Sakamoto et al. Renal Replacement Therapy (2015) 1:6
DOI 10.1186/s41100-015-0001-3

Renal Replacement Therapy

RESEARCH Open Access

Maintenance of activities of daily living despite risk from genetic polymorphism in hemodialysis patients under nutritional management who survived an average of 30 years

Kaori Sakamoto[1], Yoshihiko Kanno[2*], Mami Hiraoka[3], Matsuhiko Hayashi[4], Yoshiko Kontai[5] and Yasuo Kagawa[6]

Abstract

Background: Only 4 % of hemodialysis (HD) patients survive over 25 years after their initiation of HD even in Japan. To elucidate their clinical characteristics, we investigated their lifestyle and genetic factor. TT genotype of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism was reported as a high-risk factor for cardiovascular event and poor survival in CKD patients.

Method: Seventy-eight of Japanese patients receiving HD more than 30 years were enrolled. Their daily lifestyle and activity were evaluated with diet history questionnaires (DHQ), geriatric nutritional risk index (GNRI), and basic activity of daily living (BADL) scores. MTHFR C677T was genotyped by PCR-restriction fragment length polymorphism (RFLP).

Results: The mean dietary intake of energy was 30.6 ± 9.3 kcal/kg of ideal body weight (IBW), protein 1.1 ± 0.4 g/kg of IBW and their adequacy ratios for Japanese guideline 2007 were 97.7 and 101.9 %, respectively. BADL was 90, and daily activities were highly maintained in patients. The frequency of TT genotype was 26.9 % and it was almost twice as that in the general population. The patients with TT genotype had lower serum folate and higher serum homocysteine than those with the CC or CT genotypes, though there was no significant difference in dietary folate intake among them.

Conclusion: Although the frequency of TT genotype was higher than healthy population, our patients showed longer survival with high QOL and nutritional status. It is suggested that the proper lifestyle might overcome the genetic risk factors in patients receiving HD.

Keywords: Long survival, Genetic factor, RFLP

Background

Increasing importance has been attached to the complication of a nutritional disorder in dialysis patients as a risk factor that leads to a poor outcome [1]. Moreover, many dialysis patients are in a protein-energy wasting (PEW) state [2], and this condition has been reported to be a factor that strongly influences the survival of elderly and long-term dialysis patients [1, 3–5]. The survival rate after introduction of dialysis has been increasing with advances in dialysis techniques in Japan, but the 5–10- and 25-year survival rates are only 60.3, 36.2 and 14.1 %, respectively, and patients under long-term dialysis treatment for 25 years or longer account for only 4 % (11,802 patients) of the all dialysis patients [6]. "Long-term" outcomes represent outcomes at around 5 years in reports from other countries, and patients on hemodialysis for a short time (less than 10 years) were investigated in most studies in Japan. There have been only a

* Correspondence: kannoyh@tokyo-med.ac.jp
[2]Department of Nephrology, Tokyo Medical University, 6-7-1 Nishi-Shinjuku, Shinjuku, Tokyo 160-0023, Japan
Full list of author information is available at the end of the article

few studies in which outcomes and nutritional condition were investigated in patients on hemodialysis for more than 25 years. Moreover, it is not easy to perform a prospective study of long-term outcomes, nutritional condition, and nutritional management. Therefore, it is important to clarify the nutritional condition and food intake of patients who have been receiving hemodialysis for more than 25 years.

In Japan, the causes of death of dialysis patients are heart failure (26.6 %), infection (20.3 %), malignant tumor (9.1 %), cerebrovascular disorder (7.7 %), myocardial infarction (4.4 %), and hyperkalemia (2.9 %). Cardiovascular diseases account for about 40 % of the causes of death and contribute to the poor prognosis of dialysis patients [6]. Especially, ischemic heart disease is a significant obstacle to the maintenance of a favorable quality of life (QOL). Bachmann et al. [7] confirmed that hyperhomocysteinemia is a risk factor for cardiovascular disease in hemodialysis patients. Homocysteine (Hcy) is an amino acid produced through metabolism of an essential amino acid, methionine. Dietary folate-derived 5-methyltetrahydrofolate is required for this metabolism as a methyl-group donor. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The *MTHFR* C677T polymorphisms is a C to T transition at position 677 (exon4), which causes the substitution of alanine with valine and leads to about 35 % decrease in enzyme activity in CT heterozygotes and 60 % decrease in TT homozygotes [8]. This reduced enzyme activity causes an elevating serum Hcy level [8]. The associations of this polymorphism to hyperhomocysteinemia and cardiovascular disease have been reported. In a study reported in Japan, frequency of T allele was 33 % and the frequency of TT genotype was 10.2 % in Japanese population, and the T allele frequency was higher in ischemic heart disease and cerebral infarction patients than in normal controls. The serum Hcy level was higher in patients with TT genotype than in those with other genotypes, and the relationship between Hcy and genotype was stronger in the low folate intake [9]. The frequency of the TT genotype was 13.7 % in a study of hemodialysis patients, and it was higher (23.8 %) in patients with a cardiovascular disorder, showing that the *MTHFR* polymorphism is an important factor that influences the serum Hcy level [10, 11]. However, there have been only a few studies of Japanese patient with renal failure and hemodialysis patients, and the *MTHFR* C677T polymorphism has not been investigated in long-term hemodialysis patients.

This study was performed to clarify the actual nutrition state and food intake of long-term hemodialysis patients after more than 25 years of dialysis. We also investigated nutritional management during long-term hemodialysis which is considered as a risk factor for mortality, as association between *MTHFR* C677T polymorphism, which was based on the risks of renal dysfunction and introduction of dialysis.

Methods

Subjects

Ninety-five patients who had received hemodialysis for more than 25 years or longer were selected from outpatients undergoing hemodialysis three times a week at 14 institutions including Keio University Hospital and related facilities. Documents explaining the objective of the survey and protection of personal information were handed to the subjects, and written consent was obtained from 90 patients after an explanation had been given by physicians. Three patients with missing laboratory test values, one with missing dietary survey result, one with renal transplantation, and one who died during the survey period were excluded. Another six patients were excluded because of extreme under/over-reporting, which was assessed based on the method reported by Sasaki, the developer of brief-type self-administered diet history questionnaire (BDHQ) [12]. The exclusion criteria of the dietary survey are as follows, based on the Dietary Reference Intakes for Japanese, 2010 edition: "an energy intake lower than 0.5 times the estimated energy requirement for physical activity level I, and an energy intake of 1.5 times or higher than the estimated energy requirement for physical activity level III [13]". Subjects in this study finally consisted of 78 patients (31 male and 47 females). The survey period was from March to November in 2012.

This study was approved by Keio University School of Medicine Ethics Committee (approval number 2011-271, dated January 11, 2012) and Experimental Study Ethics Committee of Kagawa Education Institute of Nutrition (approval number 201-G, dated March 14, 2012).

Measurements

The subject characteristics (sex, age, duration of dialysis therapy, age at the time of introduction of dialysis, and primary disease for renal failure), physical conditions (height, dry weight [DW], and body mass index [BMI]) were assessed. For blood chemistry, blood samples were collected at the beginning of the first dialysis session of the week (Monday or Tuesday) immediately before dialysis, and serum albumin (Alb), blood urea nitrogen (BUN), serum creatinine (Cr), serum potassium (K), serum inorganic phosphorus (IP), serum calcium (Ca), hemoglobin (Hb), hematocrit (Hct), serum folate, serum homocysteine (Hcy), and serum vitamin B_{12} (VB_{12}) were analyzed. The concentrations of serum folate and vitamin B_{12} were measured using chemiluminescent enzyme immunoassay (CLEIA) on UniCel DxI 800 with Access Folate and Access Vitamin B12, respectively (Beckman

Table 1 Subject characteristics

Variable		Total ($n = 78$)
Age	Years	632 ± 8.1[a]
Gender (Male/female)	*n* (%)	31/47 (39.7/60.3 %)[b]
Duration of hemodialysis	Years	305 (27.0–34.3)[c]
Age of initiation of hemodialysis	Years	32.4 ± 8.0
Primary disease		
Chronic glomerulonephritis	*n* (%)	55 (70.5 %)
Chronic pyelonephritis	*n* (%)	1 (1.3 %)
Nephropathy of pregnancy	*n* (%)	3 (3.8 %)
Polycystic kidney	*n* (%)	2 (2.6 %)
Other nephritides that cannot be classified	*n* (%)	4 (5.1 %)
Other	*n* (%)	2 (2.6 %)
Unknown	*n* (%)	11 (14.1 %)
MTHFR C677T (CC/CT/TT)	n (%)	23/34/21 (29.5/43.6/26.9 %)
Height	cm	157.6 ± 8.3
Weight (dry weight)	kg	46.3 (41.7–57.0)
Body mass index (BMI)	kg/m^2	197.7 ± 2.7
BMI < 18.5	*n* (male/female) (%)	32 (7/25) (41.0 %)
18.5 ≦ BMI < 25	*n* (male/female) (%)	42 (21/21) (53.8 %)
25 ≦ BMI	*n* (male/female) (%)	4 (3/1) (5.1 %)
Geriatric nutrition index (GNRI)		93 ± 6
≧ GNRI 91 (without risk of malnutrition)	*n* (%)	46 (59.0 %)
<GNRI 91 (with risk of malnutrition)	*n* (%)	32 (41.0 %)
Barthel Index (BADL)		90.0 (78–100)
BADL (independent/some help is necessary/with help)	*n* (%)	74/4/3 (91/5/4 %)
Weight change rate between dialysis[d]	%	5.1 ± 1.9
Kt/Vsp[e]		1.67 ± 0.2
Biochemical parameters		
Serum albumin (alb)	g/dL	3.70 ± 0.3
Total protein (TP)	g/dL	6.60 ± 0.4
Blood urea nitrogen (BUN)	mg/dL	65.00 ± 17.1
Serum creatinine (Cr)	mg/dL	10.70 ± 2.2
Urea acid (UA)	mg/dL	7.10 ± 1.5
Serum sodium (Na)	mEq/L	140.00 ± 2.5
Serum potassium (K)	mEq/L	5.10 ± 0.7
Serum inorganic phosphorous (IP)	mg/dL	5.40 ± 1.4
Serum calcium (Ca)	mg/dL	9.10 ± 0.7
Hemoglobin (Hb)	g/dL	10.30 (9.6–11.2)
Hematocrit (Hb)	%	33.00 ± 3.4
Serum folate	ng/mL	4.60 (3.8–5.4)
Serum homocystein (Hcy)	μmol/L	30.80 (23.6–38.4)
Serum vitamin in B12 (VB_{12})	pmol/L	541.00 (340–1250)

Abbreviations: *ESRD* end stage renal disease, *MTHFR* methylene tetrahydrofolate reductase, *BMI* body mass index, *GNRI* geriatric nutrition risk index, *BADL* Barthel index

[a]Values are means ± standard deviation (all such values)

[b]Values are number of all patients (%)

[c]Values are median (25th and 75th percentiles), (all such values)

[d]Body weight change rate between hemodialysis; 55 subjects with data before and after hemodialysis. (values are means ± standard deviation)

[e]Standardized dialysis volume (Kt/Vsp); 55 subjects with data before and after hemodialysis. (values are means ± standard deviation)

Coulter). Serum Hcy concentrations were measured by high-performance liquid chromatography (HPLC). These measurements before and after dialysis were available in 55 patients, and the index of dialysis efficiency, Kt/Vsp, was calculated using Shinzato's formula [14]. Blood chemistry was analyzed by SRL Tokyo Medical (Tokyo, Japan).

Habitual energy, nutrient, and food group intakes were surveyed using BDHQ [15, 16] which is a simplified version of the self-administered diet history questionnaire (DHQ) developed by Sasaki et al. [17]. To assess activities of daily living, basic activities of daily living were evaluated using BADL published by Mahoney and Barthel in 1965 [18]. The geriatric nutrition risk index (GNRI) used for nutrition screening of dialysis patients was calculated using the formula below. GNRI is a nutrition screening tool for the elderly developed by Bouillanne et al. [19]. Yamada et al. [20] applied GNRI in a study of dialysis patients and reported its usefulness.

DNA was extracted from EDTA-added whole-blood samples collected as described above using a fully automated nucleic acid extraction device, Magtration® System 6GC, and a reagent that is used exclusively with this device, MagDEA DNA 200 (GC) (Precision System Science Co., Ltd., Japan). The *MTHFR* C677T polymorphism was genotyped using polymerase chain reaction (PCR) technique and restriction fragment length polymorphism (RFLP) analysis by PaGE (Tokyo, Japan). The patients were divided into three groups based on the *MTHFR* C677T genotype, and the association with each index (physical condition, laboratory test values, food intake, daily living activities, and GNRI) was investigated.

Statistical analysis

Variables detected in the measurements and survey items were confirmed using the Shapiro–Wilk test of normality and histograms. When the distribution was not normal, logarithmic transformation or nonparametric test was employed. Log-transformed serum Hcy was examined using linear regression analysis. Data sets of variables with normality were presented as means ±

Table 2 Habitual dietary intakes

Variable		Total ($n = 78$)
Intakes of nutrients and energy		
Energy	kcal	1635 (1247–1993)
Energy	kcal/kgIBW	29.2 (22.8–35.8)
Protein	g	57.6 (44.0–72.7)
Protein	g/kgIBW	1.1 (0.8–1.3)
Ratio of animal protein	%	56.2 (46.1–64.0)
NPC/N[a]		147 (126–175)
Fat	%E	27.5 (22.5–30.7)
Carbohydrate	%E	56.6 (51.6–61.1)
Retinol	μg	556 (398–878)
α-tocopherol	mg	6.7 (5.1–8.7)
Potassium	mg	1946 (1463–2586)
Calcium	mg	386 (248–502)
Phosphorus	mg	841 (609–1062)
Iron	mg	6.6 (4.6–8.3)
VitaminB_6	mg	1.0 (0.7–1.3)
VitaminB_{12}	μg	7.1 (4.6–10.1)
Folate	μg	285 (200–370)
VitaminC	mg	99 (71–136)
Salt	g	8.9 (7.4–10.8)
Energy-adjusted nutrient intakes		
Retinol	μg/1000 kcal	350 (255–490)
α-tocopherol	mg/1000 kcal	4.2 (3.6–4.9)
Potassium	mg/1000 kcal	1211 (1021–1469)
Calcium	mg/1000 kcal	225 (179–294)
Phosphorus	mg/1000 kcal	523 (453–599)
Iron	mg/1000 kcal	4.0 (3.4–4.6)
VitaminB_6	mg/1000 kcal	0.6 (0.5–0.7)
VitaminB_{12}	μg/1000 kcal	4.5 (3.2–6.4)
Folate	μg/1000 kcal	169 (137–219)
VitaminC	mg/1000 kcal	64 (44–77)
Salt	g/1000 kcal	5.5 (4.9–6.3)
Energy-adjusted food group intakes		
Cereals	g/1000 kcal	220 (185–297)
Potatoes	g/1000 kcal	18 (8–43)
Sugar and confectioneries	g/1000 kcal	2.9 (1.7–3.7)
Nuts and pulses	g/1000 kcal	18 (8–28)
Green and yellow vegetables	g/1000 kcal	52 (32–79)
Other vegetables	g/1000 kcal	68 (49–101)
Fruits	g/1000 kcal	43 (18–85)
Fish and shellfish	g/1000 kcal	39 (24–54)
Meat	g/1000 kcal	35 (25–52)
Eggs	g/1000 kcal	18 (7–31)
Daily products	g/1000 kcal	24 (7–64)
Fats and oils	g/1000 kcal	6.4b (4.4–8.6)
Confectioneries	g/1000 kcal	24 (13–50)
Alcoholic beverages and non-alcoholic beverages	g/1000 kcal	212 (138–336)
Seasoning and spice	g/1000 kcal	81 (53–139)

All values are median (25–75percentiles)
[a]NPC/N, Ratio of non-protein energy/nitrogen

standard deviations and those without normality were presented as medians (25th and 75th percentiles). Regarding food intake, the measured energy intake, animal protein ratio, lipid energy ratio, iron intake, and energy-adjusted potassium and B_6 intakes showed normal distributions, but the others did not. Since the median and mean are similar when the distribution is normal, all data were presented as medians (25th and 75th percentiles).

In the comparison of the three *MTHFR*C 677T genotype-based groups (CC, CT, and TT groups), the interval and ratio scales were compared using one-way analysis of variance and multiple comparison (homogeneous variance: Tukey's test; non-homogeneous variance: Games–Howell test), or the Kruskal–Wallis test and multiple comparison (Bonferroni correction; Mann–Whitney U test). For the nominal scale, the χ^2 test was employed.

Statistical analysis was performed using a statistical package, IBM SPSS Statistics Ver. 20 (IBM, Tokyo), and the significance level was set at less than 5 %. When a missing value was present, the item was deleted entirely.

Results

Subject characteristics

1. Clinical profile
   The characteristics, physical condition, and biochemical parameters of the study subjects were shown in Table 1.
   The mean score of the nutritional disorder risk index, GNRI, was 93 ± 6, and the percentage of patients with a nutritional disorder risk with a GNRI score of less than 91 was 41.0 %.
2. Habitual dietary intakes
   The median (25th and 75th percentiles) daily habitual energy, nutrient, energy-adjusted nutrient, and food group intakes of the patients are shown in Table 2.
3. Adequacy ratio for "Dietary recommendations for chronic kidney disease (CKD), 2007 (guidelines) [21]".
   The energy, protein, potassium, and phosphorus intake sufficiency rates were 97.7, 101.9, 103.6, and 96.7 %, respectively, which met the recommendations of the guidelines, but the salt intake sufficiency rate was 152.6 %, indicating excessive ingestion, and only 12.8 % of patients met the recommendation of less than 6 g/day.

Table 3 Clinical characteristics of patients (*MTHFR* C677T genotype)

Variable		Genotype			
		CC	CT	TT	
		(*n* = 23) 29.5 %	(*n* = 34) 43.6 %	(*n* = 21) 26.9 %	*P* value
Age	years	62.7 ± 6.9[a]	63.6 ± 8.7	63.2 ± 8.7	0.920
Gender(Male/Female)	n	11/12	10/29	10/11	0.261
Duration of hemodialysis	years	28.0 (25.0-31.0)[b]	31 (27.0-34.3)	32.0 (28.5-35.0)	0.276
Age at initiation of hemodialysis	years	33.0 ± 7.3	32.7 ± 8.5	31.3 ± 8.2	0.760
Height	cm	159.7 ± 9.1	155.7 ± 8.1	158.5 ± 7.5	0.170
Dry weight(DW)	kg	50.0[d] (43.8-59.5)	44.0[e] (39.1-50.9)	52.6[d] (44.5-59.3)	0.014*
Body mass index(BMI)	kg/m^2	20.2 ± 19.7	18.8 ± 2.8	20.5 ± 2.6	0.050
BMI<18.5	n (%)	7 (21.9 %)[c]	19 (59.4 %)	6 (18.8 %)	
18.5≦BMI<25	n (%)	15 (35.7 %)	14(33.3 %)	13 (31.0 %)	
25≦BMI	n (%)	1 (25.0 %)	1 (25.0 %)	2 (50.0 %)	
Bathel Index(BADL)		90 (74-100)	98 (84-100)	85 (78-100)	0.253
BADL(independent/some help is necessary/with help)	n	20/2/1	33/1/0	18/1/2	<0.001*
Geriatric nutrition risk index(GNRI)		9489-90	9088-96	9491-99	0.200
≧GNRI 91(without risk of malnutrition)	n(%)	16 (34.8 %)	15 (32.6 %)	15 (32.6 %)	
<GNRI 91(with risk of malnutrition)	n(%)	7 (21.9 %)	19 (59.4 %)	6 (18.8 %)	
Biochemical parameter					
serum albumin (Alb)	g/dL	3.7 ± 0.3	3.8 ± 0.3	3.7 ± 0.3	0.887
total protein (TP)	g/dL	6.4[d] ± 0.3	6.6 ± 0.5	6.7[e] ± 0.5	0.045*
blood urea nitrogen (BUN)	mg/dL	63.8 ± 14	64.2 ± 6.6	67.6 ± 21.2	0.718
serum creatinine (Cr)	mg/dL	10.8 ± 2.3	10.5 ± 2.2	10.8 ± 2.2	0.873
urea acid (UA)	mg/dL	7.1 ± 1.3	6.9 ± 1.7	7.5 ± 1.5	0.341
serum sodium (Na)	mEq/L	141 ± 2	140 ± 2.6	140 ± 2.9	0.847
serum potassium (K)	mEq/L	5.1 ± 0.6	5.2 ± 0.7	5.1 ± 0.6	0.864
serum inorganic phosphorus (IP)	mg/dL	5.7 ± 1.1	5.1 ± 1.2	5.6 ± 1.8	0.164
serum calcium (Ca)	mg/dL	8.9 ± 0.7	9.3 ± 0.7	9 ± 0.6	0.096
Hemoglobin (Hb)	g/dL	10.4 (9.1-11.5)	10.3 (10.1-11.7)	10.2 (9.5-11.1)	0.789
Hematocrit (Ht)	%	33.5 ± 4.3	32.9 ± 3.3	32.7 ± 2.9	0.164
serum folate	ng/mL	4.7[d] (4.3-5.6)	4.7[d] (3.8-6.0)	3.9[e] (3.2-4.5)	0.005*
serum homocystein (Hcy)	μmol/L	30.5[d] (25.2-37.6)	25.1[d] (22.1-36.9)	35.4[e] (27.3-58.3)	0.029*
serum vitaminB_{12} (VB_{12})	pmol/L	545 (314-1200)	630 (383-1328)	456 (291-1463)	0.763

Abbreviations: *MTHFR* Methylene tetrahydrofolate reductase, *BMI* body mass index, *GNRI* geriatric nutrition risk index, *BADL* Barthel index
[a]Values are means ± Standard deviation(all such values)
[b]Values are median(25th and 75th percentiles)(all such values)
[c]Values are number of all patients (%)
The normality of the data was first assessed using the Shapiro-Wilks test
The values are compared between the groups by the χ2 test, one-way analysis of variance and Tukey's multiple comparison test of or Games-Howell's test, and Kruskal-Wallis test and Bonferroni's multiple comparison test as appropriate. *$p < 0.05$
[d-e]Multiple comparison; A different alphabet shows that there is a significant difference

4. Frequency of genetic polymorphism
   The frequencies of *MTHFR* C677T genotype were 29.5, 43.6, and 26.9 % for CC, CT, and TT genotypes, respectively. This showed that the frequency of TT type was significantly higher than that in Japanese hemodialysis patients as reported by Morimoto et al. (13.7 %) [11] and Kimura et al. (17.4 %) [22] ($P < 0.05$), and this was also significantly higher than that in healthy Japanese (about 15 %) [23, 24] ($P < 0.05$).
5. Activities of daily living
   The median score of BADL, which evaluates the performance of activities of daily living, was 90 (range, 78–100). Notably, 91 % of subjects were "independent" while 5 % "required partial assistance" and 4 % "required assistance".

Table 4 Nutrient intakes and food group intakes of subjects with 3 different genotype of *MTHFR* C677T polymorphism

Variable		CC(*n* = 23)29.5 %		CT(*n* = 34)43.6 %		TT(*n* = 21)26.9 %		*P* value
Energy	kcal	1897	(1299-2283)	1452	(1158-1812)	1584	(1365-1945)	0.088
Energy	kcal/kgIBW	33.0	(23.5-40.0)	27.6	(21.6-34.7)	30	(23.7-34.6)	0.155
Protein	g	62.2[b]	(49.4-85.2)	49.7[c]	(38.5-66.6)	58.9	(42.6-72.6)	0.036*
Protein	g/kgIBW	1.1	(1.0-1.6)	0.9	(0.8-1.3)	1.1	(0.8-1.2)	0.086
ratio of animal protein	%	58	(52-63)	54	(42-66)	55	(43-63)	0.332
NPC/N[a]		144	(126-154)	147	(129-191)	152	(120-200)	0.383
Fat	%E	28.5	(26.2-33.2)	27	(21.5-30.5)	23.7	(19.5-29.8)	0.067
Carbohydrate	%E	54.8	(51.7-57.2)	57.6	(50.7-65.8)	57.9	(54.0-64.3)	0.210
Retinol	μg	620	(457-1143)	538	(328-791)	552	(371-828)	0.184
α-tocopherol	mg	8.2[b]	(6.0-10.4)	5.8[c]	(4.3-7.4)	6.8	(5.0-8.7)	0.025*
Potassium	mg	2069	(1539-2945)	1802	(1359-2185)	2104	(1541-2618)	0.208
Calcium	mg	398	(290-573)	373	(230-473)	386	(249-476)	0.399
Phosphorus	mg	987	(677-1212)	725	(577-998)	847	(657-1048)	0.073
Iron	mg	7.7	(5.7-9.0)	6.0	(4.2-8.1)	6.5	(4.2-8.1)	0.114
VitaminB_6	mg	1.0	(0.8-1.5)	0.9	(0.7-1.2)	1.0	(0.8-1.5)	0.157
VitaminB_{12}	μg	8.4	(6.7-12.2)	6.5	(3.8-9.8)	5.4	(4.3-11.2)	0.123
Folate	μg	285	(219-436)	279	(183-364)	285	(183-368)	0.465
VitaminC	mg	100	(73-166)	93	(71-119)	103	(51-137)	0.475
Salt	g	10.3[b]	(8.7-12.0)	7.6[c]	(6.4-9.7)	9.3[d]	(7.8-11.3)	0.001*
Retinol	μg/1000 kcal	366	(237-556)	350	(266-492)	329	(235-461)	0.519
α-tocopherol	mg/1000 kcal	4.3	(3.9-5.1)	4.2	(3.5-4.8)	4.2	(3.2-5.3)	0.476
Potassium	mg/1000 kcal	1220	(1014-1373)	1211	(1075-1469)	1210	(936-1558)	0.992
Calcium	mg/1000 kcal	250	(185-292)	227	(184-300)	222	(164-292)	0.359
Phosphorus	mg/1000 kcal	543	(496-588)	516	(434-628)	486	(405-647)	0.614
Iron	mg/1000 kcal	4	(3.5-4.5)	4.3	(3.6-4.6)	3.7	(3.0-4.7)	0.407
VitaminB_6	mg/1000 kcal	0.6	(0.50-0.70)	0.7	(0.50-0.73)	0.6	(0.50-0.80)	0.969
VitaminB_{12}	μg/1000 kcal	4.7	(4.0-5.7)	4.5	(3.0-6.2)	4.2	(2.7-6.7)	0.609
Folate	μg/1000 kcal	165	(138-219)	190	(153-223)	164	(113-212)	0.445
VitaminC	mg/1000 kcal	60	(47-81)	64	(49-77)	65	(32-70)	0.808
Salt	g/1000 kcal	5.7	(5.2-6.4)	5.3	(4.5-6.2)	5.4	(4.4-6.5)	0.164
Cereals	g/1000 kcal	201	(184-284)	220	(187-297)	230	(185-318)	0.638
Potatos	g/1000 kcal	12	(7-44)	17	(7-41)	31	(10-53)	0.303
Sugar and confectioneries	g/1000 kcal	3.5	(1.8-4.5)	2.3	(1.6-3.2)	3.2	(1.7-3.6)	0.149
Nuts and pulses	g/1000 kcal	19	(11-29)	19	(11-29)	16	(6-29)	0.522
Green and yellow vegetables	g/1000 kcal	51	(27-65)	62	(42-83)	49	(26-78)	0.343
Other vegetables	g/1000 kcal	65	(49-100)	73	(44-101)	80	(49-119)	0.933
Fruits	g/1000 kcal	51	(32-107)	32	(15-67)	50	(17-86)	0.197
Fish and shellfish	g/1000 kcal	44	(26-51)	37	(21-52)	41	(20-60)	0.717
Meat	g/1000 kcal	42	(29-70)	31	(18-50)	36	(25-47)	0.087
Eggs	g/1000 kcal	24[b]	(11-35)	17	(6-28)	8[c]	(4-21)	0.028*
Daily products	g/1000 kcal	28	(9-47)	25	(8-73)	16	(0-61)	0.380
Fats and oils	g/1000 kcal	7.5	(5.4-9.7)	5	(3.7-7.)8	6.5	(5.8-8.7)	0.055

Table 4 Nutrient intakes and food group intakes of subjects with 3 different genotype of *MTHFR* C677T polymorphism *(Continued)*

confectioney	g/1000 kcal	23	(12-46)	24	(12-52)	24	(14-48)	0.962
Alcoholic bevarages and Non-Alcoholic bevarage	g/1000 kcal	176	(114-304)	207	(143-408)	246	(154-399)	0.401
seasoning and spice	g/1000 kcal	81	(61-150)	82	(51-135)	61	(36-121)	0.281

Abbreviations: *MTHFR* methylenetetrahydrofolate reductase
[a]*NPC/N* ratio of non-protein energy per nitroden
All values are median (25th and 75th percentiles)
The normality of the data was first assessed using the Shapiro-Wilks test
The values are compared between the groups by Kruskal-Wallis test and Bonferroni's multiple comparison test as appropriate. *$p < 0.05$
[b-c]Multiple comparison; A different alphabet shows that there is a significant difference

Comparison of the genotypes of *MTHFR* C677T polymorphism

The characteristics of patients, the physical condition, biochemical parameters and dietary intake, according the *MTHFR* C677T genotypes (CC, CT, and TT), are shown in Tables 3 and 4. No significant differences were observed in age, duration of dialysis therapy, age at the introduction of dialysis, physical condition, BADL or GNRI among the different genotypes. There were significant differences in TP ($P = 0.045$), serum folate ($P = 0.005$), and serum Hcy ($P = 0.029$), among the genotypes. The serum folate level was significantly lower and the serum Hcy level was significantly higher in the subjects with TT genotype than in those CC and CT genotypes. No significant differences were present in serum Alb, BUN, Cr, K, IP, Hb, Ht, and VB_{12} among the genotypes.

Regarding dietary intake, no significant differences were observed in the energy or protein intake per kg IBW, animal protein ratio, NPC/N, lipid energy ratio, or carbohydrate energy ratio, nor were there significant differences in the energy-adjusted potassium, phosphorus, folate, vitamin B_6, or B_{12} intake among the genotypes.

Associations between the serum Hcy level and physical condition, biochemical parameters, and food intakes

The results of analysis of correlations between the serum Hcy level and physical condition, biochemical parameters and food intake are shown in Tables 5 and 6. No correlation was observed between the serum Hcy level and age, duration of dialysis therapy, BMI or BADL. A positive significant correlation was noted with GNRI ($r = 0.33$, $P = 0.003$). Regarding the association with biochemical parameters, positive correlations were noted with serum Alb ($r = 0.271$, $P = 0.009$) and IP ($r = 0.334$, $P = 0.002$) levels, and inverse correlations were noted with serum folate ($r = -0.384$, $P < 0.001$) and serum VB_{12} ($r = -0.495$, $P < 0.001$) levels. Regarding the association with food intake, inverse correlations were noted with animal protein ratio ($r = -0.252$, $P = 0.013$), energy-adjusted vitamin B_6 intake ($r = -0.192$, $p = 0.048$), and B_{12} intake ($r = -0.242$, $P = 0.017$). No correlation was noted with energy-adjusted food group intakes.

Factors that may influence the serum Hcy level

The serum Hcy level was analyzed after testing the normality of the variable using the Shapiro–Wilk test and confirming the distribution by examining the histogram, followed by logarithmic transformation. To investigate factors influencing the serum Hcy level, the correlation matrix was examined with serum Hcy level as a response variable, but no variable with $|r| > 0.9$ was present. Thus, multiple regression analysis was performed employing the stepwise method, mainly regarding factors with

Table 5 Pearson's correlation coefficients between serum homocystein and laboratory test values

			($n = 77$)
		r	*P* value
Age	year	-0.112	0.382
Gender		-0.237	0.038*
Duration of hemodialysis	year	-0.072	0.568
Age at initiation of hemodialysis	year	-0.074	0.524
MTHFR C677T		-0.249	0.029*
Height	cm	0.179	0.119
Weight(dry weight)	kg	0.205	0.073
BMI	kg/m^2	0.139	0.227
GNRI		0.330	0.003*
BADL		0.035	0.760
serum albumin	g/dL	0.271	0.017*
blood urea nitrogen (BUN)	mg/dL	0.040	0.732
serum creatinine (Cr)	mg/dL	0.200	0.081
urea acid (UA)	mg/dL	0.211	0.065
serum sodium (Na)	mEq/L	0.081	0.486
serum potassium (K)	mEq/L	0.026	0.822
serum inorganic phosphorus (IP)	mg/dL	0.334	0.003*
serum calcium (Ca)	mg/dL	-0.088	0.445
Hemoglobin (Hb)	g/dL	0.071	0.541
Hematocrit (Ht)	%	-0.025	0.830
serum folate	ng/mL	-0.384	0.001*
serum homocystein (Hcy)	pmol/L	-0.495	<0.001*

Abbreviations: *MTHFR* methylenetetrahydrofolate reductase, *BMI* body mass index, *GNRI* geriatric nutrition risk index, *BADL* Barthel index
Data not regularly distributed were log transformed for futher statistical analysis. *$P < 0.05$
Adjusted for gender, age

Table 6 Peason's correlation coefficients between serum homocystein consentration and dietary intake

			(*n* = 77)
		r	*P* value
Energy	kcal/kgIBW	-0.062	0.592
Protein	g/kgIBW	-0.149	0.194
ratio of animal protein	%	-0.252	0.027*
NPC/N[a]		0.176	0.125
Fat	%	-0.135	0.241
Carbohydrate	%	0.191	0.097
Potassium	mg	-0.103	0.372
Calcium	mg	-0.164	0.153
Phosphorus	mg	-0.110	0.342
Iron	mg	-0.100	0.388
VitaminB_6	mg	-0.101	0.382
VitaminB_{12}	μg	-0.207	0.071
Folate	μg	-0.090	0.435
Salt	g	-0.092	0.425
Energy-adjusted nutrient intakes			
Potassium	mg/1000 kcal	-0.127	0.272
Calcium	mg/1000 kcal	-0.165	0.152
Phosphorus	mg/1000 kcal	-0.175	0.127
Iron	mg/1000 kcal	-0.137	0.236
VitaminB_6	mg/1000 kcal	-0.192	0.095
VitaminH_{12}	μg/1000 kcal	-0.242	0.034*
Folate	μg/1000 kcal	-0.093	0.422
Salt	g/1000 kcal	-0.151	0.19
Energy-adjusted food group intakes			
Cereals	g/1000 kcal	0.164	0.155
Potatoes	g/1000 kcal	0.136	0.239
Sugar and confectioneries	g/1000 kcal	0.010	0.932
Nuts and pulses	g/1000 kcal	-0.062	0.590
Green and yellow vegetables	g/1000 kcal	-0.041	0.726
Other vegetables	g/1000 kcal	-0.080	0.492
Fruits	g/1000 kcal	-0.187	0.104
Fish and shellfish	g/1000 kcal	-0.095	0.413
Meat	g/1000 kcal	-0.109	0.346
Eggs	g/1000 kcal	-0.147	0.202
Daily products	g/1000 kcal	-0.109	0.347
Fats and oils	g/1000 kcal	-0.092	0.426
Confectioneries	g/1000 kcal	0.403	0.403
Alcoholic beverages and Non-Alcoholic beverage	g/1000 kcal	0.138	0.231
seasoning and spice	g/1000 kcal	0.009	0.941

[a]*NPC/N,* ratio of non-protein energy per nitrogen
Data not regularly distributed were log transformed for further statistical analysis. *$p < 0.05$
Adjusted for gender, age

which association was noted as response variables. The results are shown in Table 7.

On analysis using age and sex as adjustment factors, factors influencing the serum Hcy level were the serum VB_{12}, folate, and IP levels as well as animal protein intake ratio and serum Alb level. The results in the analysis of variance table was significant ($P < 0.001$) with $R^2 = 0.552$ and adjusted $R^2 = 0.513$. The Durbin–Watson ratio was 1.716, being non-problematic, and there was no outlying predicted value exceeding ±3SD of the measured value.

Discussion

In this study, the actual state of nutritional management of long-term hemodialysis patients was investigated at first. Nutritional management contributed to the maintenance of a high quality of life for these long-term hemodialysis patients, i.e., high level of independence in ADL was maintained. In particular, it was clarified that the influence of *MTHFR* C677T polymorphism—which has attracted international attention as a risk gene for cardiovascular disease that were major reasons for poor outcome in hemodialysis patient could be overcome. Regarding nutritional condition, mean BMI was 19.7 ± 2.7 kg/m^2, mean serum Alb level was 3.7 ± 0.3 g/dL, and median GNRI was 93 ± 6. Since the reported BMI of Japanese hemodialysis patients is 21.4 ± 4.1 kg/m^2 [5], their nutritional condition was favorable.

The frequencies of CC, CT, and TT genotypes of *MTHFR* C677T were 29.5, 43.6, and 26.9 %, respectively, and the frequency of TT genotype was significantly higher than the frequency of that in Japanese hemodialysis patients as reported by Morimoto et al. (13.7 %) [11] and Kimura et al. (17.4 %) [22] and healthy Japanese (about 15 %) [23, 24]. Many studies reported that *MTHFR* C677T polymorphism is a risk factor for nephropathy, and a significantly high frequency of the TT genotype in nephropathy patients was also detected in a meta-analysis conducted by Yang et al. [25]. Similar results were obtained in studies of Asians reported by Sun et al. [26] and Mtiraoui et al. [27]. In studies of Japanese hemodialysis patients, Kimura et al. [22] investigated the association between *MTHFR* C677T and hyperhomocysteinemia, and Morimoto et al. [11] investigated *MTHFR* C677T, hyperhomocysteinemia, and risk of cardiovascular disease, but they did not investigate whether the polymorphism is a risk factor for nephropathy. However, they investigated the genotype frequency of *MTHFR* C677T in hemodialysis patients, and the frequency of the TT genotype was similar to those reported by Sun et al. [26] and Mtiraoui et al. [27]. The TT genotype have been reported as a risk factor for cardiovascular disease by Morimoto et al. [11], and for death in end-stage renal failure patients by Jamison et al. [28], which cannot be explained by the results of our study. It was assumed that

Table 7 Multiple regression analyses to test the effects of serum components on serum homocysteine concentration

Response variable; serum Hcy					
Explanatory variable	Standardized partial regression coefficient (β)	Standard error	*P* value	95 % confidential interval	
Serum albumin (g/dL)	0.228	0.048	0.007	0.037	0.229
Serum inorganic phosphorus (mg/dL)	0.242	0.012	0.006	0.009	0.056
Serum folate (ng/mL)	−0.351	0.007	<0.0001	−0.043	−0.015
Serum VitaminB_{12} (pmol/L)	−0.367	0.0001	<0.0001	0	0
Ratio of animal protein (%)	−0.213	0.001	0.016	−0.005	−0.001

$R = 0.743$ $R^2 = 0.552$
Multiple regression analyses by stepwise method
Adjusted for gender, age
Explanatory variable; MTHFRC677T, BMI, GNRI, serum potassium, energy intake (kcal/kgIBW), protein intake (g/kgIBW), energy-adjusted potassium intake, energy-adjusted phosphate intake, energy-adjusted vitaminB_6, B_{12} intake, energy-adjusted folate intake

the frequency of the TT genotype at the time of introduction of dialysis was higher than the reported frequency because the duration of dialysis therapy was 7–10 years in the reports described above, which was far shorter than that in our study. Serum folate (5-methyltetrahydrofolate) decreases with the TT genotype because of a 70 % decrease in the MTHFR enzyme activity level compared with the activity with the CC genotype, which inhibits the pathway of conversion of homocysteine to methionine and elevates the serum Hcy level. Hyperhomocysteinemia promotes renal dysfunction, as reported by Wollesen et al. [29]. It has also been reported that the prevalence of hyperhomocysteinemia is high in hemodialysis patients, and hyperhomocysteinemia is also a risk factor for death. Similarly, hyperhomocysteinemia was noted in most patients (96 %) in our study. The physical condition, biochemical parameters, and dietary intake were compared among the *MTHFR* C677T genotypes to clarify the influence of this polymorphism. No significant differences in age, duration of dialysis therapy, age at introduction of dialysis, BMI, serum Alb level, or GNRI among different genotypes were noted. BADL was also not significantly different, and the percentages of independent patients were high with each genotype. Two of three patients "requiring assistance" harbored the TT genotype, but as described above, they were elderly, the duration of dialysis therapy was long (38 years) and they were independent in their activities of daily living, although some patients used a wheelchair. There were no significant differences in nutrient or food group intake among the different genotypes. The energy, protein, potassium, and phosphorus intakes met the nutritional levels recommended in the guidelines in all three genotypes. The salt intake of patients markedly exceeded the amount specified in the guidelines, indicating excessive consumption. However, their body weight was controlled, based on the examination of body weight changes between dialysis sessions. As reported by several preceding studies, the serum folate level was low and the serum Hcy level was high with the TT genotype. In addition, folate and vitamin B_{12} intakes met the Dietary Reference Intakes of Japanese dietary recommendations, while vitamin B_6 was slightly insufficient. Based on these findings, although the serum folate and Hcy levels were influenced by the genetic polymorphism, nutritional management was appropriate.

Four limitations of this study exist. First, the sample size was small, and investigation of sex differences was not performed. Second, the causal relationships between the factors influencing the serum Hcy level could not be identified because this was a cross-sectional study. Third, the dietary survey was capable of investigating habitual food intake over 1 month, but it is unclear whether the same pattern of food intake was maintained over a long period after introduction of dialysis. Fourth, the study results are not applicable to the present hemodialysis patients in Japan because diabetic nephropathy patients were absent in this study. Fifth, this is not a comparative study, the impact of some items that investigated in this study cannot be able to evaluate exactly. This study might be just a presentation of clinical and genetic profile of Japanese long-term dialysis patients.

A positive result was obtained in most studies that examined the association between serum Hcy level and arteriosclerotic disease. It has been clarified that hyperhomocysteinemia is a predictive factor for high mortality that is independent of other risk factors in coronary arterial disease patients [30]. Hyperhomocysteinemia was shown to be an independent risk factor in a study of 750 vascular disease patients at 19 institutions in 9 European countries [31]. It was also shown to be a strong cardiovascular disease risk factor in non-insulin dependent diabetes patients [32]. These findings are of interest with regard to the synergistic effect of risk factors. Inverse correlations of folate and vitamin B_{12} levels with serum Hcy level in healthy subjects [23] have been reported, as well as the decrease in serum Hcy level after increased ingestion of these vitamins, but it remains to be investigated whether these findings are applicable to hemodialysis patients.

Conclusion

In conclusion, it is natural that the frequency of the TT genotype of *MTHFR* C677T was very high (26.9 %) in patients because the TT genotype is a risk factor for renal failure and there might be many patients with the TT genotype at the time of introduction of dialysis. However, the frequency of the TT genotype was not high in long-term dialysis patients in preceding studies because the mortality rate during dialysis therapy was high with the TT genotype. The results of our study suggested that appropriate nutritional management decreases the high mortality rate in patients with the TT genotype.

Competing interests
This study was supported by a grant from Japanese Association of Dialysis Physicians.

Authors' contributions
KS and YKan visited each facilities, and collected questionnaires and blood samples. MH and YKag carried out RFLP analysis. MH evaluated clinical data of patients. YKon evaluated and analyzed BDHQ food questionnaires. All authors read and approved the final manuscript.

Acknowledgement
The authors gratefully thank to the physicians and all medical staff in dialysis facilities as follow, Yoshizawa Iin (Mamoru Yoshizawa), Ikenaga Jin Clinic (Hideki Ikenaga), Kameido Nephrology Clinic (Yoshiaki Itaya), Fureai Machida Hospital (Waichi Kitajima), Bosei Hospital (Tateki Kitaoka, Tetsuo Shirai, Kyoko Kino), Meguro Building Clinic (Tsuneo Takenaka), Matsumoto Clinic (Go Matsumoto, Takaomi Tanaka), Yotsuya Jin Clinic (Akiko Iwata), Namikibashi Clinic (Masaru Ogawa), Kikuna Memorial Clinic (Hideki Uchimura), Saitama Tsukinomori Clinic (Satoshi Kurihara, Naoaki Hayama), Kidney Clinic Setagaya (Shinya Suganuma), Tokorozawa Jin Clinic (Hiroshi Nagaura). A part of this study was presented in the 46th annual meeting of American Society of Nephrology (Philadelphia, 2014).

Sources of support
Fund from Japanese Association of Dialysis Physicians.

Author details
[1]Department of Clinical Nutrition and Dietetics, Kagawa Nutrition University, Sakado, Japan. [2]Department of Nephrology, Tokyo Medical University, 6-7-1 Nishi-Shinjuku, Shinjuku, Tokyo 160-0023, Japan. [3]Department of Nutrition, School of Nursing and Nutrition, Shukutoku University, Chiba, Japan. [4]Apheresis and Dialysis Center, School of Medicine, Keio University, Tokyo, Japan. [5]Department of Health and Nutrition, Faculty of Human Life Studies, University of Niigata Prefecture, Niigata, Japan. [6]Department of Medical Chemistry, Kagawa Nutrition University, Sakado, Japan.

Received: 3 July 2015 Accepted: 24 September 2015
Published online: 24 November 2015

References

1. Pifer TB, McCullough KP, Port FK, Goodkin DA, Maroni BJ, Held PJ, et al. Mortality risk in hemodialysis patients and changes in nutritional indicators: DOPPS. Kidney Int. 2002;62:2238–45.
2. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, et al. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney Int. 2008;73:391–8.
3. de Mutsert R, Snijder MB, van der Sman-de Beer F, Seidell JC, Boeschoten EW, Krediet RT, et al. Association between body mass index and mortality is similar in the hemodialysis population and the general population at high age and equal duration of follow-up. J Am Soc Nephrol. 2007;18:967–74.
4. Iseki K, Kawazoe N, Fukiyama K. Serum albumin is a strong predictor of death in chronic dialysis patients. Kidney Int. 1993;44:115–9.
5. Leavey SF, McCullough K, Hecking E, Goodkin D, Port FK, Young EW. Body mass index and mortality in 'healthier' as compared with 'sicker' haemodialysis patients: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephrol Dial Transplant. 2001;16:2386–94.
6. Nakai S, Watanabe Y, Masakane I, Wada A, Shoji T, Hasegawa T, et al. Overview of regular dialysis treatment in Japan (as of 31 December 2011). Therap Apher Dial. 2013;17:567–611.
7. Bachmann J, Tepel M, Raidt H, Riezler R, Graefe U, Langer K, et al. Hyperhomocysteinemia and the risk for vascular disease in hemodialysis patients. J Am Soc Nephrol. 1995;6:121–5.
8. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111–3.
9. Morita H, Taguchi J, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, et al. Genetic polymorphism of 5, 10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. Circulation. 1997;95:2032–6.
10. Fodinger M, Mannhalter C, Wolfl G, Pabinger I, Muller E, Schmid R, et al. Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. Kidney Int. 1997;52:517–23.
11. Morimoto K, Haneda T, Okamoto K, Ishida H, Kikuchi K. Methylenetetrahydrofolate reductase gene polymorphism, hyperhomocysteinemia, and cardiovascular diseases in chronic hemodialysis patients. Nephron. 2002;90:43–50.
12. Sasaki S, Katagiri A, Tsuji T, Shimoda T, Amano K. Self-reported rate of eating correlates with body mass index in 18-y-old Japanese women. Int J Obes Relat Metab Disord. 2003;27:1405–10.
13. Okubo H, Sasaki S, Rafamantanantsoa HH, Ishikawa-Takata K, Okazaki H, Tabata I. Validation of self-reported energy intake by a self-administered diet history questionnaire using the doubly labeled water method in 140 Japanese adults. Eur J Clin Nutr. 2008;62:1343–50.
14. Shinzato T, Nakai S, Fujita Y, Takai I, Morita H, Nakane K, et al. Determination of Kt/V and protein catabolic rate using pre- and postdialysis blood urea nitrogen concentrations. Nephron. 1994;67:280–90.
15. Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, Hirota N, et al. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol. 2012;22:151–9.
16. Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, et al. Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr. 2011;14:1200–11.
17. Sasaki S, Yanagibori R, Amano K. Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. J Epidemiol. 1998;8:203–15.
18. Mahoney FI, Barthel DW. Functional evaluation: the Barthel index. Md State Med J. 1965;14:61–5.
19. Bouillanne O, Morineau G, Dupont C, Coulombel I, Vincent JP, Nicolis I, et al. Geriatric nutritional risk index: a new index for evaluating at-risk elderly medical patients. Am J Clin Nutr. 2005;82:777–83.
20. Yamada K, Furuya R, Takita T, Maruyama Y, Yamaguchi Y, Ohkawa S, et al. Simplified nutritional screening tools for patients on maintenance hemodialysis. Am J Clin Nutr. 2008;87:106–13.
21. Nakao T, Sanaka T, Tsubakihara Y, Hattori M, Honda M, Mizuiri S, et al. Dietary recommendations for chronic kidney disease, 2007. Jpn J Nephrol. 2007;49:871–8.
22. Kimura H, Gejyo F, Suzuki S, Miyazaki R. The C677T methylenetetrahydrofolate reductase gene mutation in hemodialysis patients. J Am Soc Nephrol. 2000;11:885–93.
23. Hiraoka M, Kato K, Saito Y, Yasuda K, Kagawa Y. Gene–nutrient and gene–gene interactions of controlled folate intake by Japanese women. Biochem Biophys Research Commun. 2004;316:1210–6.
24. Sadewa AH, Sunarti, Sutomo R, Hayashi C, Lee MJ, Ayaki H, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene among the Indonesian Javanese population. Kobe J Med Sci. 2002;48:137–44.
25. Yang S, Zhang J, Feng C, Huang G. MTHFR 677T variant contributes to diabetic nephropathy risk in Caucasian individuals with type 2 diabetes: a meta-analysis. Metabolism. 2013;62:586–94.
26. Sun J, Xu Y, Zhu Y, Lu H. Genetic polymorphism of methylenetetrahydrofolate reductase as a risk factor for diabetic nephropathy in Chinese type 2 diabetic patients. Diabetes Res Clin Pract. 2004;64:185–90.
27. Mtiraoui N, Ezzidi I, Chaieb M, Marmouche H, Aouni Z, Chaieb A, et al. MTHFR C677T and A1298C gene polymorphisms and

hyperhomocysteinemia as risk factors of diabetic nephropathy in type 2 diabetes patients. Diabetes Res Clin Pract. 2007;75:99–106.
28. Jamison RL, Shih MC, Humphries DE, Guarino PD, Kaufman JS, Goldfarb DS, et al. Effect of the MTHFR C677T and A1298C polymorphisms on survival in patients with advanced CKD and ESRD: a prospective study. Am J Kidney Dis. 2009;53:779–89.
29. Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int. 1999;55:1028–35.
30. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 1997;337:230–6.
31. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA. 1997;277:1775–81.
32. Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs C, Bouter LM, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. Arterioscler Thromb Vasc Biol. 1998;18:133–8.