RESEARCH ARTICLE

Associations of equol-producing status with white matter lesion and amyloid-β deposition in cognitively normal elderly Japanese

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Abstract

Introduction: Equol, a metabolite of a soy isoflavone transformed by the gut micro-biome, is anti-oxidant and anti-amyloidogenic. We assessed the associations of equol with white matter lesion normalized to total brain volume (WML%) and amyloid beta (Aβ) deposition.

Methods: From 2016 to 2018, 91 cognitively normal elderly Japanese aged 75 to 89 underwent brain magnetic resonance imaging and positron emission tomography using ^{11}C-Pittsburgh compound-B. Serum equol was measured using stored samples from 2008 to 2012. Equol producers were defined as individuals with serum levels >0. Producers were further divided into high (> the median) and low ($\leq$ the median) producers.

Results: The median (interquartile range) WML% was 1.10 (0.59 to 1.61); 24.2% were Aβ positive, and 51% were equol producers. Equol-producing status (non-producers, low and high) was significantly inversely associated with WML%: 1.19, 0.89, and 0.58, respectively (trend $P < .01$). Equol-producing status was not associated with Aβ status.

Discussion: A randomized-controlled trial of equol targeting WML volume is warranted.

KEYWORDS
amyloid beta deposition, cognitively normal, epidemiology, equol, Japanese, Pittsburgh compound-B, soy isoflavones, white matter lesion

1 | INTRODUCTION

Preclinical studies have demonstrated that soy isoflavones (ISFs) possess anti-atherosclerotic,[1] anti-oxidant,[2] and anti-amyloidogenic[3] properties. Recent studies in Japan reported that a diet high in soy and ISFs is inversely associated with cognitive impairment[4] and incident dementia.[5] The Women's Isoflavone Soy Health (WISH) Trial, a randomized controlled trial (RCT) among 350 U.S. postmenopausal women, however, showed no significant effect of ISFs on cognition.[6] We posit that the discrepancy between the studies in Japan and the United States is partially due to the difference in equol-producing ability. Equol, a metabolite of the ISF daidzein bio-transformed by the microbiome, is most bioactive among all ISFs[7] and 40% to 70% of Japanese can convert daidzein to equol in contrast to 20% to 30% of Americans.[8] The subgroup analysis of WISH showed that equol producers tended to have improved cognition (standardized mean difference [95% confidence interval (CI)]: 0.34 [–0.04, 0.72], $P = .08$).[6] A recent cross-sectional study in elderly Japanese reported that equol producers had significantly higher cognitive scores and lower prevalence of mild cognitive impairment than non-producers.[9] No previous studies investigated a longitudinal association of equol-producing status with white matter lesion (WML) or amyloid beta (Aβ) deposition in the brain, both of which are significant predictors of cognitive decline and dementia.[10,11] We hypothesize that equol producers have significantly lower WML and Aβ deposition than equol non-producers in cognitively normal elderly Japanese.

2 | MATERIALS AND METHODS

2.1 | Study population

The current study was nested with the Suita Study, a population-based prospective-cohort study at the National Cerebral and Cardiovascular Center (NCVC) in Japan.[12] Two hundred ten participants aged 75 to 89 who met our screening criteria (no history of stroke, neurological disorders, depression under treatment, or other conditions) were randomly selected from the Suita cohort and were administered a neuropsychological battery. Among these 210 subjects, 102 subjects identified as cognitively normal underwent the imaging study (see next section). Among 102 subjects, 11 subjects were excluded (nine due to technical difficulties with imaging or intracranial mass, one due to lack of blood samples, and one due to WML volume being >5 standard deviations [SDs]), yielding our final sample size of 91. This study was approved by the Institutional Review Boards of the University of Pittsburgh and the NCVC. Informed consent was obtained from all participants.

2.2 | Selection of elderly with normal cognition

The Montreal Cognitive Assessment was used for screening. Participants with a score <21 were excluded. Then, to identify cognitively normal individuals the neuropsychological battery was administered, consisting of the Wechsler Adult Intelligence Scale-III (WAIS-III) digit span, WAIS-III block design, Trail Making Tests A and B, Wechsler Memory Scale-Revised logical memory delayed, word fluency category (animals and vegetables), word fluency letter (start with "ka"), Alzheimer's Disease Assessment Scale-cognitive subscale word list (immediate and delayed), Rey Complex Figure Test (immediate, recall, copy), Raven's Coloured Progressive Matrices, Boston Naming Test, and Stroop Test. The result of each test in the battery was classified as normal or abnormal (1.5 SD below the mean value among individuals with comparable age and education) based on normative data.[13–15] Normal cognition was defined as ≤1 abnormal test result over all domains of the neuropsychological battery.

HIGHLIGHTS

- Equol is a metabolite of a soy isoflavone daidzein transformed by the gut bacteria.
- White matter lesion (WML) volume was compared in cognitively normal elderly by equol.
- Serum equol was measured 6 to 9 years before the imaging study.
- Fifty-one percent were equol producers who were divided into high and low producers with the median.
- WML volume in high producers was >50% lower than in non-producers.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional (eg, PubMed) sources. Preclinical studies show that equol is the most bioactive among all soy isoflavones and their metabolites (equol hypothesis). While some studies in humans suggest a benefit of equol on cognition, no previous studies have examined the association of equol with white matter lesions (WMLs) in the brain.
2. **Interpretation**: Our findings agree with the equol hypothesis and indicate that equol is a strong determinant of the progression of WML volume. Contributions of traditional cardiovascular risk factors (hypertension, diabetes, and dyslipidemia) to progression of WML have been reported to be small. Equol is reported to improve arterial stiffness, a significant determinant of WML volume in the elderly. Thus, the effect of equol on arterial stiffness may be one potential mechanism.
3. **Future directions**: A randomized-controlled trial of equol (available as nutritional supplement) targeting WML volume, arterial stiffness, and cognition is warranted.

2.3 | Imaging study

2.3.1 | Magnetic resonance imaging (MRI)

Participants were scanned on a 3-Tesla Siemens MAGNETOM Trio scanner. A structural T1-weighted magnetization prepared rapid gradient echo (MPARAGE, TR/TE = 2300/2.98 ms, T1 = 900 ms, 1 mm × 1.2 mm sagittal acquisition) sequence was used for positron emission tomography-magnetic resonance (PET-MR) image registration, brain segmentation, and parcellation for PET image sampling. For assessing white matter hyperintensities, we used a T2-weighted fluid-attenuated inversion recovery (FLAIR-T2) sequence (TR/TE = 9002/56 ms Ef; TI = 2200 ms, NEX = 1) with an interleaved acquisition; 48 slices (3 mm, no gap). To obtain a good signal-to-noise ratio, the average of four acquisitions was used. A fuzzy-connectedness algorithm was used to segment the WML from each individual's FLAIR-T2 images.[16] The volume of WML is presented as the proportion of the total brain volume. Acquired images were analyzed at the University of Pittsburgh.

2.3.2 | Aβ PET

Participants were intravenously given 15 mCi ^{11}C-Pittsburgh compound B (PiB) over 20 seconds. A 20-minute PET scan (4 × 5-minute frames) was acquired beginning 50 minutes after PiB injection using a Siemens Biograph mCT PET/computed tomography (CT) scanner (4 ring 22.1 cm axial field-of-view, reconstructed image resolution ~5 mm full width half maximum [FWHM]). All scans were acquired in 3D-mode and reconstructed using filtered back-projection. A low-dose (<20 mrem) non-diagnostic CT scan (19 mAs, 120 kVp, 1.0 mm pitch) was acquired for attenuation correction of PET emission data. Other standard PET data corrections were applied during the reconstruction process.

PET images were processed and analyzed using a semi-automated analysis pipeline based on FreeSurfer (v5.3) software.[17] Specific PiB retention was indexed by the standardized uptake value ratio using cerebellum as reference.[18] A global cortical index of total Aβ load was determined based upon a weighted average of nine subregions relevant to Aβ pathology (anterior cingulate, posterior cingulate, insula, superior frontal cortex, orbitofrontal cortex, lateral temporal cortex, parietal, precuneus, and ventral striatum). Aβ positivity was defined as a global cortical index ≥1.346.[19] All PET images were analyzed at the University of Pittsburgh. The results were highly reproducible.[18]

2.4 | Measurements of daidzein, genistein, equol, and other clinical characteristics

The serum collected in 2008 to 2012 (6 to 9 years before the imaging study) and stored at –80°C as well as collected at the time of the imaging study was used to determine fasting levels of two major ISFs (daidzein and genistein) and equol at a commercial laboratory (LSI

Medicine Corporation, Tokyo, Japan). Coefficients of variation for these measurements were <5%. Polymorphisms of the apolipoprotein E (APOE) gene were determined by GTS-7000 system (Shimadzu, Kyoto, Japan) at NCVC.[20] Hypertension was defined as systolic blood pressure (BP) ≥ 140 mmHg, diastolic BP ≥ 90 mmHg or under anti-hypertensive medication. Diabetes was defined as fasting blood glucose ≥ 7 mmol/L or on anti-diabetic medications. Dyslipidemia was defined as fasting serum total cholesterol ≥ 5.69 mmol/L or on lipid-lowering medications.[21] Body mass index (BMI) was defined as body weight (kg) divided by the square of the body height (cm^2).

2.5 | Statistical analysis

Equol non-producers were defined as those whose serum level of equol was zero. Among individuals whose serum levels of equol were > zero 6 to 9 years before the imaging study, we defined high- and low-equol producers as those whose levels of equol were > the median and ≤ the median, respectively. The associations of equol-producing status (non-producers, low, and high producers) with WML% and with Aβ positivity were assessed by linear and logistic regressions, respectively. The analyses were first adjusted for age, sex, and BMI (Model I), further adjusted for hypertension, diabetes, dyslipidemia, and coronary heart disease (CHD; Model II), additionally adjusted for APOE$\varepsilon 2\varepsilon 4$, $\varepsilon 3\varepsilon 4$ or $\varepsilon 4\varepsilon 4$ and years of education (Model III) and further adjusted for blood levels of daidzein (Model IV) or genistein instead of daidzein (Model V). For each equol-producing status, the adjusted means of WML% were presented and the odds ratio of Aβ positivity was presented using equol non-producers as a reference group. Similarly, using the tertiles of blood levels of daidzein and genistein, associations of WML% and Aβ positivity were analyzed. Due to skewed distribution, we log-transformed WML% and the estimates were transformed to the original scale for presentation. In addition, using the same cutoff point of serum levels of equol as described above, we analyzed cross-sectional associations of equol-producing status with WML% and with Aβ deposition. Furthermore, we divided participants into three groups: continuous non-producers (whose serum level of equol was zero at both times), non-continuous equol producers (whose serum level of equol was zero only at one time) and continuous equol producers (whose serum levels of equol were > zero at both times) and analyzed the association with WML% and Aβ positivity. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp, Armonk, NY, USA). A P value of <.05 was considered to represent statistical significance.

3 | RESULTS

The mean (SD) age of the 91 participants was 81.6 (3.1) years; 51% were females; 54.9%, 13.2%, and 54.9% had hypertension, diabetes, and dyslipidemia, respectively. The median (interquartile range) WML% was 1.10 (0.59 to 1.61) and 24.2% were Aβ positive (Table 1). Basic characteristics between 11 subjects who were excluded from

TABLE 1 Basic characteristics of the participants (n = 91)

Age, mean (SD), years	81.6 (3.1)
Sex (male/female), number	45/46
Body mass index, mean (SD), kg/m^2	22.4 (3.1)
Hypertension, number (%)	50 (54.9)
Diabetes, number (%)	12 (13.2)
Dyslipidemia, number (%)	50 (54.9)
Coronary heart disease, number (%)	4 (4.4)
Years of education, mean (SD), years	12.8 (2.4)
Apolipoprotein E4 carrier, number (%)	8 (8.8)
WML%, median (interquartile range), %	1.10 (0.59, 1.61)
Amyloid beta positive, number (%)	22 (24.2)

Abbreviation: SD, standard deviation; WML%, white matter lesion volume normalized to the total brain volume.

the study and the 91 participants were similar (Table S1 in supporting information).

Forty-nine percent of participants (45/91) were equol non-producers. Basic characteristics of the participants were similar across equol-producing status (Table S2 in supporting information). Among 45 non-producers, the median (interquartile range) serum level of daidzein, a precursor of equol, was 82.3 (37.9 to 315.1) μmol/L, and two participants had zero value (Table 2). In these two participants, the serum level of genistein was also zero. Serum levels of daidzein and genistein were significantly correlated (Spearman correlation of 0.876, $P < .01$). Serum levels of equol were not significantly correlated with either daidzein or genistein (Spearman correlations of 0.103 [$P = .329$] and 0.200 [$P = .057$], respectively).

Equol-producing status 6 to 9 years before and at the time of the imaging study had a fair concordance (Table S3A in supporting information). Among 46 equol producers identified 6 to 9 years before the imaging study, equol was detected in 26 at the time of imaging study while among 45 non-producers, 30 remained non-producers over the same time interval (kappa statistics 0.231 [$P = .026$]). The results were similar after excluding participants whose serum daidzein was zero (Table S3B).

Equol-producing status (non-producers, low, and high producers) 6 to 9 years before the imaging study was significantly inversely associated with WML% after adjusting for age, sex, and BMI. WML% in high producers was >50% lower than in non-producers (Model I in Table 3).

The association remained significant after further adjusting for comorbidities, APOE$\varepsilon2\varepsilon4$, $\varepsilon3\varepsilon4$ or $\varepsilon4\varepsilon4$ and years of education (Model III). An additional adjustment for either serum levels of daidzein (Model IV) or genistein (Model V) did not attenuate the association (Table 3). We excluded one participant whose WML volume was >5 SDs. Including this participant in the analysis did not materially change the results (Table S4 in supporting information). Equol-producing status was not significantly associated with Aβ deposition (Table 4).

Sex-specific analyses showed that in both sexes, equol-producing status was significantly inversely associated with WML% and that in either sex, equol-producing status was not significantly associated with Aβ deposition (Table S5 in supporting information).

Equol-producing status at the time of imaging study was significantly inversely associated with WML% after adjusting for age, sex, BMI, and comorbidities. However, the association was attenuated and became non-significant after further adjusting for APOE$\varepsilon2\varepsilon4$, $\varepsilon3\varepsilon4$ or $\varepsilon4\varepsilon4$ and years of education (Table S6 in supporting information).

Equol-producing status taking into account of equol levels 6 to 9 years before and at the time of the imaging study (continuous non-producers, non-continuous, and continuous producers) were significantly inversely associated with WML% even after the full adjustment (Table S7 in supporting information). Continuous producers had >50% lower WML% than continuous non-producers. Equol-producing status was not significantly associated with Aβ deposition in cross-sectional or this analysis.

Serum levels of daidzein were not significantly associated with WML% (Table 5A), whereas those of genistein were significantly inversely associated with WML% (Table 5B). This association remained significant even after adjusting for comorbidities, APOE$\varepsilon2\varepsilon4$, $\varepsilon3\varepsilon4$ or $\varepsilon4\varepsilon4$, and years of education (Model III). However, after further adjusting for equol-producing status, the association was attenuated and became non-significant (Model IV). Serum levels of neither daidzein nor genistein had any significant association with Aβ deposition (Table 5C and 5D).

4 | DISCUSSION

The major finding of the current study was that among cognitively normal elderly Japanese, equol-producing status determined 6 to 9 years before the imaging study was significantly inversely associated with WML%. The significant inverse association remained

TABLE 2 Serum levels of equol, daidzein, and genistein in all participants and by equol-producing status 6 to 9 years before the imaging study

	All participants (n = 91)	Equol non-producer (n = 45)	Low producer (n = 23)	High producer (n = 23)
Equol, μmol/L	4.3 (0, 48.7)	0	22.8 (9.2, 31.4)	166.1 (109.0, 256.4)
Daidzein, μmol/L	94.4 (35.3, 247.0)	82.3 (37.9, 315.1)	51.8 (23.6, 87.6)	134.1 (110.6, 250.5)
Genistein, μmol/L	276.6 (86.7, 522.1)	276.6 (61.1, 586.6)	161.4 (73.9, 219.4)	358.6 (288.7, 647.6)

Notes: Values are expressed as median (interquartile range).
Equol non-producers and producers were defined as participants whose serum levels of equol were 0 or > 0, respectively. Using the median of serum equol levels among equol producers, equol producers were further divided into low (≤ the median) and high producers (> the median).

TABLE 3 Association of WML% with equol-producing status 6 to 9 years before the imaging study (structural brain magnetic resonance imaging; %)

	Non-producers (n = 45)	Low producers (n = 23)	High producers (n = 23)	Trend *P*
Model I	1.19 (0.97, 1.49)	0.89 (0.67, 1.17)	0.58 (0.44, 0.72)	<.01
Model II	1.16 (0.94, 1.42)	0.92 (0.69, 1.23)	0.59 (0.44, 0.78)	<.01
Model III	1.13 (0.92, 1.37)	0.93 (0.69, 1.12)	0.63 (0.48, 0.83)	<.01
Model IV	1.13 (0.93, 1.38)	0.88 (0.67, 1.15)	0.64 (0.49, 0.85)	<.01
Model V	1.11 (0.93, 1.38)	0.88 (0.67, 1.17)	0.65 (0.49, 0.85)	<.01

Abbreviation: WML%, white matter lesion volume normalized to the total brain volume.
Notes: Values are expressed as adjusted mean (95% confidence interval).
Model I: adjusted for age, sex, and body mass index; Model II: further adjusted for hypertension, diabetes, dyslipidemia, and coronary heart disease; Model III: further adjusted for apolipoprotein ε4 and years of education; Model IV: additionally adjusted for daidzein; Model V: adjusted for genistein instead of daidzein to Model III.

TABLE 4 Association of Aβ positivity (odds ratio of Aβ positive in low and high equol producers as compared to equol non-producers) with equol producing status 6 to 9 years before the imaging study (Aβ positron emission tomography)

	Non-producers (n = 45)	Low producers (n = 23)	High producers (n = 23)	Trend *P*
Model I	1	0.85 (0.22, 3,27)	1.35 (0.41, 4.45)	.667
Model II	1	0.75 (0.19, 3.29)	1.34 (0.39, 4.58)	.670
Model III	1	0.81 (0.16, 3.98)	1.33 (0.32, 5.60)	.714

Abbreviation: Aβ, amyloid beta.
Notes: Values are expressed as odds ratio (95% confidence interval).
Model I: adjusted for age, sex, and body mass index; Model II: further adjusted for hypertension, diabetes, dyslipidemia, and coronary heart disease; Model III: further adjusted for apolipoprotein ε4 and years of education.

even after adjusting for hypertension, APOEε2ε4, ε3ε4 or ε4ε4, and other covariates. High equol producers had >50% lower WML% than non-producers. Moreover, the significant inverse association was observed in both sexes. Equol-producing status was not significantly associated with Aβ deposition. A precursor of equol, daidzein, was not significantly associated with WML%. Although the ISF genistein was significantly inversely associated with WML%, this association became non-significant after further adjusting for equol-producing status. Our results indicate that equol is a strong protective factor against the occurrence of WMLs.

Equol is a metabolite of the ISF daidzein bio-transformed by the gut microbiome.[22] Thus, unless one consumes daidzein, equol cannot be produced. Furthermore, even after one consumes daidzein, equol cannot be produced without the presence of specific gut bacteria.[23,24] The reported prevalence of equol producers in Japan was 40% to 70%,[8] although the definition of equol producers differed between studies. The current study also detected equol in 51% of the participants. In Western countries in which ISFs are not part of the regular diet, equol-producing status is determined typically after a 3-day soy challenge and 20% to 30% of adults are reported to be equol producers.[8] The difference in the prevalence is speculated to be due to differences in bacteria species[23] and complexities of the microbiome in the gut,[24] forms of ISF (aglycon form in Asian countries vs glycoside in Western countries)[25] and to a lesser degree, genetic factors.[26]

Although the stability of equol-producing status is reported for a few years,[27,28] no previous studies reported the stability for over 5 years. The current study found a fair concordance of equol-producing status 6 to 9 years apart. The cross-sectional analysis showed that equol-producing status at the time of the imaging study was significantly inversely associated with WML%, which was attenuated and became nonsignificant after the full adjustment. However, when taking equol-producing status at both times (6 to 9 before and at the time of the imaging study) into account, equol-producing status (continuous non-producers, non-continuous, and continuous producers) was significantly inversely associated with WML%. Equol-producing status was not significantly associated with Aβ deposition in either analysis. These results indicate that long-term exposure to equol is associated with lower WML% independent of amyloidogenic processes.

We observed a significant inverse association of equol-producing status with WML% but not with Aβ deposition. Some observational studies suggest that WML may interact with Aβ deposition in the brain.[29] However, current evidence supports an additive role of WML rather than a synergetic interaction.[10,30,31]

Limitations of the study warrant discussion. First, blood levels of ISFs reflect dietary intake over the previous few days. However, fasting serum levels of ISFs have a reasonably good correlation with ISF intake assessed by 28-day dietary records[32] because soy and ISFs are a component of the Japanese diet.[33] Additionally, the seasonal

TABLE 5 Associations of WML% (%) with serum levels of (A) daidzein and (B) genistein and of Aβ positivity (%) with serum levels of (C) daidzein and (D) genistein 6 to 9 years before the imaging study (structural brain magnetic resonance and Aβ positron emission tomography)

WML%				
(A) Daidzein	**Lowest tertile (n = 30)**	**Middle tertile (n = 31)**	**Highest tertile (n = 30)**	**Trend P**
Model I	0.92 (0.68, 1.19)	1.03 (0.79, 1.34)	0.83 (0.64, 1.08)	.593
Model II	0.97 (0.74, 1.28)	1.02 (0.78, 1.32)	0.79 (0.61, 1.03)	.285
Model III	1.10 (0.77, 1.29)	1.00 (0.78, 1.29)	0.78 (0.61, 1.01)	.198
Model IV	0.99 (0.78, 1.26)	1.04 (0.81, 1.33)	0.76 (0.64, 1.03)	.382
(B) Genistein	**Lowest tertile (n = 30)**	**Middle tertile (n = 31)**	**Highest tertile (n = 30)**	**Trend P**
Model I	1.07 (0.83, 1.38)	1.04 (0.81, 1.34)	0.70 (0.54, 0.91)	.026
Model II	1.098(0.84, 1.40)	1.02 (0.79, 1.32)	0.71 (0.55, 0.92)	.025
Model III	1.10 (0.86, 1.41)	0.94 (0.73, 1.21)	0.76 (0.59, 0.97)	.036
Model IV	1.00 (0.78, 1.26)	1.03 (0.81, 1.32)	0.76 (0.60, 0.96)	.112
Aβ status (positive)				
(C) Daidzein	**Lowest tertile (n = 30)**	**Middle tertile (n = 31)**	**Highest tertile (n = 30)**	**Trend P**
Model I	1	0.56 (0.15, 2.10)	0.99 (0.30, 3.22)	.999
Model II	1	0.53 (0.13, 2.10)	0.93 (0.27, 3.17)	.937
Model III	1	0.31 (0.06, 1.58)	0.59 (0.14, 2.47)	.548
(D) Genistein	**Lowest tertile (n = 30)**	**Middle tertile (n = 31)**	**Highest tertile (n = 30)**	**Trend P**
Model I	1	0.68 (0.19, 2.41)	1.02 (0.30, 3.45)	.974
Model II	1	0.60 (0.16, 2.20)	1.02 (0.30, 3.47)	.988
Model III	1	0.70 (0.16, 3.13)	0.68 (0.16, 2.80)	.587

Abbreviations: Aβ, amyloid beta; WML%, white matter lesion volume normalized to the total brain volume.

Notes: Values are expressed as adjusted mean (95% confidence interval) for WML% and odds ratio for Aβ status.

Model I: adjusted for age, sex, and body mass index; Model II: further adjusted for hypertension, diabetes, dyslipidemia, and coronary heart disease; Model III: additionally adjusted for apolipoprotein ε4 and years of education; Model IV (only for white matter lesions%): additionally adjusted for equol producing status.

variation of ISF intake is reported to be small.[32] Second, we lacked several potentially important covariates including physical activity,[34] inflammation,[35] and sleep.[36] Reported effect sizes of these factors on WML, however, are very minimal. Finally, although basic characteristics were very similar by equol-producing status, we cannot rule out the possibility that the association with WML is not mediated by equol itself but rather some phenotypes related to equol-producing status.

Our observation that more than 50% lower WML% in high equol producers as compared to non- producers has important implications for future RCTs. Equol has been tested in RCTs on post-menopausal symptoms, skin aging, and arterial stiffness,[37–40] but has never been tested on WML%. Thus, we will discuss the design, target population, primary, and other potential outcomes, and sample size of such an RCT. Study design will be a parallel, randomized, double-blind placebo-controlled trial with a 24-month or longer intervention. Dose of equol will be 20 mg/d. A pharmacokinetics study of equol in humans[41] shows that supplementation of 20 mg/d of equol will achieve blood levels of equol observed in high equol producers in the current study. Study population will be elderly aged 75 and older without dementia. Exclusion criteria include subjects who regularly eat soy products containing ISF. However, such subjects will be miniscule in the United States because dietary intake of ISF in Western countries is very low (< 3 mg/d) compared to Japan and other East Asian countries (30 to 50 mg/d).[42]

Primary outcome will be progression of WML%. Secondary outcomes will be arterial stiffness and cognitive decline. Arterial stiffness rather than hypertension or BP may be more critical for developing WML.[43] We have shown that arterial stiffness is significantly associated with increased WML independent of BP.[44] Our systematic review and meta-analysis of RCTs of ISF on arterial stiffness showed that ISFs significantly improved arterial stiffness, although the maximum duration of intervention was short (12 weeks).[45] An RCT in the UK showed that ISF supplementation significantly improved arterial stiffness and the effect of ISF was more prominent in equol producers.[46] An RCT in Japan showed that equol supplementation significantly improved arterial stiffness and the effect was more prominent in equol non-producers.[40] Taken together, an effect of equol on arterial stiffness may

be one potential mechanism linking equol and reduced WML. Our systematic review and meta-analysis of RCTs of ISFs on cognition showed that supplementation of ISF improved overall cognition.[47] However, most of these RCTs were conducted in postmenopausal women and effect of equol on cognition in the elderly has not been examined.

Imaging biomarker other than WML% may include amyloid and tau PETs. Amyloid and tau are hallmarks of Alzheimer's disease (AD) pathology and preclinical studies show that ISF possesses anti-amyloidogenic[3] and anti-tau phosphorylation[48] properties. Practically, these scans are expensive. Moreover, our results did not support anti-amyloidogenic properties of equol. Alternative to tau PET, an option would be recently reported plasma phospho-tau 217,[49] which is a better diagnostic biomarker of AD than other blood-based biomarkers and distinguishes AD from other neurodegenerative disorders similarly with tau-PET and cerebrospinal fluid tau.

Other blood biomarkers may include markers of inflammation and endothelial function. Observational studies reported associations of blood biomarkers of inflammation (C-reactive protein, interleukin-6, glial fibrillary acidic protein, etc.)[35] and endothelial function (E-selectin, intercellular adhesion molecule-1, etc.) [50] with increased WML. Preclinical studies and some studies in humans show that ISF is anti-inflammatory[51] and improves blood biomarkers related to endothelial function.[52]

Another potential biomarker of interest is mitochondrial function. Mitochondria function declines with age and mitochondria dysfunction is considered one of the intracellular processes severely compromised in AD.[53] Estrogen receptor-β (ERβ) is found within mitochondria and preclinical studies reported that activation of ERβ stimulates mitochondria function.[54] Equol is an ERβ agonist.[8] In fact, supplementation of 20 mg/d equol for 2 weeks in 15 patients with AD improved, although not statistically significantly, mitochondria cytochrome oxidase activity.[55]

Sample size of 240 would be sufficient to conduct such an RCT targeting WML%. Our prospective cohort study among dementia-free elderly (mean age of 86) in Pittsburgh[10] showed that the mean (SD) annual progression of WML% was 0.28% (0.34; unpublished data). Assuming a 50% slower progression of WML% in the intervention group based on our results, we would have >80% power to detect this difference in a sample size of 120 subjects per arm at $\alpha = 0.05$ (two tailed). With a conservative estimate of 20% attrition over 2 years, we will achieve >80% power to detect a reasonable effect size (50%) with a sample size of 120 participants in each arm at baseline with 80% completers.

In conclusion, in cognitively normal elderly Japanese, equol-producing status determined 6 to 9 years before the imaging study was significantly inversely associated with WML% but not with Aβ deposition. WML% in high producers was >50% lower than in non-producers. An RCT of equol on WML volume is warranted.

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CONFLICT OF INTEREST

FUNDING INFORMATION

DATA AVAILABILITY STATEMENT

Akira Sekikawa and Chendi Cui had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Akira Sekikawa, Chendi Cui, and Yuefang Chan, University of Pittsburgh, conducted and are responsible for the data analysis.

AUTHOR CONTRIBUTIONS

Study concept and design: Sekikawa, Miyamoto, Ihara, Kuller. Acquisition, analysis, or interpretation of data: Higashiyama, Watanabe, Kokubo, Kakuta, Ihara, Fukuda, Miyamoto, Lopez, Yu, Mathis, Klunk, Lopresti, Aizenstein, Chang, Cui, Sekikawa. Drafting the manuscript: Sekikawa. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: Sekikawa, Cui, Chang. Obtaining funding: Sekikawa.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.