VC(-) SMP30/GNL KO mice, amounts of GSH were increased by 44.5%, 32.8% and 30.8%, respectively, above levels in livers from VC(+) SMP30/GNL KO, VC(+) WT and VC(-) WT mice, but the differences among the four groups were not statistically significant (Fig. 7). On the other hand, GSH levels in the soleus muscles and plasma from VC(-) SMP30/GNL KO mice were 16.4% and 45.5%, respectively, lower than in VC(+) SMP30/GNL KO mice, although the GSH content in heart tissues from VC(-) SMP30/GNL KO mice were not changed when compared to the other three groups.

DISCUSSION

Despite the traditional belief that VC is an essential cofactor for the activation of γ-BBD and TMLD in the carnitine biosynthesis pathway *in vivo*, results from the present study using VC-depleted SMP30/GNL KO mice challenge this assumption. Our conclusion to the contrary stems from experiments presented here in which these VC(-) SMP30/GNL KO mice successfully produced carnitine despite the animals' VC insufficiency.

SMP30 is an age-associated protein that decreases with aging in the liver, kidney and lungs in an androgen-independent manner (41-43). SMP30 was first discovered in 1992 by using a proteomic analysis that compared soluble proteins from the livers of young and old rats (41). Although the decreased expression of SMP30 during aging was immediately apparent, the physiological functions of SMP30 remained obscure for more than ten years (44). To resolve this issue, we developed SMP30 KO mice by gene targeting (36). During subsequent experiments performed *in vivo* and *in vitro*, SMP30 KO mice proved far more susceptible to TNF-α- and Fas-mediated apoptosis than their WT counterparts (36) and showed abnormal accumulations of lipids including triglycerides, cholesterol and phospholipids in the liver (45,46). Recently we found that SMP30 is a GNL involved in the VC biosynthetic pathway. Further study showed that SMP30/GNL KO mice could not synthesize VC *in vivo* and that they developed symptoms of scurvy when fed a VC-deficient diet (35).

The SMP30/GNL KO mice used here were fed a VC- and carnitine-deficient diet (Table 1) and water containing no VC for 75 days after weaning, which began when the animals were 40-days-old. Initially, these VC(-) SMP30/GNL KO mice grew as well as matching VC(+) mice as evident from their increased body weight for the first 40 days followed by a gradual decrease (Fig. 2). After another 30 days, the VC(-) KO mice weighed 32% less than the VC(+) controls given supplementary VC. Additionally, multiple tissues and plasma of the SMP30/GNL KO mice had less than 2% the total VC content of VC(+) SMP30/GNL KO, VC(+) WT and VC(-) WT mice (Fig. 3). However, total carnitine levels in various tissues, except the heart, unexpectedly showed no difference among these four groups (Fig. 4). The abundance of carnitine in the heart and skeletal muscle has been attributed to these tissues' production of energy via β-oxidation from long-chain fatty acids for muscle expansion (7). In the present study, total carnitine levels in hearts from VC(-) SMP30/GNL KO and VC(-) WT mice were about half the amounts in VC(+) SMP30/GNL KO and VC(+) WT mice. Still unclear is whether VC(+) groups generated an increased quantity of carnitine or the VC(-) group underwent a decrease of carnitine; however, this phenomenon may be related to the requirement for extra carnitine storage in the heart for use in energy production.

In mammals, carnitine homeostasis is maintained by adsorption from dietary sources, endogenous synthesis and efficient tubular reabsorption by the kidney. *In vivo*, VC depletion in tissues and plasma may be a manifestation of the inhibitory effects of carnitine excretion from tissues and an increase in the efficiency of tubular reabsorption from the kidney. To examine the effects of VC depletion and carnitine deficiency on the urinary excretion of carnitine, we measured total carnitine levels in urine collected for 24 h periods at 30 and 80 days after feeding of a VC- and carnitine-deficient diet. However, the total carnitine levels in urine were quite similar in all four groups of test mice at those time intervals (Fig. 5). These results denote that the VC deficiency in tissues and plasma had no influence on the urinary carnitine excretion of SMP30/GNL KO mice.

However, many reports indicate that tissue carnitine levels are significantly decreased in VC-depleted guinea pigs *in vivo* (30-32). Alkonyi *et al.* (33) reported that an increase of urinary excretion greatly contributed to the loss of carnitine in

guinea pigs during states of VC deficiency and starvation. Rebouche (34) also stated that carnitine depletion in VC-deficient guinea pigs resulted from a decreased efficiency of carnitine reabsorption in their kidneys. These reports strongly assert that subnormal levels of VC in various tissues of VC-depleted guinea pigs were caused by a rise in urinary carnitine excretion. If so, guinea pigs cannot be used to determine the involvement of VC in carnitine biosynthesis. In the present study the amount of carnitine excreted in the urine of SMP30/GNL KO mice was no different in samples from VC-depleted *versus* VC-supplemented animals (Fig. 5). Although the difference between guinea pigs and SMP30/GNL KO mice is still unclear, SMP30/GNL KO mice are a far more suitable animal model for determining the necessity of VC in carnitine biosynthesis.

We further examined carnitine biosynthesis by using VC-depleted liver and kidney tissues from SMP30/GNL KO mice. Humans, cats, cows, hamsters and rabbits can synthesize carnitine in the liver and kidney because they have γ-BBD activity in those tissues; however, mice, rats, sheep, dogs and guinea pigs synthesize carnitine only in the liver, because their kidneys lack γ-BBD activity entirely or contain only very low levels (22-24). In the present study of liver homogenates from VC-depleted SMP30/GNL KO mice with and without 1 mM VC, there was no difference in carnitine production (Fig. 6A), In addition, no carnitine production was detectable in the kidney samples according to the same assay system (Fig. 6B). These results strongly suggest that VC is not essential for carnitine biosynthesis *in vitro*. Vlies *et al.* (24) reported that both TMLD and γ-BBD activities were reduced when VC was removed from their complete assay mixture *in vitro*. However, Punekar *et al.* (29) observed a small amount of carnitine synthesis, but not VC, in the presence of GSH, in their assay mixture. Further, a combination of GSH and glutathione peroxidase yielded a large amount of carnitine synthesis, suggesting that GSH may effectively replace VC in the carnitine biosynthetic pathway. In fact, in the livers from our VC(-) SMP30/GNL KO mice, GSH levels were higher by 31 to 45% than in those from the other three groups tested, although the difference was not statistically significant (Fig. 7). Conversely, GSH levels in soleus muscles and plasma of VC(-) SMP30/GNL KO mice were lower than that from the other three groups. These results reinforce the likelihood that GSH may replace VC in the carnitine biosynthetic pathway *in vivo* when VC is depleted in liver.

In conclusion, our results indicate that VC is not essential for carnitine biosynthesis *in vivo*, as verified by the clearcut presence of carnitine in VC-depleted SMP30/GNL KO mice. Additionally, GSH may compensate for VC in the event of the latter's depletion. Finally, our model of SMP30/GNL KO mice provides an optimal opportunity for investigating the necessity of VC in carnitine biosynthesis.

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FOOTNOTES

*This study is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan (to A.I., S.H. and N.M.), and a Grant-in-Aid for Smoking Research Foundation, Japan (to A.I.) and the Suzuken Memorial Foundation, Japan (to A.I.). We thank Ms. P. Minick for the excellent English editorial assistance. Vitamin C powder was kindly provided by DSM Nutrition Japan.

The abbreviations used are: γ-BB, γ-butyrobetaine; γ-BBD, γ-BB dioxygenase; DHA, dehydroascorbic acid; DTT, dithiothreitol; EDL, extensor digitorum longus muscle; GNL, gluconolactonase; GSH, reduced glutathione; HPLC, high-performance liquid chromatography; KO, knockout; PCA, perchloric acid; SMP30, senescence marker protein-30; TMABA, 4-N-trimethylaminobutyraldehyde; TML, 6-N-trimethyllysine; TMLD, TML dioxygenase; HTML, 3-hydroxy-6-N-trimethyllysine; VC, vitamin C; WT, wild type

FIGURE LEGENDS

Fig. 1. The pathway of carnitine biosynthesis with proposed involvement of VC. L-lysine residues in proteins are trimethylated by specific methyltransferases that use S-adenosyl-L-methionine as the methyl donor to TML. After its release by protein degradation, TML is hydroxylated by TMLD to HTML. HTML is cleaved by 3-hydroxy-6-N-trimethyllysine aldolase to TMABA and glycine. TMABA is oxidized to γ-BB by 4-N-trimethylaminobutyraldehyde dehydrogenase. In the last step, γ-BB is hydroxylated to L-carnitine by γ-BBD. In the present study, we found VC is not an essential cofactor for the activation of γ-BBD and TMLD in the carnitine biosynthesis pathway.

Fig. 2. Body weight changes of VC(+) and VC(-) groups composed of WT and SMP30/GNL KO mice. After the mice were weaned at 40 days of age (indicated at day 0), their body weights were measured for 75 days, and the mean body weight changes (difference from the mean body weight at day 0) were plotted. The final body weights of VC(+) SMP30/GNL KO, VC(-) SMP30/GNL KO, VC(+) WT and VC(-) WT mice at day 70 were 35.2 ± 1.7 g, 23.9 ± 1.9 g, 33.9 ± 0.5 g and 39.1 ± 0.5 g, respectively. Values are expressed as means ± SEM of five animals.

Fig. 3. Total VC levels in the cerebrum, cerebellum, liver, kidney, soleus muscle, extensor digitorum longus muscle (EDL), heart and plasma from VC(+) and VC(-) groups from WT and SMP30/GNL KO mice. Mice were supplied with or deprived of VC in drinking water for 75 days, starting when they were weaned at 40 days of age. Bars represent DHA (pink column) and ascorbic acid (green column). Values of total VC (DHA plus ascorbic acid) are expressed as means ± SEM of five animals.

Fig. 4. Total carnitine levels in the cerebrum, cerebellum, liver, kidney, soleus muscle, extensor digitorum longus muscle (EDL), heart and serum from VC(+) and VC(-) groups of WT and SMP30/GNL KO mice. Mice were supplied with or deprived of VC in drinking water for 75 days, starting when they were weaned at 40 days of age. Values of total carnitine are expressed as means ± SEM of five animals.

Fig. 5. Excretion of total carnitine in the urine at (A) 30 days and (B) 80 days after weaning of VC(+) and VC(-) groups of WT and SMP30/GNL KO mice. Each mouse was housed in a metabolic cage, and urine was collected for 24 h. Values of total carnitine were normalized by creatinin values and expressed as means ± SEM of five animals.

Fig. 6. *In vitro* carnitine biosynthesis assay. VC-depleted liver and kidney homogenates were prepared as described in "Experimental Procedures." For the carnitine biosynthesis assay, homogenates were incubated with 1 mM VC (pink circle) or without VC (green square) at 37°C for the indicated times. (A) Total carnitine concentrations in liver homogenates during incubation. (B) Total carnitine concentrations in kidney homogenates during incubation. (C) Total carnitine concentrations in VC-depleted liver homogenates heated at 95°C for 5 min then incubated at 37°C for 60 and 120 min. Values are expressed as means ± SEM of four samples.

Fig. 7. GSH levels in the liver, kidney, soleus muscle, heart and plasma from VC(+) and VC(-) groups from WT and SMP30/GNL KO mice. Mice were supplied with or deprived of VC in drinking water for 75

days, starting when they were weaned at 40 days of age. Values of GSH are expressed as means ± SEM of five animals.

Table I
Diet composition of CLEA-purified diet

Nutritional component (in 100 g)	
Moisture (g)	8.0
Crude protein (g)	20.4
Crude fat (g)	6.0
Crude fiber	3.0
Crude ash (g)	6.2
Nitrogen-free extract (NFE) (g)	56.4
Calorie (kcal)	361.2

Minerals (in 100 g)	
Ca (g)	0.89
P (g)	0.66
Mg (g)	0.08
Na (g)	0.23
K (g)	0.50
Fe (mg)	31.70
Cu (mg)	0.32
Zn (mg)	3.46
Co (mg)	0.10
Mn (mg)	3.51
Ca/P	1.35
Ca/Mg	11.13
K/Na	2.17

Vitamins (in 100 g)	
Vitamin A (mg)	1.65
Vitamin D_3 (μg)	25
Vitamin E (mg)	20
Vitamin K_3 (mg)	0.3
Vitamin B_1 (mg)	1.5
Vitamin B_2 (mg)	1.6
Total vitamin C (mg)*	ND
Vitamin B_6 (mg)	1.0
Vitamin B_{12} (μg)	0.5
Pantothenic acid (mg)	4.0
Niacin (Nicotinic acid) (mg)	10.2
Folic acid (mg)	0.2
Choline (mg)	300
Biotin (μg)	500
Inositol (mg)	15

Other component (in 100 g)	
Total carnitine (mg)*	ND

ND, not detectable.
Data from CLEA Japan, Tokyo, Japan.
*Total VC and total carnitine were measured as described in "Experimental Procedures."

Figure 1

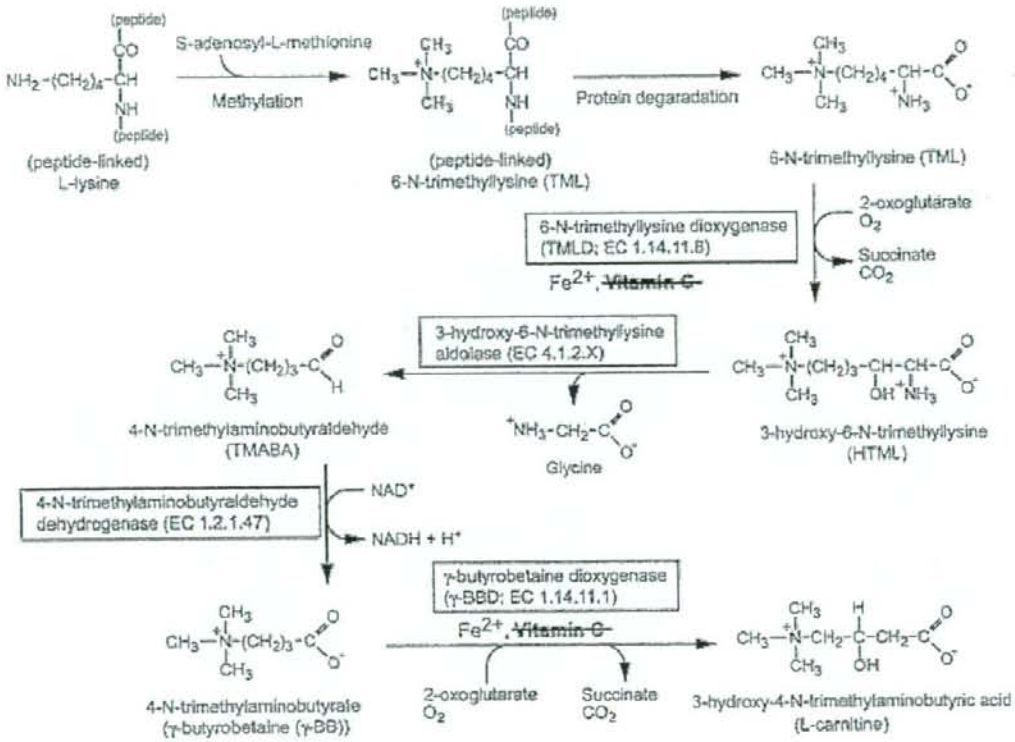

(peptide)
S-adenosyl-L-methionine
Methylation
Protein degaradation
(peptide-linked) L-lysine
(peptide-linked) 6-N-trimethyllysine (TML)
6-N-trimethyllysine (TML)
6-N-trimethyllysine dioxygenase (TMLD; EC 1.14.11.8)
Fe2+, Vitamin C
2-oxoglutarate
O2
Succinate
CO2
3-hydroxy-6-N-trimethyllysine aldolase (EC 4.1.2.X)
4-N-trimethylaminobutyraldehyde (TMABA)
Glycine
3-hydroxy-6-N-trimethyllysine (HTML)
4-N-trimethylaminobutyraldehyde dehydrogenase (EC 1.2.1.47)
NAD+
NADH + H+
γ-butyrobetaine dioxygenase (γ-BBD; EC 1.14.11.1)
Fe2+, Vitamin C
2-oxoglutarate
O2
Succinate
CO2
4-N-trimethylaminobutyrate (γ-butyrobetaine (γ-BB))
3-hydroxy-4-N-trimethylaminobutyric acid (L-carnitine)

Figure 2

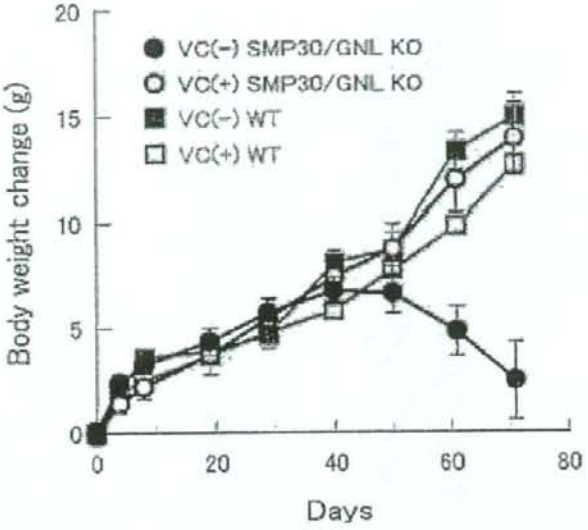

VC(−) SMP30/GNL KO
VC(+) SMP30/GNL KO
VC(−) WT
VC(+) WT
Body weight change (g)
20
15
10
5
0
0
20
40
60
80
Days

Figure 3

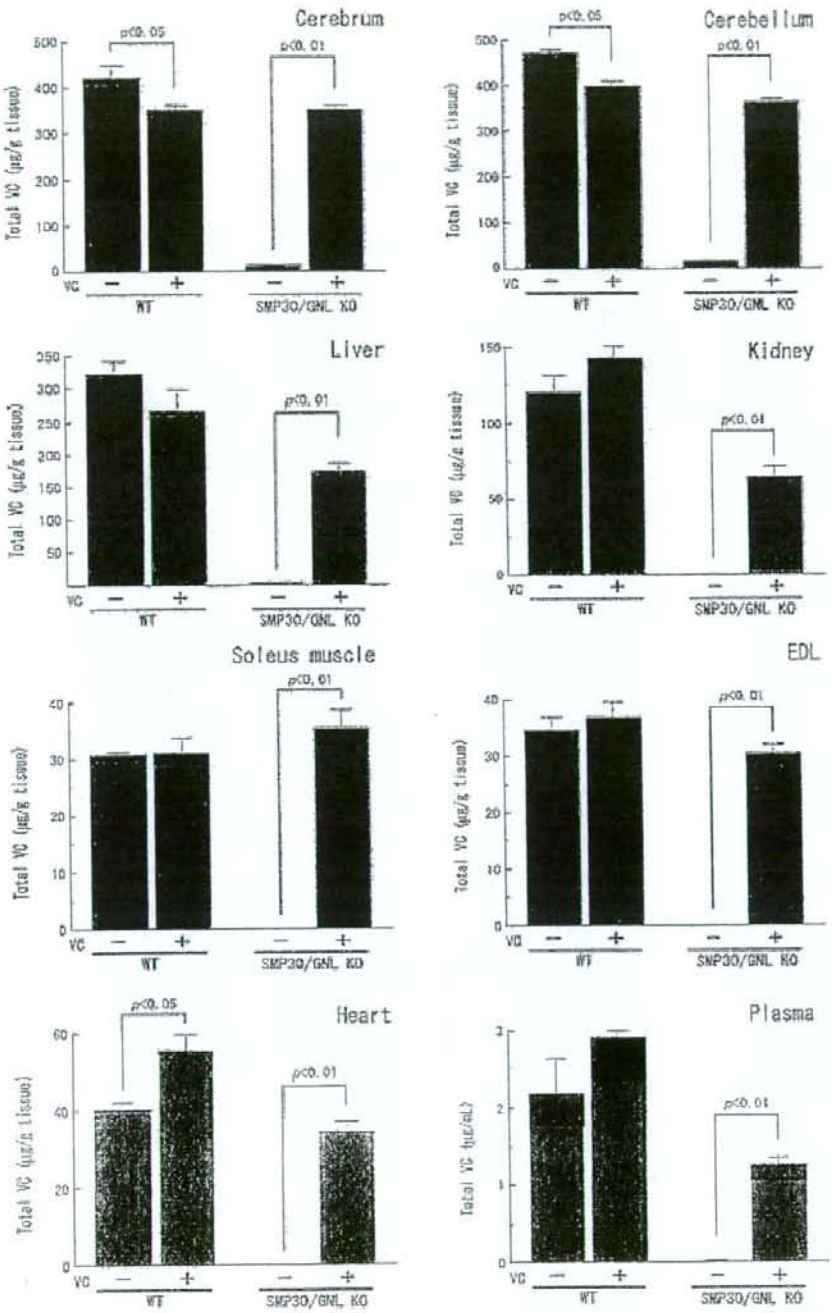

Cerebrum
Cerebellum
Liver
Kidney
Soleus muscle
EDL
Heart
Plasma
p<0.05
p<0.01
Total VC (μg/g tissue)
Total VC (μg/mL)
VC
WT
SMP30/GNL KO

Figure 4

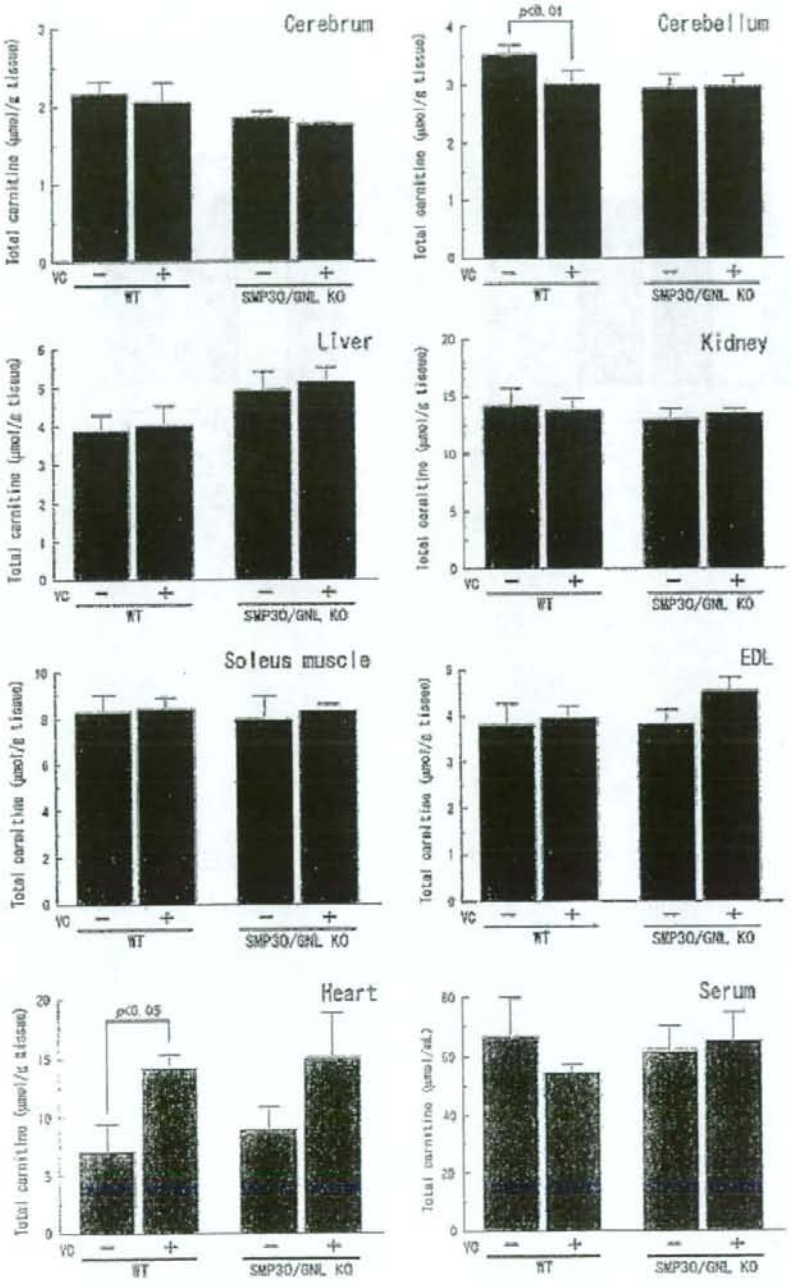

Cerebrum
Cerebellum
p<0.01
Liver
Kidney
Soleus muscle
EDL
Heart
p<0.05
Serum
Total carnitine (μmol/g tissue)
Total carnitine (μmol/mL)
VC
WT
SMP30/GNL KO

Figure 5

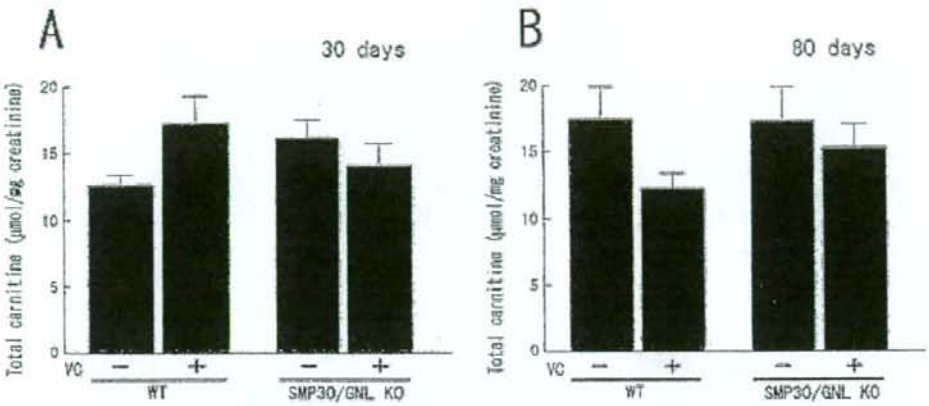

A
30 days
Total carnitine (μmol/mg creatinine)
20
15
10
5
0
VC
−
+
−
+
WT
SMP30/GNL KO
B
80 days
Total carnitine (μmol/mg creatinine)
20
15
10
5
0
VC
−
+
−
+
WT
SMP30/GNL KO

Figure 6

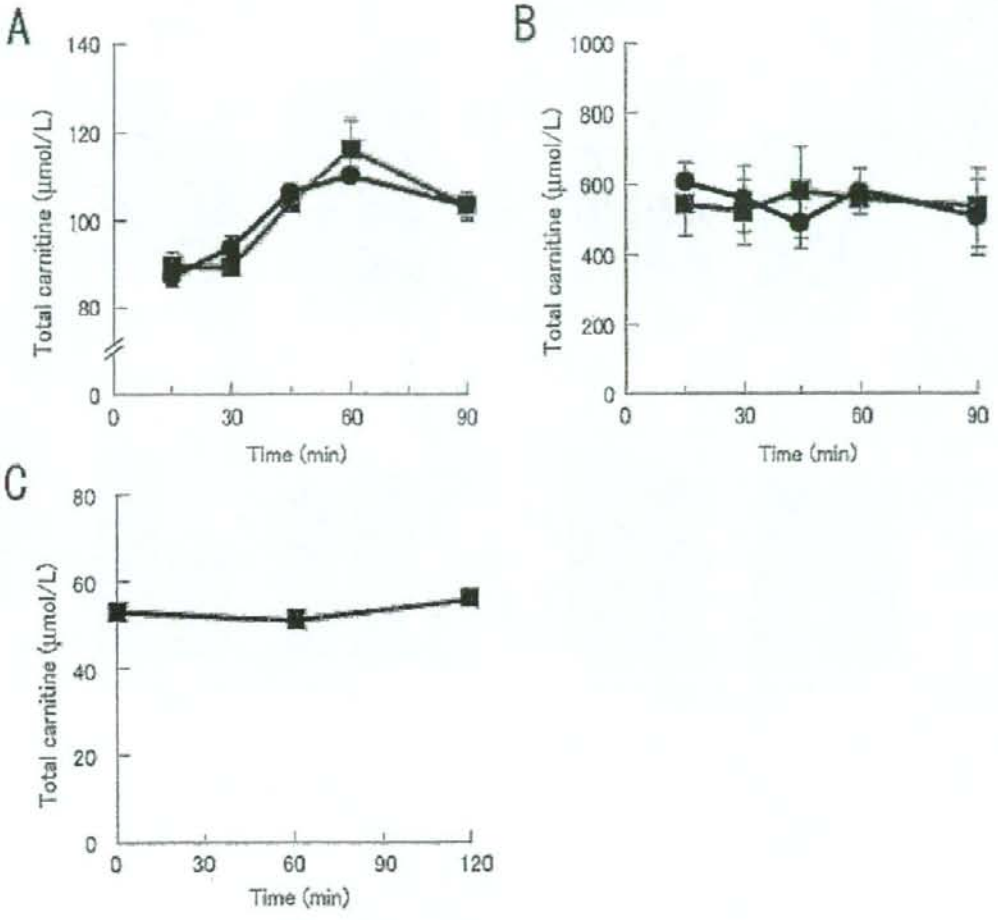
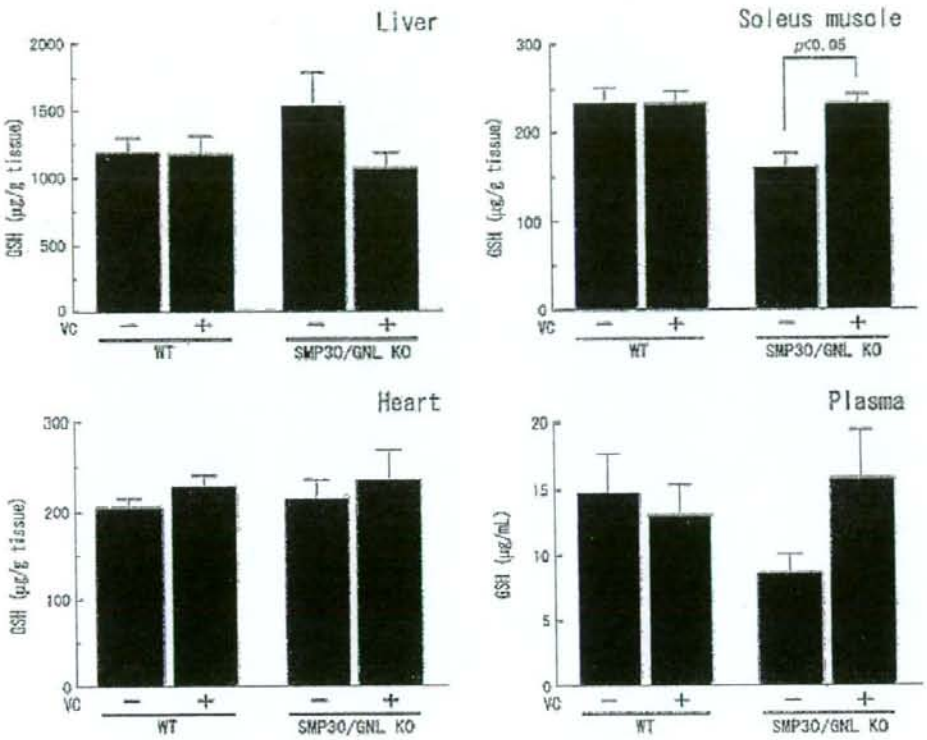

A
Total carnitine (μmol/L)
140
120
100
80
0
0
30
60
90
Time (min)
B
Total carnitine (μmol/L)
1000
800
600
400
200
0
0
30
60
90
Time (min)
C
Total carnitine (μmol/L)
80
60
40
20
0
0
30
60
90
120
Time (min)

Figure 7


Liver
GSH (μg/g tissue)
2000
1500
1000
500
0
VC
−
+
−
+
WT
SMP30/GNL KO
Soleus muscle
p<0.05
GSH (μg/g tissue)
300
200
100
0
VC
WT
SMP30/GNL KO
Heart
GSH (μg/g tissue)
300
200
100
0
VC
WT
SMP30/GNL KO
Plasma
GSH (μg/mL)
20
15
10
5
0
VC
WT
SMP30/GNL KO

Geriatr Gerontol Int 2008; 8: 93–100

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

White matter lesions as a feature of cognitive impairment, low vitality and other symptoms of geriatric syndrome in the elderly

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Aim: White matter lesions (WML) are common findings on magnetic resonance imaging (MRI) in elderly persons. In this study, we analyzed the relation of WML with global cognitive function, depression, vitality/volition, and 19 symptoms of geriatric syndrome in Japanese elderly patients who attended three university geriatric outpatient clinics.

Methods: Two hundred and eighty-six subjects (103 men and 183 women; mean ± standard deviation age, 74.5 ± 7.8 years) were included in this study. MRI scans were performed for the diagnosis of WML, and the severity of periventricular and deep white matter hyperintensities (PVH and DWMH) was rated semiquantitatively. Concurrently, all subjects underwent tests of cognitive function, depressive state and vitality, and were examined for 19 symptoms of geriatric syndrome.

Results: The study subjects showed cognitive decline, depression and low vitality, all to a mild extent. Univariate linear regression analysis showed a negative correlation between the severity of WML and cognitive function or vitality. Multiple logistic analysis revealed that the severity of WML was a significant determinant of cognitive impairment and low vitality, after adjustment for confounding factors such as age, sex and concomitant diseases. PVH and/or DWMH score was significantly greater in subjects who exhibited 13 out of 19 symptoms of geriatric syndrome. Logistic regression analysis indicated that WML were associated with psychological disorders, gait disturbance, urinary problems and parkinsonism.

Conclusion: WML were associated with various symptoms of functional decline in older persons. Evaluating WML in relation to functional decline would be important for preventing disability in elderly people.

Keywords: deep white matter hyperintensity, geriatric syndrome, periventricular hyperintensity, white matter lesion.

Accepted for publication 10 December 2007.

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doi: 10.1111/j.1447-0594.2008.00454.x

Introduction

Brain magnetic resonance imaging (MRI) has markedly enhanced the chance of detecting characteristic hyperintense signals in the periventricular and subcortical areas on T2-weighted images, even in asymptomatic older persons.[1] These lesions are known as white matter lesions (WML), leukoaraiosis or white matter (periventricular and subcortical) hyperintensities.[2–4] WML, which accompany symptoms of gait abnormalities,[5–7] urinary symptoms[5,8,9] and cognitive impairment,[4,10,11] are reported to be associated with aging,[12–14] hypertension,[14] diabetes[15] and atherosclerosis.[5] There is poor understanding of the pathogenesis of the lesions, and it remains unknown whether WML are mere innocuous radiological changes that appear as a result of the aging process,[2,3,10] or whether they are one of the causal factors of the functional decline in elderly people.

Geriatric syndrome is a group of symptoms that are related to daily life, and the comorbidity triggers the loss of independence of elderly persons. Hence, evaluation of geriatric syndrome is important for the physical and mental care of the elderly. To address the pathological significance of WML in the global cognitive and psychological functions, and in geriatric syndrome in representative Japanese elderly subjects, we organized a group of geriatric outpatient clinics, and investigated the clinical manifestations of WML in those patients. Especially, we analyzed the relation of WML with global cognitive function, depressive state, vitality/volition and 19 symptoms of geriatric syndrome.

Methods

Subjects

This was a multicenter study performed at three different university geriatric outpatient clinics in Japan under the organization of a Longevity Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (H15-Choju-013). Two hundred and eighty-six consecutive subjects (103 men and 183 women; mean ± standard deviation [SD] age, 74.5 ± 7.8 years) were included in this study: 187 at Kyorin University Hospital, 74 at Chiba University Hospital, and 25 at Nagoya University Hospital, from January 2004 to January 2005.

The diagnosis of dementia was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The definition of hypertension was systolic blood pressure (BP) of more than 140 mmHg or diastolic BP of more than 90 mmHg, or receiving antihypertensive drugs. The definition of diabetes was glycosylated hemoglobin A1c of more than 6.5%, or receiving antidiabetic drugs. The definition of hyperlipidemia was total cholesterol of more than 5.72 mmol/L, triglyceride of more than 1.70 mmol/L, or receiving antihyperlipidemic drugs.

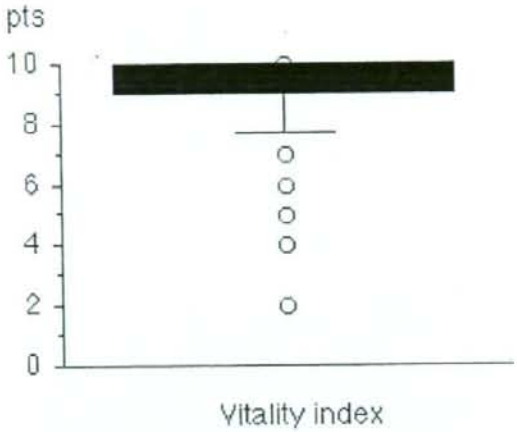

Figure 1 Distribution of vitality index. All subjects underwent assessment of vitality index as a measure of vitality related to activities of daily living (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0–10).

All subjects underwent the following assessment of global cognitive and psychological function. Cognitive function was evaluated by Mini-Mental State Examination (MMSE).[16] In this examination, we focused on calculation (serial subtraction of 7 from 100) to evaluate attention and working memory (part of the frontal lobe function). We also performed verbal fluency or word recollection test by asking the subjects to name as many vegetables as possible, which is also indicative of the frontal lobe function. Depression was evaluated by the 15-item Geriatric Depression Scale (GDS-15), which consists of 15 dichotomous questions for screening depressive symptoms in elderly subjects (range, 0–15).[17] Vitality index was used to measure vitality or volition in daily life (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0–10).[18] A full score can be maintained until one is severely disabled in cognition or function. The distribution of vitality index in the subjects of this study is shown in Figure 1.

We examined symptoms of geriatric syndrome: 19 dichotomous questions about hallucinations, delusions, insomnia, vertigo, paralysis, numbness, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, constipation, decreased appetite, weight loss, apathy, speech impairment, swallowing difficulty, tremor and muscle stiffness.

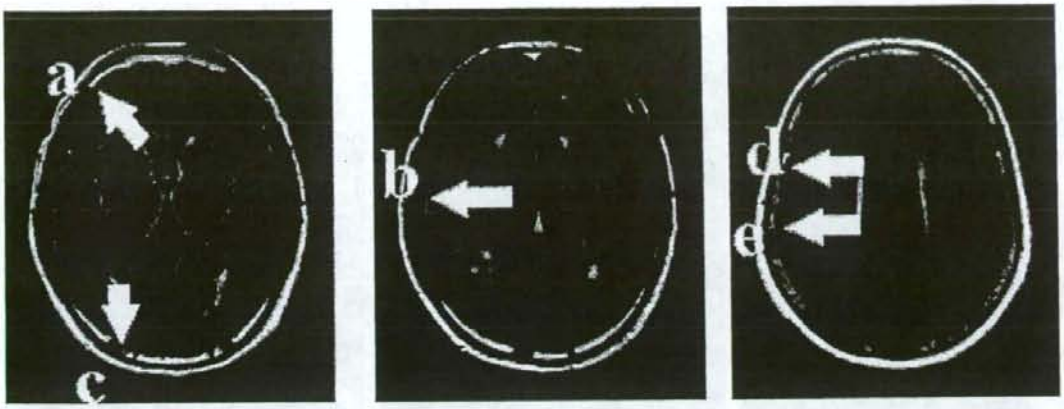

Figure 2 Evaluation of periventricular hyperintensity (PVH). PVH were evaluated in six regions in three slices: (a) adjacent to the frontal horns, (b) lateral ventricular body, (c) occipital horns, (d) frontal central semiovale in the parietal region and (e) occipital centrum semiovale in the parietal region in both hemispheres. Each area was rated as five grades according to the method of Junque *et al.*: (0) no hyperintensities; (1) <25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) >75%.[11] The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Magnetic resonance imaging

Magnetic resonance imaging scans were performed for the diagnosis of WML and cerebral infarction on 1.5-T scanners (Toshiba, Nasu, Japan). T1-weighted images (repetition time [TR], 496 ms; echo time [TE], 12 ms), T2-weighted images (TR, 4280 ms; TE, 105 ms), and fluid-attenuated inversion-recovery (FLAIR)-weighted images (TR, 8000 ms; TE, 105 ms; 5-mm slice thickness) were obtained in the axial plane. MRI images were examined to differentiate between WML, characterized by isointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images, and cerebral infarction, characterized by hypointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images.

White matter lesions were classified as periventricular hyperintensities (PVH), which adjoined the lateral ventricle, and deep white matter hyperintensities (DWMH), located in the deep white matter apart from the lateral ventricles.

Periventricular and deep white matter hyperintensity scores

Periventricular hyperintensities were evaluated in six regions in three slices: adjacent to the frontal horns, lateral ventricular body, occipital horns, frontal central semiovale in the parietal region, and occipital centrum semiovale in the parietal region in both hemispheres (Fig. 2). Each area was rated as five grades according to the systematic quantification method developed by Junque *et al.*: (0) no hyperintensities; (1) less than 25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) more than 75%.[11] The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Deep white matter hyperintensities were evaluated in the frontal, temporal, parietal and occipital lobes, and in the basal ganglia in both hemispheres (Fig. 3). Each lesion was rated as three grades according to the diameter by the study of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) more than 10 mm. The sum of all grades in five regions in both hemispheres was defined as the DWMH score.[4] Analysis was performed assuming that the white matter scores of PVH and DWMH were quantitative interval scales.

Statistical analysis

The relationship between two continuous variables such as MMSE, GDS-15 or vitality index, and WML (PVH or DWMH) score was analyzed by univariate linear regression analysis, and the correlation was analyzed by means of Pearson's simple correlation coefficients. Statistical significance was set at $P < 0.05$.

The relation of cognitive impairment or low vitality with PVH score or DWMH score was assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. Cognitive impairment and low vitality were defined as an MMSE score of 23 or less[19] and a vitality index of 9 or less, respectively. Odds ratios and 95% confidence interval were calculated from the, coefficients and their standard errors.

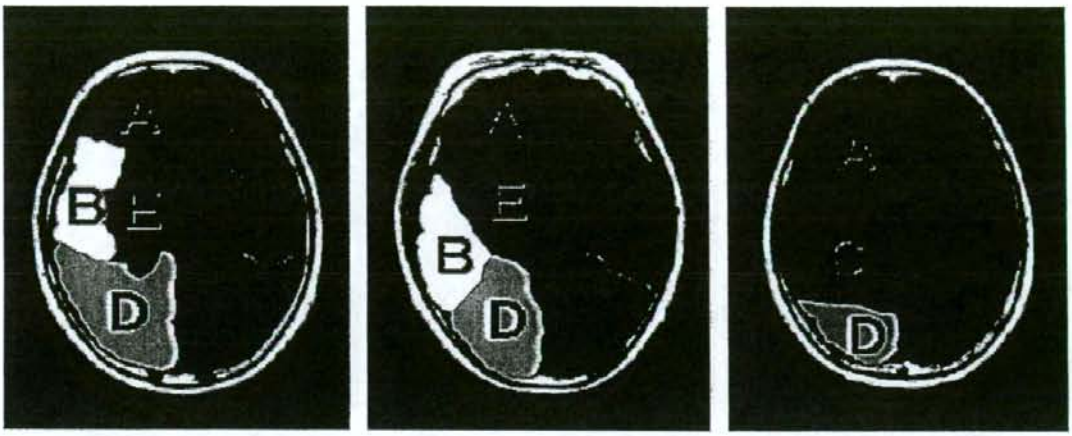

Figure 3 Evaluation of deep white matter hyperintensities (DWMH). DWMH were evaluated in the (A) frontal, (B) temporal, (C) parietal and (D) occipital lobes, and (E) in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to diameter by the method of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) >10 mm.[4] The sum of all grades in five regions in both hemispheres was defined as the DWMH score.

Periventricular hyperintensity score or DWMH score was compared between subjects who did or did not exhibit each symptom of geriatric syndrome and analyzed by Student's *t*-test. When the difference was considered to be significant ($P < 0.05$), the difference was further assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease.

Ethical considerations

This study was approved by the ethical committees of the institutes involved in this project. We explained this study clearly, and obtained written consent from all participants and their guardians (mainly family members). All the data were stored and analyzed carefully to preserve the subjects' anonymity and protect their privacy.

Results

Clinical data

The clinical characteristics of the study subjects are shown in Table 1. The mean age of subjects was 74.5 ± 7.8 years (mean ± SD), and subjects aged 65 or older comprised 88.1%. The mean body mass index was 21.8 ± 3.3 kg/m^2 and none of the subjects were obese. Of the subjects, 10.1% had experienced stroke or other cerebrovascular disease and 22.7% were smokers. Hypertension, diabetes and hyperlipidemia were present in 50.7%, 27.3% and 50.0% of the subjects, respectively.

White matter lesions

Periventricular hyperintensities and DWMH were observed in 77.7% and 96.7% of the total subjects, respectively. The mean score of PVH and DWMH was 5.5 ± 4.8 and 35.5 ± 39.8, respectively (Table 1). Pearson's correlation analysis showed a strong positive correlation between PVH score and DWHM score ($r = 0.56$, $P < 0.0001$). In relation to aging, a positive correlation was found between PVH score and age ($r = 0.34$, $P < 0.0001$), and between DWMH score and age ($r = 0.28$, $P < 0.0001$).

Cognitive and psychological assessment

The mean score of MMSE, GDS-15 and vitality index was 23.1 ± 5.3, 5.0 ± 3.5 and 9.4 ± 1.2 points, respectively, indicating that the subjects showed cognitive decline, depression and decreased vitality, all to a mild extent. Given that a score of 23 or below on MMSE is regarded as the presence of cognitive impairment,[19] 47.5% of the subjects fell into this category. The causes of cognitive impairment were Alzheimer disease (AD; 53.3%), vascular dementia (VaD; 16.4%), combined dementia of AD and VaD (9.0%) and other types of dementia (21.3%). Pearson's correlation analysis revealed a negative correlation between PVH score and MMSE, PVH score and vitality index, DWMH score and MMSE, and DWMH score and vitality index,

Table 1 Clinical characteristics of study subjects

	Prevalence ($n = 286$)	Mean ± standard deviation
Clinical characteristics		
Age (years)		74.5 ± 7.8
Women (%)	74.0	
Height (m)		1.55 ± 0.08
Bodyweight (kg)		52.4 ± 10.6
Body mass index (kg/m^2)		21.8 ± 3.3
Systolic blood pressure (mmHg)		135.3 ± 20.2
Diastolic blood pressure (mmHg)		76.3 ± 11.8
Prevalence of complications		
Hypertension (%)	50.7	
Diabetes (%)	27.3	
Hyperlipidemia (%)	50.0	
Past history of cerebrovascular disease (%)	10.1	
Smoking (%)	22.7	
Cognitive and psychological assessment		
Mini-Mental State Examination (0–30 points)		23.1 ± 5.3
Geriatric depression scale (0–15 points)		5.0 ± 3.5
Vitality index (0–10 points)		9.4 ± 1.2
White matter lesions		
Periventricular hyperintensities (points)	5.5 ± 4.8	
Deep white matter hyperintensities (points)	35.5 ± 39.8	

Table 2 Relationship between white matter lesions and global cognition (MMSE), depressive state (GDS-15) and vitality (vitality index)

	Linear regression PVH score	DWMH score
MMSE	−0.380**	−0.272**
GDS-15	0.022	−0.066
Vitality index	−0.432**	−0.184*

Univariate linear regression analysis: *$P < 0.01$, **$P < 0.0001$. DVMH, deep white matter hyperintensity; GDS-15, 15-item Geriatric Depression Scale; MMSE, Mini-Mental State Examination; PVH, periventricular hyperintensity.

respectively (Table 2). It was also found that calculation (serial subtraction of 7 from 100) was negatively correlated with PVH score ($r = -0.156$, $P = 0.04$, data not shown), and verbal fluency (naming as many vegetables as possible) was negatively correlated with PVH score ($r = -0.216$, $P < 0.01$, data not shown). On the other hand, no significant correlation was found between PVH score and GDS-15, or between DWMH score and GDS-15. Multiple logistic analysis revealed that PVH score and DWMH score remained significant determinants of cognitive impairment (MMSE, ≤23) and low vitality (vitality index, ≤9) after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 3).

One hundred and ninety subjects reported symptoms of geriatric syndrome. The frequency is shown in Table 4. Frequent symptoms (>20%) were tripping (32.1%), constipation (26.3%), gait disturbance (23.2%) and pollakiuria (22.1%). Student's *t*-test showed that PVH score was significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, weight loss, apathy, swallowing difficulty, tremor and muscle stiffness. Multiple logistic analysis revealed that PVH score remained a significant determinant of hallucinations, tripping, pollakiuria, urinary incontinence, weight loss, apathy and swallowing difficulty after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 5). By the same method, DWMH score was

Table 3 Periventricular hyperintensity and deep white matter hyperintensity scores as determinants of cognitive impairment and low vitality

	PVH score			DWMH score		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Cognitive impairment	1.185	1.084–1.295	<0.001	1.010	1.001–1.021	<0.05
Low vitality	1.260	1.133–1.401	<0.0001	1.025	1.012–1.039	<0.001

Cognitive impairment and low vitality were defined as MMSE ≤23 and vitality index ≤9, respectively. Multiple logistic analysis was performed after adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; DVMH, deep white matter hyperintensity; OR, odds ratio; PVH, periventricular hyperintensity.

significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence and constipation. Multiple logistic analysis revealed that DWMH score remained a significant determinant of hallucinations, delusions, tripping, urinary incontinence and constipation after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 6).

Discussion

Elderly persons are affected by multiple chronic diseases. Once they are affected by serious illness, full recovery cannot be expected with medical treatment, because elderly patients are often trapped in a vicious circle of illness and poor quality of life (QOL). This is the reason why care and welfare contribute to the total well-being of the elderly. Physicians need to pay great attention to improving QOL as well as treating illness. Thus, it is important to comprehend the whole picture of their life by means of comprehensive geriatric assessment, which evaluates multiple aspects of an elderly person's life, such as activities of daily living, cognition, mood, vitality, communication and social environment.

The present study confirmed a negative correlation between the severity of WML and MMSE score. Multivariate analysis showed that the presence of WML was a significant risk factor for cognitive impairment, even after adjustment for confounding factors of age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease. The mechanism and the size and location of WML that impair cognitive function are not yet clear. However, from previous studies, it seems convincing that a reduction of blood flow in the frontal lobe plays an important role in cognitive impairment in elderly people who exhibit WML.[20,21] Clinical manifestations of WML include attention deficit and a decline in information-processing ability.[4,13,22] Junque *et al.* reported the reappearance of primitive reflexes, one of the symptoms of frontal lobe dysfunction, in patients with WML.[11] In this study, patients with PVH showed attention deficit (incapability of calculation) and verbal inarticulacy (naming less vegetables), implying the impairment of frontal lobe function. WML, as reported previously,[6,23] were negatively correlated with vitality. Multiple logistic regression analysis, using potential risk factors including advanced age as confounding variables, found that the presence of WML was an independent risk factor for low vitality. Additionally, a relation between PVH score and apathy, a significant symptom of geriatric syndrome, was also found. From previous studies showing the importance of frontal lobe function in vitality,[24–26] we assume that blood flow reduction in the frontal lobe may account for the apathy and low vitality in patients with WML. More precisely, WML disrupting the frontal-subcortical circuit may result in dysfunction in the anterior cingulate and dorsolateral prefrontal circuits, thereby leading to apathy and decreased vitality.[5,6,20] Increase in PVH score or DWMH score was not apparently correlated with depression, probably because depression is associated with many factors such as aging, female sex, hyperlipidemia and medication.[27–29] The subjects in this study were mostly elderly (88.1%) and female (74.0%). We assume that these confounding conditions made it difficult to prove a true relation between WML and depression. From analysis of the association of WML with geriatric syndrome, it appears that WML have a relation to psychiatric symptoms (hallucinations and delusions), gait abnormalities (gait disturbance, tripping and falls), urinary symptoms (pollakiuria and urinary incontinence) and possibly with parkinsonism (swallowing difficulty, tremor and muscle stiffness). It was reported that WML were related to gait abnormalities,[5–7] presumably caused by disruption of the frontal-subcortical circuit.[30] Some other studies suggested that parkinsonism is also a contributing factor to gait disturbance in patients with WML.[6,31] Interestingly, we found that both gait abnormalities and symptoms of parkinsonism were associated with WML.

The present study confirmed an association between WML and voiding dysfunction (pollakiuria and incontinence). It was reported that urinary dysfunction was derived from damage to the frontal-subcortical

Table 4 Comparison of periventricular hyperintensity and deep white matter hyperintensity scores between subjects who did or did not exhibit each symptom of geriatric syndrome

Geriatric syndrome	Prevalence (%)	PVH score Symptom Present	Absent	*P*-value	DWMH score Symptom Present	Absent	*P*-value
Hallucination	6.8	**8.5 ± 5.9**	**4.4 ± 4.7**	**<0.01**	**59.8 ± 43.9**	**28.6 ± 35.4**	**<0.01**
Delusion	9.5	**7.6 ± 5.2**	**4.4 ± 4.8**	**0.01**	**56.1 ± 37.6**	**28.2 ± 35.9**	**<0.01**
Insomnia	18.9	4.2 ± 3.6	4.7 ± 4.9	0.56	31.4 ± 36.0	31.3 ± 37.6	0.98
Vertigo	18.9	6.1 ± 6.5	4.4 ± 4.4	0.06	33.4 ± 38.1	30.7 ± 37.0	0.70
Paralysis	2.1	8.5 ± 4.8	4.6 ± 4.9	0.12	59.5 ± 47.2	30.1 ± 36.3	0.11
Numbness	16.6	5.1 ± 4.6	4.6 ± 4.8	0.62	34.6 ± 40.0	29.9 ± 36,0	0.52
Gait disturbance	23.2	**6.7 ± 5.1**	**4.2 ± 4.7**	**<0.01**	**43.3 ± 41.7**	**27.5 ± 34.9**	**0.01**
Tripping	32.1	**6.4 ± 4.5**	**3.9 ± 4.9**	**<0.01**	**42.1 ± 43.7**	**25.9 ± 32.4**	**<0.01**
Falls	17.9	**6.6 ± 4.9**	**4.3 ± 4.8**	**0.01**	**45.8 ± 43.1**	**28.0 ± 35.0**	**0.01**
Pollakiuria	22.1	**8.0 ± 5.8**	**3.8 ± 4.2**	**<0.01**	**41.5 ± 41.0**	**41.5 ± 41.0**	**0.04**
Urinary incontinence	13.8	**7.5 ± 5.1**	**4.3 ± 4.8**	**<0.01**	**52.4 ± 44.9**	**52.4 ± 44.9**	**<0.01**
Constipation	26.3	5.8 ± 4.3	4.4 ± 5.1	0.08	**44.5 ± 45.1**	**44.5 ± 45.1**	**<0.01**
Decreased appetite	14.7	6.1 ± 4.4	4.5 ± 5.0	0.12	42.1 ± 42.6	42.1 ± 42.6	0.11
Weight loss	14.2	**6.9 ± 4.1**	**4.4 ± 5.0**	**0.01**	40.7 ± 41.3	40.7 ± 41.3	0.15
Apathy	7.6	**7.4 ± 3.6**	**4.4 ± 5.0**	**0.03**	30.7 ± 28.1	30.7 ± 28.1	0.97
Speech impairment	2.7	5.6 ± 5.2	4.5 ± 4.7	0.62	35.3 ± 48.0	35.3 ± 48.0	0.80
Swallowing difficulty	14.7	**12.2 ± 4.4**	**4.5 ± 4.8**	**<0.01**	44.6 ± 34.6	44.6 ± 34.6	0.40
Tremor	5.3	**9.1 ± 6.5**	**4.4 ± 4.7**	**<0.01**	45.0 ± 38.1	45.0 ± 38.1	0.24
Muscle stiffness	3.2	**9.2 ± 4.8**	**4.5 ± 4.9**	**0.02**	48.7 ± 43.4	48.7 ± 43.4	0.23

PVH and DWMH score are shown as mean ± SD. Boldface values are statistically significant ($P < 0.05$ by Student's *t*-test). DVMH, deep white matter hyperintensity; PVH, periventricular hyperintensity.

Table 5 Periventricular hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.12	0.043	1.004–1.248
Tripping	1.11	0.005	1.032–1.194
Pollakiuria	1.17	0.001	1.067–1.278
Urinary incontinence	1.11	0.022	1.015–1.207
Weight loss	1.14	0.007	1.036–1.246
Apathy	1.14	0.027	1.015–1.276
Swallowing difficulty	1.35	0.019	1.050–1.741

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

Table 6 Deep white matter hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.017	0.020	1.003–1.032
Delusion	1.016	0.024	1.002–1.030
Tripping	1.011	0.020	1.002–1.020
Urinary incontinence	1.016	0.008	1.004–1.028
Constipation	1.011	0.025	1.001–1.021

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

circuit.[5,20] In relation to the symptoms of parkinsonism (swallowing difficulty, tremor and muscle stiffness), this association was previously explained by dysfunction of the frontal-subcortical circuit.[6,31] The importance of this lesion was also suggested by a study showing that swallowing difficulty occurs with dysfunction of internuncial neurons that link the brainstem to the cerebral cortex.[32]

Considering the cause of manifestation of geriatric syndrome in patients with WML, it appears that damage to associative pathways in the frontal and subcortical regions due to ischemic hypoperfusion is an important mechanism.[5,20,21] It is necessary to localize the responsible connecting pathway for each symptom by a sophisticated approach in the future.

In conclusion, we showed that WML were associated with cognitive impairment, low vitality and geriatric syndrome of psychological disorders, gait disturbance,