphorylcholine; CCI, Charlson comorbidity index

3.2.5 The association between alpha-GPC and stroke among subpopulation underwent health screening examinations

Table 13 shows hazard ratios for all factors in the prediction model for total stroke according to Cox proportional hazards regression among those who underwent health screening examinations. The risk of stroke was significantly higher in alpha-GPC user than non-user, men than women, lower income than higher income, ever-smoker than never-smoker, higher CCI than lower CCI, lower than higher physical activity, higher than lower alcohol use, and presence of diabetes mellitus, hypertension, or dyslipidemia than those without each disease.

Table 13. Hazard ratios for all factors in the prediction model for total stroke according to Cox proportional hazards regression among those who underwent health screening examinations.

	Total	Men	Women
Alpha-GPC Non-user	1.00 (reference)	1.00 (reference)	1.00 (reference)
User	1.48 (1.43-1.52)	1.53 (1.47-1.60)	1.44 (1.39-1.49)
Age, per 1 year increase	1.07 (1.07-1.07)	1.07 (1.07-1.07)	1.07 (1.07-1.07)
Sex			
Men	1.00 (reference)	-	-
Women	0.89 (0.88-0.90)	-	-
Income			
1st (highest)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2nd	1.06 (1.05-1.07)	1.07 (1.06-1.09)	1.05 (1.04-1.06)
3rd	1.09 (1.07-1.10)	1.10 (1.08-1.11)	1.07 (1.05-1.08)
4th (lowest)	1.08 (1.08-1.09)	1.12 (1.10-1.13)	1.04 (1.02-1.05)
CCI, per 1 unit increase	1.08 (1.08-1.08)	1.08 (1.08-1.08)	1.09 (1.08-1.09)
Smoking			
Never smoker	1.00 (reference)	1.00 (reference)	1.00 (reference)
Past smoker	1.00 (0.98-1.01)	0.99 (0.97-1.00)	1.20 (1.14-1.26)
Current smoker	1.46 (1.44-1.47)	1.45 (1.44-1.47)	1.53 (1.49-1.57)
Physical activity, times per week			
0	1.00 (reference)	1.00 (reference)	1.00 (reference)
1-2	0.85 (0.84-0.86)	0.84 (0.83-0.85)	0.86 (0.85-0.87)
3-4	0.81 (0.80-0.82)	0.81 (0.80-0.82)	0.81 (0.80-0.83)
5-6	0.80 (0.78-0.82)	0.80 (0.78-0.82)	0.80 (0.77-0.82)
≥7	0.90 (0.89-0.91)	0.90 (0.88-0.91)	0.90 (0.88-0.92)
Alcohol intake, times per week			
0	1.00 (reference)	1.00 (reference)	1.00 (reference)
0-1	0.97 (0.96-0.98)	0.94 (0.93-0.96)	1.01 (0.99-1.03)
1-2	1.00 (0.99-1.01)	0.98 (0.97-1.00)	1.07 (1.04-1.10)
3-4	1.04 (1.03-1.06)	1.03 (1.01-1.06)	1.07 (1.01-1.13)
≥5	1.16 (1.14-1.18)	1.14 (1.12-1.16)	1.17 (1.10-1.24)

Body mass index, per 1 kg/m^2 increase	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.01 (1.00-1.01)
Diabetes			
No	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	1.35 (1.34-1.36)	1.41 (1.39-1.43)	1.28 (1.25-1.30)
Hypertension			
No	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	1.30 (1.29-1.31)	1.32 (1.31-1.34)	1.27 (1.26-1.28)
Dyslipidemia			
No	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	1.05 (1.04-1.07)	1.13 (1.11-1.15)	1.01 (1.00-1.02)

Acronyms: aHR, adjusted hazard ratio; CI, confidence interval.

The association of alpha-GPC use with the risk of stroke among those who underwent health screening examinations are depicted in Table 14. After multivariable adjustments for health behaviours and health status, including age, sex, household income, Charlson comorbidity index, smoking, alcohol consumption, physical activity, body mass index, diabetes, hypertension, and dyslipidemia, the association between alpha-GPC use and the development of stroke were in line with main results. Users had a higher risk for total stroke (aHR 1.48, 95% CI 1.43-1.52), ischemic stroke (aHR 1.40, 95% CI 1.35-1.46), and hemorrhagic stroke (aHR 1.41, 95% CI 1.27-1.56) than alpha-GPC non-users.

The association of alpha-GPC the risk of stroke among those who underwent health screening examinations are depicted in Table 15. Users had a higher risk for total stroke (aHR 1.34, 95% CI 1.31-1.37), ischemic stroke (aHR 1.26, 95% CI 1.22-1.30), and hemorrhagic stroke (aHR 1.27, 95% CI 1.17-1.39) after excluding those with stroke events within the first 4 years of follow-up.

Table 14. Sensitivity analysis on the association of alpha-GPC use with stroke risk among participants who underwent health screening examinations

	Total		Men		Women	
	Non-user	User	Non-user	User	Non-user	User
Total stroke	1.00 (reference)	1.48 (1.43-1.52)	1.00 (reference)	1.53 (1.47-1.60)	1.00 (reference)	1.44 (1.39-1.49)
Ischemic stroke	1.00 (reference)	1.40 (1.35-1.46)	1.00 (reference)	1.44 (1.37-1.53)	1.00 (reference)	1.36 (1.30-1.43)
Hemorrhagic stroke	1.00 (reference)	1.41 (1.27-1.56)	1.00 (reference)	1.40 (1.19-1.64)	1.00 (reference)	1.40 (1.23-1.60)

Adjusted hazard ratios calculated by Cox proportional hazards regression after adjustments for age, sex, household income, Charlson comorbidity index, smoking, alcohol consumption, physical activity, body mass index, diabetes, hypertension, and dyslipidemia.

Diabetes defined as being prescribed anti-diabetic medication for diabetes (ICD-10 codes: E11-E14) or having fasting serum glucose levels of ≥126 mg/dL.

Hypertension defined as being prescribed anti-hypertensive medication for hypertension (ICD-10 codes: I10) or having blood pressure levels of ≥140/90 mmHg.

Dyslipidemia defined as being prescribed statin medication for dyslipidemia (ICD-10 codes: E78) or having total cholesterol levels of ≥240 mg/dL.

Table 15. Sensitivity analysis on the association of alpha-GPC use with stroke risk after excluding participants with stroke events within the first 1 to 4 years of follow-up within the total cohort.

	Total		Men		Women	
	Non-user	User	Non-user	User	Non-user	User
Total stroke						
1-year	1.00 (reference)	1.41 (1.39-1.44)	1.00 (reference)	1.50 (1.46-1.55)	1.00 (reference)	1.36 (1.33-1.39)
2-year	1.00 (reference)	1.37 (1.35-1.40)	1.00 (reference)	1.46 (1.41-1.51)	1.00 (reference)	1.32 (1.29-1.35)
3-year	1.00 (reference)	1.36 (1.33-1.39)	1.00 (reference)	1.44 (1.39-1.49)	1.00 (reference)	1.31 (1.27-1.34)
4-year	1.00 (reference)	1.34 (1.31-1.37)	1.00 (reference)	1.40 (1.35-1.46)	1.00 (reference)	1.30 (1.26-1.34)
Ischemic stroke						
1-year	1.00 (reference)	1.32 (1.29-1.35)	1.00 (reference)	1.38 (1.33-1.44)	1.00 (reference)	1.26 (1.22-1.30)
2-year	1.00 (reference)	1.29 (1.25-1.32)	1.00 (reference)	1.35 (1.30-1.41)	1.00 (reference)	1.23 (1.19-1.27)
3-year	1.00 (reference)	1.28 (1.24-1.31)	1.00 (reference)	1.35 (1.29-1.41)	1.00 (reference)	1.22 (1.17-1.26)
4-year	1.00 (reference)	1.26 (1.22-1.30)	1.00 (reference)	1.31 (1.25-1.38)	1.00 (reference)	1.21 (1.16-1.26)
Hemorrhagic stroke						
1-year	1.00 (reference)	1.32 (1.24-1.41)	1.00 (reference)	1.34 (1.20-1.49)	1.00 (reference)	1.30 (1.20-1.42)
2-year	1.00 (reference)	1.30 (1.21-1.39)	1.00 (reference)	1.34 (1.19-1.51)	1.00 (reference)	1.27 (1.16-1.39)
3-year	1.00 (reference)	1.29 (1.20-1.40)	1.00 (reference)	1.32 (1.16-1.50)	1.00 (reference)	1.27 (1.15-1.49(
4-year	1.00 (reference)	1.27 (1.17-1.39)	1.00 (reference)	1.24 (1.07-1.43)	1.00 (reference)	1.29 (1.16-1.43)

Adjusted hazard ratios calculated by Cox proportional hazards regression after adjustments for age, sex, household income, and Charlson comorbidity index.

After 1:1 matching, the risk for total stroke, ischemic stroke, and hemorrhagic stroke was significantly higher among alpha-GPC users than in alpha-GPC non-users (Table 16).

Finally, Table 17 depicts the risk of stroke per one IQR increase in alpha-GPC prescription days among those who were prescribed alpha-GPC. For one IQR increase in alpha-GPC prescription days, the risk for total stroke (aHR 1.09, 95% CI 1.07-1.10), ischemic stroke (aHR 1.09, 95% CI 1.07-1.10), and hemorrhagic stroke (aHR 1.06, 95% CI 1.02-1.11) was significantly increased.

Table 16. Hazard ratios for stroke according to alpha-GPC use after 1:1 exact matching

	Total		Men		Women	
	Non-user	**User**	**Non-user**	**User**	**Non-user**	**User**
Number of participants	108,721	108,721	38,779	38,779	69,962	69,9642
Total stroke						
Events	10,198	14,119	3,644	5,418	6,554	8,701
Person-years	895,689	868,874	307,263	294,758	588,426	574,116
aHR (95% CI)	1.00 (reference)	1.43 (1.39-1.47)	1.00 (reference)	1.55 (1.49-1.62)	1.00 (reference)	1.36 (1.32-1.41)
Ischemic stroke						
Events	6,484	8,332	2,435	3,307	4,049	5,025
Person-years	895,689	868,874	307,263	294,758	588,426	574,116
aHR (95% CI)	1.00 (reference)	1.33 (1.29-1.37)	1.00 (reference)	1.42 (1.35-1.50)	1.00 (reference)	1.27 (1.22-1.33)
Hemorrhagic stroke						
Events	834	1,087	316	411	518	676
Person-years	895,689	868,874	307,263	294,758	588,426	574,116
aHR (95% CI)	1.00 (reference)	1.35 (1.23-1.48)	1.00 (reference)	1.36 (1.17-1.57)	1.00 (reference)	1.34 (1.20-1.51)

Alpha-GPC non-users were matched with users via 1:1 exact matching for age, sex, household income, and Charlson comorbidity index.
Adjusted hazard ratios calculated by Cox proportional hazards regression after adjustments for age, sex, household income, and Charlson comorbidity index.
Acronyms: aHR, adjusted hazard ratio; CI, confidence interval.
This result was generated with S. Choi.

Table 17. Hazard ratios for stroke per 1 interquartile range increase in alpha-GPC prescription days among those who were prescribed alpha-GPC

	Total stroke	Ischemic stroke	Hemorrhagic stroke
Number of subjects	108,721	108,721	108,721
Events	14,119	8,332	1,087
Person-years	868,874	868,874	868,874
aHR (95% CI)	1.09 (1.07-1.10)	1.09 (1.07-1.10)	1.06 (1.02-1.11)
p value	<0.001	<0.001	0.004

Adjusted hazard ratios calculated by Cox proportional hazards regression after adjustments for age, sex, household income, and Charlson comorbidity index.
Acronyms: aHR, adjusted hazard ratio; CI, confidence interval.

3.2.6 Alpha-GPC prescription ICD-10 codes during 2006-2008 among those without dementia, stroke, or TIA

Table 18 depicts ICD-10 codes for alpha-GPC prescription among individuals with neither dementia nor cerebrovascular diseases, including stroke and TIA, during 2006-2008.

Table 18. ICD-10 codes for alpha-GPC prescription among individuals without dementia or cerebrovascular diseases, including stroke and TIA, during 2006-2008.

ICD-10	Count	Percentage	Diagnosis
I10	18,068	8.37	Hypertension
K29	16,811	7.79	Gastritis
R42	13,253	6.14	Dizziness and giddiness
E78	7,496	3.47	Dyslipidemia
H81	7,065	3.27	Disorders of vestibular function
I73	6,282	2.91	Other peripheral vascular diseases
G44	5,741	2.66	Other headache syndromes
M54	5,522	2.56	Low back pain
E11	5,112	2.37	Non-insulin-dependent diabetes mellitus
R51	4,980	2.31	Headache
R41	4,795	2.22	Other symptoms and signs involving cognitive functions and awarenes
M17	3,770	1.75	Osteoarthritis of knee
S06	3,525	1.63	Intracranial injury
G43	3,216	1.49	Migraine
G40	3,059	1.42	Epilepsy and recurrent seizures
K25	2,855	1.32	Gastric ulcer
M13	2,615	1.21	Other arthritis
M81	2,512	1.16	Osteoporosis without current pathological fracture
K21	2,378	1.10	Gastro-esophageal reflux disease
M79	2,207	1.02	Other and unspecified soft tissue disorders, not elsewhere classified
K59	2,165	1.00	Constipation, unspecified
K30	2,065	0.96	Functional dyspepsia.
G47	1,934	0.90	Insomnia
K27	1,920	0.89	Peptic ulcer, site unspecified

H93	1,917	0.89	Tinnitus
G64	1,880	0.87	Other disorders of peripheral nervous system.
F48	1,810	0.84	Other nonpsychotic mental disorders
M48	1,803	0.84	Spinal stenosis
M47	1,742	0.81	Spondylosis
E14	1,673	0.78	Unspecified diabetes mellitus
I70	1,661	0.77	Atherosclerosis
M51	1,623	0.75	Thoracic, thoracolumbar, and lumbosacral intervertebral disc disorders
J30	1,615	0.75	Vasomotor and allergic rhinitis
K76	1,563	0.72	Fatty (change of) liver, not elsewhere classified
R11	1,510	0.70	Nausea and vomiting
I11	1,437	0.67	Hypertensive heart disease
I20	1,414	0.66	Angina pectoris
G46	1,386	0.64	Vascular syndromes of brain in cerebrovascular diseases
J20	1,311	0.61	Other acute lower respiratory infections
M75	1,211	0.56	Shoulder lesions
M15	1,190	0.55	Primary generalized (osteo)arthritis
K58	1,135	0.53	Irritable bowel syndrome
M25	1,095	0.51	Other joint disorder, not elsewhere classified
J45	1,085	0.50	Asthma

Table 19. ICD-10 codes for alpha-GPC among individuals without dementia or cerebrovascular diseases, including stroke and TIA, during 2006-2008 (Minor indications less than 0.5%).

ICD-10	Count	Percentage	ICD-10	Count	Percentage	ICD-10	Count	Percentage
G93	1,040	0.48	**M80**	321	0.15	**Z03**	180	0.08
G63	1,007	0.47	**N95**	319	0.15	**R81**	179	0.08
J06	945	0.44	**R10**	319	0.15	**R47**	175	0.08
M19	916	0.42	**E10**	316	0.15	**G59**	174	0.08
G25	867	0.40	**N31**	314	0.15	**H35**	173	0.08
G51	861	0.40	**D33**	304	0.14	**J32**	170	0.08
F45	819	0.38	**J04**	301	0.14	**K75**	165	0.08
J00	791	0.37	**S01**	297	0.14	**G54**	160	0.07
R12	791	0.37	**D64**	296	0.14	**J18**	159	0.07
F51	740	0.34	**N30**	288	0.13	**L03**	159	0.07
N40	704	0.33	**I79**	282	0.13	**L30**	159	0.07
M50	682	0.32	**J41**	272	0.13	**H53**	157	0.07
S13	676	0.31	**I87**	261	0.12	**G95**	155	0.07
M72	670	0.31	**K70**	260	0.12	**M10**	146	0.07
B35	640	0.30	**G91**	258	0.12	**G32**	145	0.07
G81	625	0.29	**L29**	254	0.12	**H36**	145	0.07
I25	623	0.29	**I99**	253	0.12	**D35**	144	0.07
S33	596	0.28	**G52**	249	0.12	**C71**	142	0.07
G62	593	0.27	**T90**	247	0.11	**B18**	141	0.07
J40	561	0.26	**S00**	236	0.11	**J22**	139	0.06
J03	551	0.26	**N39**	235	0.11	**S23**	135	0.06
J44	539	0.25	**E05**	234	0.11	**R05**	134	0.06
K31	518	0.24	**M46**	234	0.11	**R63**	134	0.06
G50	506	0.23	**H91**	231	0.11	**H40**	133	0.06

L23	496	0.23	**B02**	230	0.11	**R52**	132	0.06
K52	468	0.22	**K26**	229	0.11	**H34**	131	0.06
M06	457	0.21	**A09**	224	0.10	**R50**	129	0.06
I50	455	0.21	**G57**	218	0.10	**G58**	126	0.06
I74	453	0.21	**I48**	214	0.10	**R06**	125	0.06
S02	452	0.21	**S22**	211	0.10	**H04**	124	0.06
D32	451	0.21	**E13**	210	0.10	**E83**	121	0.06
R07	419	0.19	**F34**	210	0.10	**K05**	118	0.05
D50	404	0.19	**G53**	210	0.10	**K12**	118	0.05
R25	404	0.19	**I13**	204	0.09	**S93**	113	0.05
G56	401	0.19	**I49**	200	0.09	**E86**	112	0.05
M53	381	0.18	**S32**	200	0.09	**H65**	110	0.05
H90	373	0.17	**G24**	197	0.09	**F10**	109	0.05
R60	373	0.17	**G90**	197	0.09	**H52**	108	0.05
H82	368	0.17	**R73**	195	0.09	**M16**	108	0.05
E07	366	0.17	**R55**	194	0.09	**F33**	105	0.05
R56	363	0.17	**H68**	192	0.09	**J31**	105	0.05
K73	362	0.17	**S43**	192	0.09	**M24**	103	0.05
E03	357	0.17	**M65**	189	0.09	**F38**	102	0.05
J42	356	0.16	**M43**	188	0.09	**R20**	102	0.05
H10	350	0.16	**M62**	187	0.09	**S63**	102	0.05
H60	343	0.16	**I82**	185	0.09	**D43**	101	0.05
L50	343	0.16	**J01**	185	0.09	**M77**	100	0.05
J02	336	0.16	**H66**	183	0.08			
G21	335	0.16	**R00**	182	0.08			

Chapter 4. Discussion

4.1. The association between alpha-GPC and stroke risk

In this large cohort of over 12 million men and women aged ≥50 years who did not have underlying stroke, TIA, and AD at baseline, alpha-GPC use was associated with a higher risk of incident stroke within 10 years in a dose-responsive manner after adjusting for traditional cerebrovascular risk factors. To our knowledge, this is the first study to investigate the long-term severe adverse events of alpha-GPC use.

While no studies have examined the effect of alpha-GPC on stroke, previous studies have determined the association between TMAO from dietary choline or lecithin and stroke. This may be relevant as all oral alpha-GPC is converted to free choline through hydrolysis in the gut mucosa (Traini et al., 2013), and increased plasma levels and highly distributed liver of choline reflect the absorption and metabolism of alpha-GPC (Min et al., 2019).

A meta-analysis of 19 prospective cohorts reported that elevated concentrations of TMAO and its precursors including L-carnitine, choline, or betaine, were associated with increased risks of major adverse cardiovascular events including stroke and all-cause mortality accounting for traditional risk factors (Heianza et al., 2017). A nested case-control study reported that higher TMAO levels were linked to an increased risk of the first stroke among patients with hypertension (Nie et al., 2018). Another case-control study also

reported that patients with ischemic stroke had higher levels of TMAO, suggesting that higher TMAO levels were associated with an increased risk of the first stroke (Rexidamu et al., 2019). A multicentre prospective cohort study reported that increased TMAO levels were associated with an increased risk of new ischemic brain lesions among patients with severe carotid artery stenosis (Wu et al., 2018). A prediction model showed that TMAO was an independent predictor of ischemic stroke among patients with atrial fibrillation (Liang et al., 2019). We also found alpha-GPC use is dose-dependently associated with an increased risk of subsequent stroke after adjustment for traditional cerebrovascular risk factors.

The mechanism by which TMAO induces stroke is not well understood; it is widely established that the TMAO pathway promotes the development of atherosclerosis and thrombosis. First, a metabolomics study reported that dietary supplementation of mice with choline, TMAO, and betaine activates upregulation of expression of multiple macrophage scavenger receptors and subsequent accumulation of cholesterol and foam cells (Wang, Klipfell, Bennett, Koeth, Levison, DuGar, Feldstein, Britt, Fu, & Chung, 2011). Second, an atherogenesis study reported that TMAO promotes proinflammatory changes in arterial walls through mitogen-activated protein kinase and nuclear factor-kB signalling (Seldin et al., 2016).

Lastly, TMAO could directly contribute to platelet hyperreactivity and enhanced thrombosis (Zhu et al., 2016). Taken together, these mechanisms suggest that the alpha-GPC dose-dependent development of

ischemic stroke is plausible. However, these explanations assume that high TMAO is caused by choline but not alpha-GPC itself, although high plasma choline level induced by alpha-GPC has been confirmed (Min et al., 2019). Further studies on the biological and pharmacological mechanism of how alpha-GPC could increase the risk of stroke are warranted.

Additionally, we showed that the risk of hemorrhagic stroke was also increased with alpha-GPC use, although the association was weaker than that of ischemic stroke. Another study also reported that TMAO had a stronger positive association with hemorrhagic stroke than ischemic stroke among patients with hypertension (Nie et al., 2018). The mechanisms behind hemorrhagic stroke include lipohyalinosis of small arteries and fibrinoid necrosis induced by long-standing hypertension, followed by formation of small microaneurysms (Charcot-Bouchard aneurysms) that subsequently rupture (Sierra et al., 2011). When endothelial dysfunction like fibrinoid necrosis occurs, excessive reactive oxygen species production and inflammation are inevitable (Moskowitz et al., 2010). Additive inflammation by TMAO may accelerate hemorrhagic stroke. It is plausible that the previous study showed patients with hypertension were more likely to have hemorrhagic stroke than ischemic stroke according to baseline TMAO levels (Nie et al., 2018). We also found that development of hemorrhagic stroke was associated with the duration of alpha-GPC use, which may be because some but not all subjects had hypertension. Nevertheless, this significant but weak association needs further confirmation.

4.2. Previous randomized controlled trials on oral alpha-GPC

There were only two RCTs on the effects of oral alpha-GPC on cognitive dysfunction (Amenta et al., 2014; De Jesus Moreno Moreno, 2003). However, there is a high likelihood of bias. First, both trials may be prone to selection bias owing to unclear random sequence generation and allocation concealment. Second, Moreno et al. did not explain the placebo medication group clearly and only described it as follows (De Jesus Moreno Moreno, 2003): “Placebo was chosen as the reference treatment because, at the time the study was designed, no reference drug (including ChE inhibitors) was adequately documented as being active in the treatment of dementia.”

Third, the ASCOMALVA trial also has a high risk of attrition bias. Missing data was not imputed using appropriate methods (i.e., there was no intent-to-treat or ITT analysis). Fourth, there was no information on the population number when analyzing the relevant cognitive score after treatment. The results were only shown in figures (Amenta et al., 2014). Fifth, a safety concern of alpha-GPC was shown in the ASCOMALVA trial. The withdrawal percentage of the reference group using only donepezil was 21% (12/56 participants); conversely, that of the active treatment group using AChE inhibitors was 30% (17/57 participants). Withdrawals of severe cases, including death and worsening of AD, were found only in the active treatment group (Amenta et al., 2014). Taken together, we suggest that manufacturers producing alpha-GPC must adequately report safety issues, and post-

marketing surveillance must be performed by authorities.

4.3. South Korea approved alpha-GPC as a drug for most types of neurodegenerative disorders

The Korean Ministry of Food and Drug Safety approved alpha-GPC as a drug, based on results of some clinical trials, for symptoms associated with neurodegenerative diseases or cerebrovascular defects, covered under the national insurance. As the number of patients with AD has increased, alpha-GPC use in Korea has increased by 4.5 times over 8 years, which is provoking criticism that government approval for alpha-GPC as a drug was a hasty judgement and, consequentially, a huge waste of national medical finances because its improved efficacy was not yet confirmed.

In this study, we found that there were many people who took alpha-GPC without any other medication for AD (i.e., as an alpha-GPC user in this study). Use of alpha-GPC is originally recommended as an add-on to a drug for AD like donepezil, memantine, galantamine, or rivastigmine based on the ASCOMALVA trial. Accordingly, prescribing only alpha-GPC to individuals without AD merely benefits the pharmaceutical companies without any benefit to the public.

Indeed, many manufacturers in other countries, including the U.S., are producing alpha-GPC as dietary supplements, which means they did not submit an investigational New Drug (IND) application to the US Food and Drug Administration (FDA). However, some manufacturers were advertising

that alpha-GPC can treat or prevent AD, dementia, other neurodegenerative disorders, Parkinson's disease, and even cardiovascular diseases. Therefore, the pubic mistakenly assumes that alpha-GPC is a drug for dementia. The US FDA advises manufacturers to submit an IND application, or alternatively, remove all statements indicating that the products are intended for such uses (U.S. Food and Drug Administration, 2018).

Most dietary supplements are generally regarded as safe, although the efficacy is not that high. However, we found that alpha-GPC is not harmless. Alpha-GPC use in individuals without AD is associated with increased risk of stroke after a 10-year follow-up. Although there was no study to examine the long-term safety of alpha-GPC, this mechanism relating to stroke is explainable via the effect of high intake of choline or its metabolite, phosphatidylcholine, in previous studies.

4.4. Limitations

This study has limitations which need to be noted. First, alpha-GPC users in this study were significantly older and had more comorbidities than alpha-GPC non-users, which suggests that alpha-GPC users may already have subclinical atherosclerotic changes. To minimize this limitation, another cohort of 1:10 and 1:1 matching for age, sex, income, and comorbidity was generated. The risk of stroke increased with alpha-GPC use even after matching cohort. Nevertheless, there remains not matching variables, including smoking, alcohol use, physical activity, and BMI. Subgroup underwent health screening examinations, was possible to use diverse variables such as BMI, lifestyle factor, hypertension, diabetes mellitus, or dyslipidemia. After adjustments for these covariates, we found the positive association between alpha-GPC use and the risk of stroke. Second, it was not considered whether the individuals used alpha-GPC during follow-up duration. Some individuals who used alpha-GPC might stop to use during follow-up vice versa. In this reason, further studies are warranted to figure out the effect of alpha-GPC on the cerebrovascular disease.

Third, the development of stroke confirmed by hospitalization for two or more days with the relevant ICD-10 codes may have led to an underestimation of the actual number of stroke events. Nonetheless, a previous study showed that identifying cardiovascular disease cases using diagnostic codes from a claims database was more than 80% accurate (Park

et al., 2000). Lastly, as the target population was the elderly, it was expected that many deaths would occur, in which case death events may act as competing events for the development of stroke. However, the results of a competing risk analysis after treating death as a competing event also showed that the use of alpha-GPC was consistently associated with increased risk for stroke. Owing to the study design, it remains the possibility of the reverse causality such as consumption of alpha-GPC after some subclinical events. In this reason, we tried to conduct sensitivity analysis of subjects who received a diagnosis of stroke within the first 4 years of follow-up. The results continued to indicate an increased risk of stroke with alpha-GPC use. However, we did not conduct for more than 4 years of follow-up, which could be the limitation.

We excluded patients with TIA at baseline to remove participants with potential subclinical atherosclerotic changes, which could have confounded the association of alpha-GPC with the risk of stroke. Nevertheless, some individuals were still prescribed alpha-GPC, which may be due to the broad indications of the insurance coverage of alpha-GPC in South Korea, including secondary symptoms caused by cerebrovascular defects, changes in mood and behaviours, and senile depression. Therefore, alpha-GPC was frequently prescribed for the purpose of possibly preventing cognitive decline in the elderly even without dementia. Despite this, future studies which use cases of verified stroke events from medical chart records would be beneficial.

Chapter 5. Conclusions

Alpha-GPC use was associated with the risk of incident stroke within 10 years in a dose-responsive manner after adjustment for traditional cerebrovascular risk factors. This is the first study to examine long-term severe adverse events of alpha-GPC. Future studies are needed to determine the possible mechanisms behind the potential effects of alpha-GPC on increased cerebrovascular risk.

Acknowledgement

Part of this research (analysis using NHIS data) was accepted in *JAMA Network Open*. For receiving a doctoral degree in Seoul National University, publication of a part of the results of the thesis in an SCI or SCIE journal is required.

The author specially thanks to S. Choi for responding kindly and in detail to questions about statistical analysis of Bigdata.

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Abstract in Korean

L-alpha glycerylphosphorylcholine과 뇌졸중 발생 관련성

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서론: L-alpha glycerylphosphorylcholine (alpha-GPC, choline alfoscerate, 콜린 알포세레이트)은 인지기능에 관여하는 아세틸콜린의 전구체로서의 노인 인구에서 전세계적으로 사용되는 건강기능식품이며, 한국에서는 전문의약품으로 승인된 약제이다. 반면, 콜린은 장내 비생물에 의해 trimethyl-amine으로 대사되며, 간에서 trimethylamine-N-oxide로 변환되는데, 이 물질은 동맥경화를 유발하는 것으로 증명된 바 있다. 이 기전을 통해 콜린이 많이 들어있는 음식의 과량 섭취와 동맥경화성 질환의 관련성을 설명해 왔다. 그런데 alpha-GPC는 우리 몸에서 콜린 및 글리세롤로 대사되기 때문에, alpha-GPC의 다량 섭취 또한 콜린의 섭취를 높이므로 이에 대한 부작용을 검토할 필요가 있다. 그러나 이와 관련한 선행 연구는 전무한 실정이다. 이에 본 연구에서는 경구 alpha-GPC의 처방 여부 및 처방 기간에 따른 10년 후 뇌졸중 발생을 분석하고자 하였다.

방법: 해당 연구는 2002년부터 수집된 국민건강보험공단 빅데이터를 활

용한 후향적 코호트 연구이다. 연구대상자는 2005년 12월 31일까지 alpha-GPC를 처방받았던 사람, 치매약을 복용했던 사람, 관찰시작 시점 이전에 사망했거나, 뇌졸중 또는 일과성 허혈 발작 진단이 있는 대상자를 제외한 12,008,977명을 대상으로 하였다. 대상자들은 2006-2008년 동안 경구 alpha-GPC 처방 여부로 구분하였다. 경구 alpha-GPC 처방군과 비슷한 코호트를 구축하기 위해 나이, 성별, 소득수준, 동반질환 변수를 이용하여 1:10, 1:1 매칭 코호트를 구축하였다. 경구 alpha-GPC 사용은 약제 처방의 <2, 2-6, 6-12 및 >12개월로 더 세분화하여 구분하였다. 추적기간은 중앙값 10년 (2008-2019)이었으며, 결과지표는 뇌졸중 (모든 뇌졸중, 허혈성 뇌졸중, 출혈성 뇌졸중) 발생이었다. 분석은 Cox 비례위험 회귀 모델에 근거하였다.

결과: 연구 대상자는 50세 이상의 성인 12,008,977명 (남성: 46.7%; 여성: 53.3%)이 포함되었다. 경구 alpha-GPC 비사용 (n=11,900,100)와 비교하여 사용 (n=108,877)는 10년 후 총 뇌졸중 (보정 위험비 [95% 신뢰구간]: 1.46 [1.43-1.48]), 허혈성 뇌졸중 (1.36 [1.33-1.39]) 및 출혈성 뇌졸중 (1.36 [1.28-1.44]) 위험이 높았다. 매칭 코호트에서 분석한 결과도 alpha-GPC 사용은 10년 후 총 뇌졸중 (1.43 [1.41-1.46]), 허혈성 뇌졸중 (1.34 [1.31-1.37]) 및 출혈성 뇌졸중 (1.37 [1.29-1.46]) 위험이 높았다. 경구 alpha-GPC 사용 내에서도 약물 복용 증가는 처방기간에 비례하여 총 뇌졸중 발생 위험이 높았다 (p for trend <0.001).

결론: 치매나 뇌졸중이 없는 50세 이상의 성인에서 경구 alpha-GPC 사용은 비사용에 비해 뇌졸중 관련 공변량을 보정하였을 때, 10년 추적관찰 후 뇌졸중 발생 위험을 높았다. 경구 alpha-GPC의 잠재적인 뇌혈관 위험 상승 관련한 메커니즘을 확인하기 위한 향후 연구가 필요하다.

Keywords: L-alpha glcerylphosphorylcholine; Choline alfoscerate; Stroke; Dementia; Pharmacoepidemiology; Big data science

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