J Epidemiol 2014;24(5):379-384
doi:10.2188/jea.JE20140002

Original Article

Associations Between *hOGG1* Ser326Cys Polymorphism and Increased Body Mass Index and Fasting Glucose Level in the Japanese General Population

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Received January 8, 2014; accepted March 31, 2014; released online July 5, 2014

ABSTRACT

Background: Evidence suggests that Ser326Cys, a genetic polymorphism of human 8-oxoguanine glycosylase 1 (*hOGG1*), is associated with insulin resistance and type 2 diabetes; however, the underlying mechanism is unclear. Recently, an animal study showed a significant association between the *hOGG1* genotype and obesity, although evidence for such an association in humans is limited. The purpose of this study was to examine the association between the *hOGG1* genotype and body mass index (BMI) and fasting blood glucose (FBG) levels.

Methods: Cross-sectional analysis was conducted using the baseline survey data from a Japan Multi-Institutional Collaborative Cohort Study, which included 1793 participants aged 40–69 years. The *hOGG1* polymorphism was detected using a multiplex polymerase chain reaction-based invader assay. Multiple linear regression, analysis of covariance, and logistic regression were used to control for confounding variables.

Results: The Cys allele was significantly associated with increased BMI, FBG level, and total cholesterol (TC) level, even after adjustment for gender, age, energy intake, alcohol, smoking, physical activity, and family history of diabetes. An association with BMI was still observed after further adjustment for FBG and TC, but not for the study area (Amami or the mainland). The Cys/Cys genotype was significantly more prevalent in the participants with higher BMI (>27.5 kg/m^2). However, the impact of genotype decreased and significance disappeared after adjusting for the study area.

Conclusions: The present results suggest that the study area being inside Japan confounds the association between *hOGG1* genotype and obesity.

Key words: human 8-oxoguanine glycosylase 1 (*hOGG1*); obesity; body mass index (BMI); fasting blood glucose (FBG); polymorphism; study area

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INTRODUCTION

Reactive oxygen species (ROS) are known to play an essential role in the pathogenesis of diabetes.[1] Several studies have reported that oxidative stress associated with insulin resistance, β cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction can ultimately lead to the diabetes disease state.[2–4] ROS also cause strand breaks and base modifications in DNA, including the oxidation of guanine residues to 8-hydroxy-2′-deoxyguanine (8-OHdG). These ROS-induced mutations alter the function of various genes and influence the pathogenesis of several diseases, such as cancer, cardiovascular disease, neurodegenerative diseases, and diabetes.[1] Base-excision repair (BER) plays an important role in preventing such disease, and human 8-oxoguanine glycosylase 1 (*hOGG1*) is one of the key glycosylases involved in the BER system.[5] The Ser326Cys polymorphism of the highly polymorphic OGG1 gene has been studied the most because this polymorphism is associated with functional differences in enzyme activity[6] and loss of function.[7] However, most epidemiological studies of this polymorphism have focused on cancer susceptibility.[8–12]

In the past decade, several studies have reported that the Ser326Cys *hOGG1* polymorphism is associated with insulin resistance[13] and type 2 diabetes[14–17]; however, the underlying mechanism has not been elucidated. Obesity-associated insulin resistance is a major risk factor for type 2 diabetes,[18] and fat accumulation has been reported to be associated with systemic oxidative stress[19,20]; therefore, it may be possible to assess the risk of diabetes based on the association between the Ser326Cys *hOGG1* polymorphism and body mass index (BMI). Recently, an animal study found that *hOGG1* deficiency alters cellular substrate metabolism, which favors a sparing phenotype and increased susceptibility to obesity[21]; however, evidence for such association between *hOGG1* polymorphism and BMI in humans is limited.[14]

The purpose of this study was to determine whether the *hOGG1* Cys allele is associated with BMI and fasting glucose level. We also studied whether this association is modified by the effects of other factors.

METHODS

Study participants

The purpose of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study is to confirm and detect gene-environment interactions for lifestyle-related diseases using a large genome cohort, as previously described.[22] Briefly, the J-MICC Study, which was initiated in 2005, included volunteers aged 35–69 years from 10 areas of Japan: Chiba, Shizuoka, Okazaki, Aichi, Takashima and Kyoto, which are located in Honshu Island; Tokushima, which is located in Shikoku Island; Fukuoka and Saga, which are located in Kyushu Island; and Amami, which is located 380 km southwest of Kyushu Island. Throughout this paper, we refer to Honshu Island, Shikoku Island, and Kyushu Island as "the mainland". In this cross-sectional study, data from 4512 participants throughout these areas were collected during the period of 2005–2008.[23] Written informed consent was obtained from all participants. The study protocol was approved by the Nagoya University School of Medicine ethics committees and other participating institutions.

Questionnaire and measurements

A self-administered questionnaire was used to collect data on alcohol consumption, smoking, dietary habits, physical activity, current medication, disease history, and first-degree family history of diabetes. Details of the dietary assessment and estimation of physical activity were reported elsewhere.[24–27]

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). We defined a BMI of >27.5 kg/m^2 as obese; increased mortality has been reported above this point among East Asians.[28] The HbA1c (%) and fasting blood glucose (FBG), triglyceride, total cholesterol, and HDL cholesterol levels were measured in laboratories in each study area, and the results of these measurements were collected. The HbA1c (%) value was converted from the Japan Diabetes Society (JDS) to the National Glycohemoglobin Standardization Program (NGSP) by using the following equation published by the JDS: NGSP (%) = 1.02 × JDS (%) + 0.25%.[29]

Genotyping

Genotyping was performed as described previously.[23] Single nucleotide polymorphisms, including the *hOGG1* Ser326Cys (rs1052133), were genotyped using a multiplex polymerase chain reaction-based Invader assay (Third Wave Technologies, Madison, WI, USA)[30] at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN.

Statistical analysis

In the analysis, we excluded 2719 participants based on any of the following conditions: missing data of *hOGG1* polymorphism ($n = 11$); missing FBG ($n = 2627$) data; taking type 2 diabetes medication ($n = 89$); or a dietary energy intake greater than 4000 kcal/day ($n = 2$). Consequently, data for 976 men and 817 women aged 35–69 years were retained for analysis. Among these participants, data on alcohol consumption (17 men and 23 women) or physical activity (5 men and 5 women) were missing for some participants.

All analyses were performed with the SAS statistical software package (Ver. 9.3 for Windows; SAS Institute, Cary, NC, USA). A P value of less than 0.05 was considered statistically significant.

Table 1. Characteristics according to the *hOGG1* Ser326Cys genotype among 1793 subjects

	Ser/Ser		Ser/Cys		Cys/Cys		*P*
n (%) *n* = 1793	365	(20.4)	866	(48.3)	562	(31.3)	
Gender, women (%)	180	(49.3)	393	(45.4)	244	(43.4)	0.209
Age (y) (SD)	55.0	(8.6)	54.5	(8.9)	55.1	(8.7)	0.354
Study area, Amami area (%)	60	(16.4)	185	(21.4)	204	(36.3)	<0.001
Total energy intake (kcal/d) (SD)	1729.7	(348.4)	1752.9	(371.6)	1730.0	(376.3)	0.406
BMI (kg/m^2) (SD)	23.2	(3.0)	23.2	(3.3)	23.7	(3.5)	0.021
Physical activity level (METs·h) (SD)	13.6	(12.1)	14.7	(13.9)	15.5	(14.9)	0.775
Current alcohol drinkers, *n* (%)	192	(53.6)	485	(57.2)	333	(60.9)	0.001
Current smoking, *n* (%)	63	(17.3)	155	(17.9)	98	(17.4)	0.981
Family history of diabetes, *n* (%)	58	(15.9)	142	(16.4)	85	(15.1)	0.451
HbA1c (NGSP) (%)	5.61	(0.55)	5.54	(0.44)	5.58	(0.46)	0.199
FBG (mg/dL) (SD)	96.9	(13.3)	96.6	(14.6)	99.4	(16.9)	<0.001
TG (mg/dL) (SD)	110.8	(69.1)	114.0	(80.9)	116.7	(82.4)	0.368
TC (mg/dL) (SD)	207.1	(33.0)	210.9	(34.0)	212.3	(34.2)	0.077
HDL-C (mg/dL) (SD)	64.7	(16.5)	64.1	(16.4)	63.3	(16.5)	0.278

BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.
P values for Chi-square test or Kruskal-Wallis test.

To compare the characteristics of participants according to the *hOGG1* genotype, we used the Kruskal-Wallis test for continuous variables and χ^2 tests for categorical variables. Adjusted means and their 95% confidence intervals (CIs) of BMI, FBG, and total cholesterol according to *hOGG1* genotype were evaluated by least-squares general linear regression, and linear trends were assessed by the statistical significance of the regression coefficient of an ordinal variable for the factor under the following considerations: gender; age (continuous); energy intake (continuous); physical activity level (continuous); alcohol consumption status (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46.0 g ethanol/day); smoking status (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/day); first-degree family history of diabetes (positive, negative, or unknown); study area (Amami or the mainland); BMI (continuous, for the evaluation of FBG and total cholesterol); FBG (continuous, for BMI and total cholesterol); and total cholesterol (continuous, for BMI and FBG). Odds ratios (ORs) and 95% CIs of *hOGG1* genotype for excessive BMI (>27.5 kg/m^2) were estimated using logistic regression models adjusted for potential confounders (age, BMI, energy intake, alcohol consumption, smoking, physical activity, family history of diabetes, and study area).

RESULTS

The characteristics of study participants according to the *hOGG1* Ser326Cys genotype are shown in Table 1. The genotype distributions of the *hOGG1* Ser326Cys gene among all participants followed the Hardy-Weinberg equilibrium ($\chi^2 = 0.511$, $P = 0.475$). Genotype frequency was significantly different in the Amami area ($P < 0.001$). The Cys allele carriers had significantly higher mean BMI ($P = 0.021$) and FBG ($P < 0.001$) levels, and a higher proportion were current alcohol drinkers ($P < 0.001$). TC level tended to be higher in Cys allele carriers, although this difference was not statistically significant ($P = 0.077$).

After adjusting for possible confounding factors, such as gender, age, energy intake, physical activity level, ethanol intake, smoking, and family history of diabetes, the Cys allele was found to be significantly associated with higher BMI, FBG, and TC levels in a dose-dependent manner (all $P < 0.05$, Table 2, Model 2). The association with BMI was still significant after further adjustment for FBG and TC ($P = 0.02$, Model 3). However, the significance disappeared after adjusting for study area ($P = 0.23$, Model 4).

Data for the evaluation of the association between obesity (BMI > 27.5 kg/m^2) and *hOGG1* genotype using logistic regression analysis are shown in Table 3. The prevalence of obesity in the Cys/Cys genotype was significantly greater after adjusting for gender, age, energy intake, physical activity level, ethanol intake, smoking, family history of diabetes, FBG, and TC (Model 3). Although Cys allele carriers tended to have a higher proportion of obesity, the significance of this association disappeared after adjusting for the study area (Model 4). The OR of the Amami area for obesity was 2.44 (95% CI 1.67–3.56), which was greater than that of the *hOGG1* genotype.

DISCUSSION

In this cross-sectional study, we observed significant associations between the *hOGG1* Cys/Cys genotype and higher BMI and incidence of obesity, after adjustment for possible confounding factors other than the study area. After adjusting for study area, however, this significance disappeared, suggesting that study area is a confounding factor. Despite this lack of association, Cys allele carriers tended to have a higher proportion of obesity than Ser/Ser.

Table 2. Adjusted means of BMI, FBG, and TC according to *hOGG1* Ser326Cys genotype

	Ser/Ser		Ser/Cys		Cys/Cys		*P* for trend
	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Model 1[a]							
BMI (kg/m^2)	23.2	(22.8–23.5)	23.2	(23.0–23.4)	23.7	(23.4–24.0)	0.013
FBG (mg/dL)	97.1	(95.6–98.6)	96.6	(95.6–97.6)	99.2	(98.0–100.4)	0.013
TC (mg/dL)	206.6	(203.2–210.0)	210.9	(208.6–213.1)	212	(209.5–215.0)	0.025
Model 2[b]							
BMI (kg/m^2)	23.2	(22.9–23.5)	23.2	(23.0–23.4)	23.7	(23.4–24.0)	0.018
FBG (mg/dL)	97.3	(95.8–98.8)	96.3	(95.4–97.2)	99.1	(97.9–100.2)	0.025
TC (mg/dL)	207.0	(203.5–210.4)	210.8	(208.5–213.0)	212	(209.1–214.6)	0.040
Model 3[c]							
BMI (kg/m^2)	23.2	(22.8–23.5)	23.3	(23.1–23.5)	23.6	(23.4–23.9)	0.020
FBG (mg/dL)	97.7	(96.4–99.1)	96.6	(95.7–97.5)	98.4	(97.3–99.5)	0.260
TC (mg/dL)	207.1	(203.6–210.5)	211.0	(208.7–213.2)	212	(208.7–214.3)	0.071
Model 4[d]							
BMI (kg/m^2)	23.3	(22.9–23.6)	23.3	(23.1–23.5)	23.5	(23.2–23.8)	0.230
FBG (mg/dL)	98.1	(96.7–99.4)	96.7	(95.8–97.6)	98.1	(97.0–99.2)	0.769
TC (mg/dL)	207.1	(203.7–210.6)	211.0	(208.7–213.2)	211	(208.6–214.3)	0.078

BMI, body mass index; CI, confidence interval; FBG, fasting blood glucose; TC, total cholesterol.
[a]Adjusted for gender and age (continuous).
[b]Adjusted for Model 1 and further adjusted for energy intake (continuous), physical activity level (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/day), smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/day), and family history of diabetes (positive, negative, or unknown).
[c]Adjusted for all variables in Model 2 and further adjusted for BMI (for FBG and TC), FBG (for BMI and TC), and TC (for BMI and FBG).
[d]Adjusted for all variables in Model 3 and further adjusted for the study area (Amami or the mainland).

Table 3. Odds ratios and 95% CIs for obesity (BMI > 27.5 kg/m^2) according to *hOGG1* Ser326Cys genotype among 1793 subjects

BMI (kg/m^2)	>27.5	≤27.5	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
	n	*n*	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Ser/Ser	24	341	1.00	reference	1.00	reference	1.00	reference	1.00	reference
Ser/Cys	84	782	1.50	(0.94–2.41)	1.44	(0.90–2.32)	1.55	(0.94–2.57)	1.45	(0.87–2.41)
Cys/Cys	62	500	1.77	(1.08–2.90)	1.74	(1.06–2.86)	1.74	(1.03–2.94)	1.46	(0.86–2.48)
			P_{trend} = 0.026		P_{trend} = 0.029		P_{trend} = 0.049		P_{trend} = 0.225	

BMI, body mass index; CI, confidence interval; OR, odds ratio.
[a]Adjusted for gender and age (continuous).
[b]Adjusted for Model 1 and further adjusted for energy intake (continuous), physical activity level (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/d), smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/d), and family history of diabetes (positive, negative, or unknown).
[c]Adjusted for all variables in Model 2 and further adjusted for FBG and TC.
[d]Adjusted for all variables in Model 3 and further adjusted for the study area (Amami or the mainland).

Several epidemiological studies have examined the associations between the Ser326Cys *hOGG1* polymorphism and insulin resistance[13] and type 2 diabetes.[14–17] Wang et al reported that the Cys/Cys variant significantly decreases insulin sensitivity, even after adjustment for possible confounders, including BMI, among the Taiwanese.[13] Three studies detected significant association between Ser326Cys *hOGG1* polymorphism and diabetes.[14,16,17] Specifically, Daimon et al reported that decreased insulin secretion is associated with being a Cys allele carrier, measured by homeostatic model assessment beta cell function (HOMA-β) in a Japanese population,[14] while Gönül et al reported a significant association between the Cys allele and insulin resistance in a Turkish population, measured by HOMA-R.[17] On the other hand, one case-control study conducted in a Polish population failed to detect an association between this polymorphism and diabetes[15] due to limited sample size. Regarding obesity, an animal study showed significant association between the *hOGG1* genotype and obesity,[21] and one epidemiological study reported a positive association between BMI and this polymorphism.[14] Our study showed no evidence of an association between this polymorphism and increased BMI and FBG.

In the present study, the study area had a significant impact on the prevalence of obesity. Among the studies reporting genetic differences between the Amami and the mainland populations,[23,31,32] two used data from the J-MICC study.[23,32] Nishiyama et al found a low but significant level of genetic

differentiation between the mainland population and the population of the Amami Islands,[32] while Wakai et al reported that some polymorphisms showed a substantial difference in minor allele frequency among the participating cohorts.[23] They proposed that genetic variation among the study areas should be considered when analyzing the data from the J-MICC study. According to the Japanese Single Nucleotide Polymorphisms (JSNP) database, the frequency of *hOGG1* genotypes of Ser/Ser, Ser/Cys, and Cys/Cys was reported for 18%, 59%, and 23% of participants, respectively.[33] In this study, the genotype frequency of Ser/Ser, Ser/Cys, and Cys/Cys polymorphisms in mainland Japan was 22.7%, 50.7%, and 26.6%, while those on Amami Island were 13.4%, 41.2%, and 45.4%, respectively. We found that the variation in the *hOGG1* genotype Cys/Cys frequency between the Amami and the mainland could lead to a false-positive result if the study area was not considered. This is known as confounding by population stratification,[34] which needs to be carefully considered in genetic epidemiology, even in the relatively homogeneous Japanese population. A significantly higher BMI in the Amami area may reflect population differences in genetic or environmental factors; therefore, further investigation is needed.

This study has several methodological limitations. First, the cross-sectional nature of our study limits our ability to determine causation, even though we excluded participants who were on medication for type 2 diabetes. In addition, we did not have appropriate replication data accompanying this study. Second, although measuring fat accumulation using computed tomography scans or echograms is ideal, we used BMI to evaluate obesity. Misclassification of obesity may have therefore occurred; however, misclassification of obesity would be expected to lower estimations for the association. Third, there may be intrinsic information bias in our assessments of lifestyle-related factors, such as dietary and family history. However, if any misclassification were present, it would be non-differential by the *hOGG1* genotype and would likely underestimate the true associations. Finally, although we adjusted for potential confounding factors in the multivariate analysis, residual confounding factors by known or unknown risk factors may have been present.

In conclusion, these results suggest that the *hOGG1* Ser/Cys genotype may have some influence on obesity, although its contribution is smaller than the influence of the study area. While our study found no associations of this genotype with BMI or FBG levels, we did find evidence of confounding by population stratification for these associations. This report may provide important information for genetic association analysis in the Japanese population.

ONLINE ONLY MATERIAL

Abstract in Japanese.

ACKNOWLEDGEMENTS

The authors thank Kyota Ashikawa, Tomomi Aoi, and other members of the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN for support with genotyping and Yoko Mitsuda, Keiko Shibata, and Etsuko Kimura at the Department of Preventive Medicine, Nagoya University, Graduate School of Medicine for their technical assistance. This study was supported in part by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (nos. 25460759, 17015018, 18390182, 18790380, 20249038, 20689014, and 221S0001).

Conflicts of interest: None declared.

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RESEARCH ARTICLE

Dietary Patterns and Clinical Outcomes in Hemodialysis Patients in Japan: A Cohort Study

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OPEN ACCESS

Citation: Tsuruya K, Fukuma S, Wakita T, Ninomiya T, Nagata M, Yoshida H, et al. (2015) Dietary Patterns and Clinical Outcomes in Hemodialysis Patients in Japan: A Cohort Study. PLoS ONE 10(1): e0116677. doi:10.1371/journal.pone.0116677

Academic Editor: Manlio Vinciguerra, University College London, UNITED KINGDOM

Received: January 5, 2014

Accepted: December 11, 2014

Published: January 21, 2015

Funding: This study is supported by scientific research grants from Kyowa Hakko Kirin (Japan). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: S. Fukuhara is an advisor of epidemiology study for Kyowa Hakko Kirin (a commercial source) and receives consulting fees from Kyowa Hakko Kirin. T. Akizawa is a consultant and has grants with Kyowa Hakko Kirin and Chugai (a commercial source). This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Abstract

Background & Objectives

Little is known about actual dietary patterns and their associations with clinical outcomes in hemodialysis patients. We identified dietary patterns in hemodialysis patients in Japan and examined associations between dietary patterns and clinical outcomes.

Design, setting, participants, measurements

We used data from 3,080 general-population participants in the Hisayama study (year 2007), and data from 1,355 hemodialysis patients in the Japan Dialysis Outcomes and Practice Patterns Study (JDOPPS: years 2005–2007). Food intake was measured using a brief self-administered diet-history questionnaire (BDHQ). To identify food groups with the Hisayama population data, we used principal components analysis with Promax rotation. We adjusted the resulting food groups for total daily energy intake, and then we used those adjusted food-group scores to identify dietary patterns in the JDOPPS patients by cluster analysis (Ward's method). We then used Cox regression to examine the association between dietary patterns and a composite of adverse clinical outcomes: hospitalization due to cardiovascular disease or death due to any cause.

Results

We identified three food groups: meat, fish, and vegetables. Using those groups we then identified three dietary patterns: well-balanced, unbalanced, and other. After adjusting for potential confounders, we found an association between an unbalanced diet and important clinical events (hazard ratio 1.90, 95% C.I. 1.19–3.04).

Conclusions

Hemodialysis patients whose diet was unbalanced were more likely to have adverse clinical outcomes. Thus hemodialysis patients might benefit not only from portion control, but also from a diet that is well-balanced diet with regard to the food groups identified here as meat, fish, and vegetables.

Introduction

Dietary management is important to improve outcomes in hemodialysis patients. Clinical guidelines provide a recommended intake of micronutrients[1] to prevent hyperphosphatemia, hyperkalemia, hypertension, and water retention. Reduced intakes of protein, raw vegetables, and salt are recommended.[2–8] Excessive dietary restriction may of course result in malnutrition, but details of dietary patterns that might improve outcomes in hemodialysis patients are largely unknown.

Some previous research on nutritional epidemiology in kidney disease has focused on the absolute amounts of foods and micronutrients[7,9]. We focused instead on dietary patterns, which were identified by their balance (or unbalance) among food groups. Given that the prognosis of hemodialysis patients is better in Japan than in the US and Europe, we expected that an understanding of the relationship between dietary pattern and prognosis in hemodialysis patients in Japan would also provide useful information for hemodialysis care in other countries.

Here we report the results of a cohort study using data from hemodialysis patients participating in the Japan Dialysis Outcomes and Practice Patterns Study (JDOPPS) [10,11]. Our goals were to identify dietary patterns in those patients and to investigate relationships between dietary patterns and important clinical outcomes.

Methods

Ethics

The ethics committees of Kyushu University (Fukuoka, Japan) and Kyoto University (Kyoto, Japan) approved this study. Written informed consent was obtained from participants in the Hisayama study[12,13] and in the JDOPPS. The data were analyzed anonymously.

Participants and setting

The participants were selected from among Japanese volunteers participating in the Hisayama study[12,13] and Japanese hemodialysis patients participating in the JDOPPS.

The Hisayama study is a population-based study that has been conducted since 1961 in Hisayama-cho in the Kyushu region of Japan. Subjects are volunteers of various ages, and are considered to represent the age distribution of the population of Japan.[14,15] In the present study, we analyzed data from 3,080 people enrolled in the Hisayama study in 2007.

The JDOPPS is part of the International Dialysis Outcomes and Practice Patterns Study, an international longitudinal study of hemodialysis patients. Patients in the JDOPPS were selected randomly from among representative dialysis facilities in Japan, and they appear to represent all hemodialysis patients in Japan. The design of the DOPPS is detailed elsewhere.[16] After we excluded data from hemodialysis patients whose dietary intake was not measured and those with a daily energy intake of less than 500 kcal or more than 4,000 kcal, data from 1,355 hemodialysis patients who participated in the third phase of the JDOPPS between 2005 and 2007 were available for analysis.

The predictors

The methods regarding the predictors had four steps: (1) collection of data on food consumption, (2) identification of food groups, (3) computation of food-group scores, and (4) identification of dietary patterns. Those four steps are described in sequence below. We note that this method for identifying dietary patterns is based on foods and food groups, not on micronutrients, and that methods such as the one we used in this study are common in nutritional epidemiology.[17–20]

(1) Collection of data on food consumption (Hisayama study): Data on foods consumed were obtained using a brief self-administered diet-history questionnaire (the BDHQ).[21–23] The BDHQ is a 4-page structured questionnaire that contains questions about 58 foods and beverages, and allows the total energy intake and the intake of micronutrients to be estimated. Reports of previous studies indicate that food intake estimated using the BDHQ is consistent with intake measured using semi-weighted 16-day dietary records.[21,24] Food intake was measured with the BDHQ in the Hisayama study in 2007 and in the JDOPPS during the second year of JDOPPS enrollment, between 2006 and 2007.

(2) Identification of food groups (Hisayama study): To identify food groups, we conducted a principal components analysis (PCA). We used PCA with Promax rotation to reduce the results regarding the many foods listed in the BDHQ to a smaller set of food groups. That is, we used PCA to identify groups of foods that were eaten with approximately equal frequencies by the same people. We did those analyses with data from 3,080 participants in the Hisayama study. Here it is important to remember one similarity between PCA and other multivariate analyses: When the values of an independent variable are nearly the same among almost all participants, then that independent variable contributes little or no information to the results, and such variables should be deleted from the analyses. Therefore, in PCA it is common practice to delete items that vary by only small amounts between individuals [25], so for the PCA in this study we used 20 foods from the 58 in the BDHQ.

(3) Computation of food-group scores (Hisayama study and JDOPPS): After identifying food groups, we standardized the frequency of consumption of each food by using the mean and standard deviation in the Hisayama data. Then we used those standardized frequencies to compute food-group scores for each JDOPPS patient, and we used the residual method [26] to adjust those food-group scores for the total daily energy intake

(4) Identification of dietary patterns (JDOPPS): To identify dietary patterns in the JDOPPS patients, we used Ward's method of cluster analysis[27] on the energy-adjusted food-group scores. Thus, the patterns we identified were based on the relative amounts of foods from each food group that the JDOPPS patients actually ate.

The outcome

This study had one outcome, which was a composite of important adverse clinical events: hospitalization due to cardiovascular disease or death due to any cause. Cardiovascular disease included coronary heart disease, arrhythmia, congestive heart failure, cardiac valvular disease, cardiac myopathy, and pericarditis. The date and cause of hospitalization was ascertained approximately every 4 months in the JDOPPS.

Analyses (associations between dietary patterns and the outcome)

Cox regression analysis was used to investigate relationships between dietary patterns and the composite outcome. Those relationships were expressed as hazard ratios. The time between the second year of food-intake measurement using the BDHQ and the composite outcome was studied first. Two models were used. In Model 1, the covariates considered in estimating the hazard ratio were age, sex, and hemodialysis duration. In Model 2, the covariates were body mass index, serum albumin, total daily energy intake, and comorbid conditions (coronary artery disease, congestive heart failure, cerebrovascular disease, peripheral vascular disease, and diabetes).

In a sensitivity analysis, we adjusted for hemoglobin level, the dose of erythropoietin-stimulating agent (ESA), and Kt/V, in addition to the covariates included in Model 2. In another sensitivity analysis, we adjusted for smoking habit in addition to the covariates included in Model 2.

All analyses were done with SAS 9.2 (SAS Institute, Cary, NC) and STATA 13.1 software (STATA, College Station, TX).

Results

Population characteristics

Table 1 shows the characteristics of participants in the Hisayama study and in the JDOPPS. We included 3,080 participants from the Hisayama study. The mean of their ages was 62.7 years, and 10.6% of them had diabetes. We also included 1,355 hemodialysis patients from

Table 1. Demographic and clinical characteristics of the participants in the Hisayama study and in the JDOPPS.

	Hisayama(n = 3,080)	JDOPPS(n = 1,355)
Mean (SD) age, years	62.7 (12.0)	61.4 (11.9)
Male (%)	43.6	61.4
Mean (SD) dialysis duration, years	NA	7.6 (7.2)
Mean (SD) BMI	23.1 (3.5)	21.1 (3.2)
Comorbid conditions (%)		
Diabetes	10.6	32.1
Coronary heart disease	6.0	41.3
Cerebrovascular disease	3.7	11.5
Other cardiovascular disease	8.2	30.6
Peripheral vascular disease	0.2	15.9
Cancer	7.5	9.4
Mean (SD) albumin, g/dL	4.2 (0.3)	3.8 (0.4)

JDOPPS: Japan Dialysis Outcomes and Practice Patterns Study, NA: not applicable, BMI: body mass index.

doi:10.1371/journal.pone.0116677.t001

the JDOPPS. The mean of their ages was 61.4 years, and 32.1% of them had diabetes. The mean duration of their dialysis was 7.6 years. The proportions of comorbidities, including diabetes and cardiovascular disease, were higher in the JDOPPS group than in the Hisayama group.

Food groups (general–population results)

In the first PCA, "natto (fermented soybean)" had a moderate loading on 2 components. We therefore deleted "natto" and ran the PCA again. The first three components had eigenvalues greater than 1: 5.69, 1.53, and 1.35, which accounted for 28.4%, 7.8%, and 6.74% of the variance, respectively. As shown in Table 2, three food groups were identified. The first group included carrot & pumpkin, root vegetables, cabbage (cooked), mushrooms, seaweed, lettuce & cabbage (raw), potatoes, tofu (bean curd) & fried tofu, turnip (radish), and tomato. This we call the vegetables group. The second group included squid & octopus & shrimp & shellfish, dried fish, fatty fish, lean fish, and small fish with bones. This we call the fish group. The third group included ham, pork & beef, chicken, and eggs. This we call the meat group.

Table 2. Coefficients after Promax rotation (Principal Components Analysis, Hisayama data).

		Component		
Food Item		**1**	**2**	**3**
Carrot/pumpkin		0.798	−0.079	0.001
Root vegetables		0.754	−0.055	−0.022
Green leafy vegetables		0.705	−0.092	0.067
Cabbage (cooked)		0.686	−0.146	0.148
Mushrooms		0.636	0.058	0.013
Seaweed		0.591	0.135	−0.127
Lettuce/cabbage (raw)		0.543	−0.122	0.260
Potatoes		0.530	0.168	−0.057
Tofu (bean curd)/fried tofu		0.504	0.154	−0.026
Turnip (radish)		0.497	0.189	−0.106
Tomato		0.426	0.104	−0.003
Dried fish		−0.081	0.693	0.087
Fatty fish		0.027	0.630	0.116
Lean fish		0.103	0.594	−0.034
Small fish with bones		0.160	0.591	−0.179
Squid, octopus, shrimp, shellfish		−0.095	0.536	0.248
Ham		−0.135	0.042	0.718
Pork/beef		0.054	0.019	0.715
Chicken		0.057	0.121	0.564
Eggs		0.116	−0.012	0.474
Coefficents of correlation	1	1.000	0.430	0.267
	2		1.000	0.242
	3			1.000
Deleted food				
Natto				

Data on 20 foods were analyzed with principal components analysis (Promax rotation). The first three components had eigenvalues greater than 1: 5.69, 1.53, and 1.35, which accounted for 28.4%, 7.8%, and 6.74% of the variance.

doi:10.1371/journal.pone.0116677.t002

Table 3. Adjusted food-group scores for each cluster (JDOPPS data).

Cluster	*n*	Food-group score		
		Vegetables	Fish	Meat
Well-balanced	666 49.2%	0.297 (0.460)	0.216 (0.936)	0.319 (0.874)
Unbalanced	189 14.0%	1.522 (0.454)	0.528 (0.809)	0.315 (0.838)
Other	500 36.9%	−0.971 (0.643)	−0.488 (0.945)	−0.544 (0.980)

Each food-group score was adjusted for total daily energy intake by the residual method [20]. Values in parentheses are standard deviations.

doi:10.1371/journal.pone.0116677.t003

Dietary patterns in hemodialysis patients

Cluster analysis of the adjusted food-group scores revealed three clusters, which we call (1) "well-balanced diet", (2) "unbalanced diet," and (3) "other diet" (Table 3). Patients in the first of those three clusters, i.e. those whose diet was well-balanced, were those who ate approximately equal amounts of food from the meat, fish, and vegetable groups. Almost half of the JDOPPS patients had a well-balanced diet (49.2%). Patients in the second of the three clusters, i.e. those whose diet was unbalanced, were those who ate a much larger amount from the vegetable group than from the meat group and the fish group. They amounted to 14% of the JDOPPS patients.

Fig. 1 shows the amounts of micronutrients for each cluster of JDOPPS patients. According to clinical guidelines, protein intake was within the prescribed range among those who ate a well-balanced diet, too high among those who ate an unbalanced diet, and too low among the others. [1] The mean salt intake was more than 6 g/day in all groups, and was highest among those who ate an unbalanced diet. Potassium intake was within the prescribed range among those who ate a well-balanced diet, too high among those who ate an unbalanced diet, and too low among the others. Phosphorus intake was similar to potassium intake.

Patient characteristics by dietary pattern

Table 4 shows characteristics of the JDOPPS patients, stratified by the three dietary patterns. Patients who ate an unbalanced diet were older than those who ate a well-balanced diet, and fewer of them were male. Total daily energy intake, protein intake, salt intake, and potassium intake were highest among those whose diet was unbalanced.

Association between dietary pattern and clinical outcomes in hemodialysis patients

Table 5 shows associations between dietary patterns and the composite outcome. In Model 1, which included adjustments for age, gender, and dialysis duration, the unbalanced diet was associated with a higher event rate than the well-balanced diet (adjusted hazard ratio [HR] 1.81, 95% CI 1.15–2.85). A similar association was seen in Model 2 (adjusted HR 1.90, 95% CI 1.19–3.04), that is, after adjustment for serum albumin, BMI, and total daily energy intake, in addition to the covariates included in Model 1.

In the sensitivity analysis adjusted for the covariates included in Model 2 and also adjusted for hemoglobin level, ESA dose, and single-pool Kt/V, we also found a similar association between unbalanced diet and the composite outcome (adjusted HR 1.89, 95% CI 1.11–3.23). In the other sensitivity analysis, adjusted for the covariates included in Model 2 and also for smoking habit, we again found a similar association between unbalanced diet and adverse clinical events (adjusted HR 1.85, 95% CI 1.16–2.97).

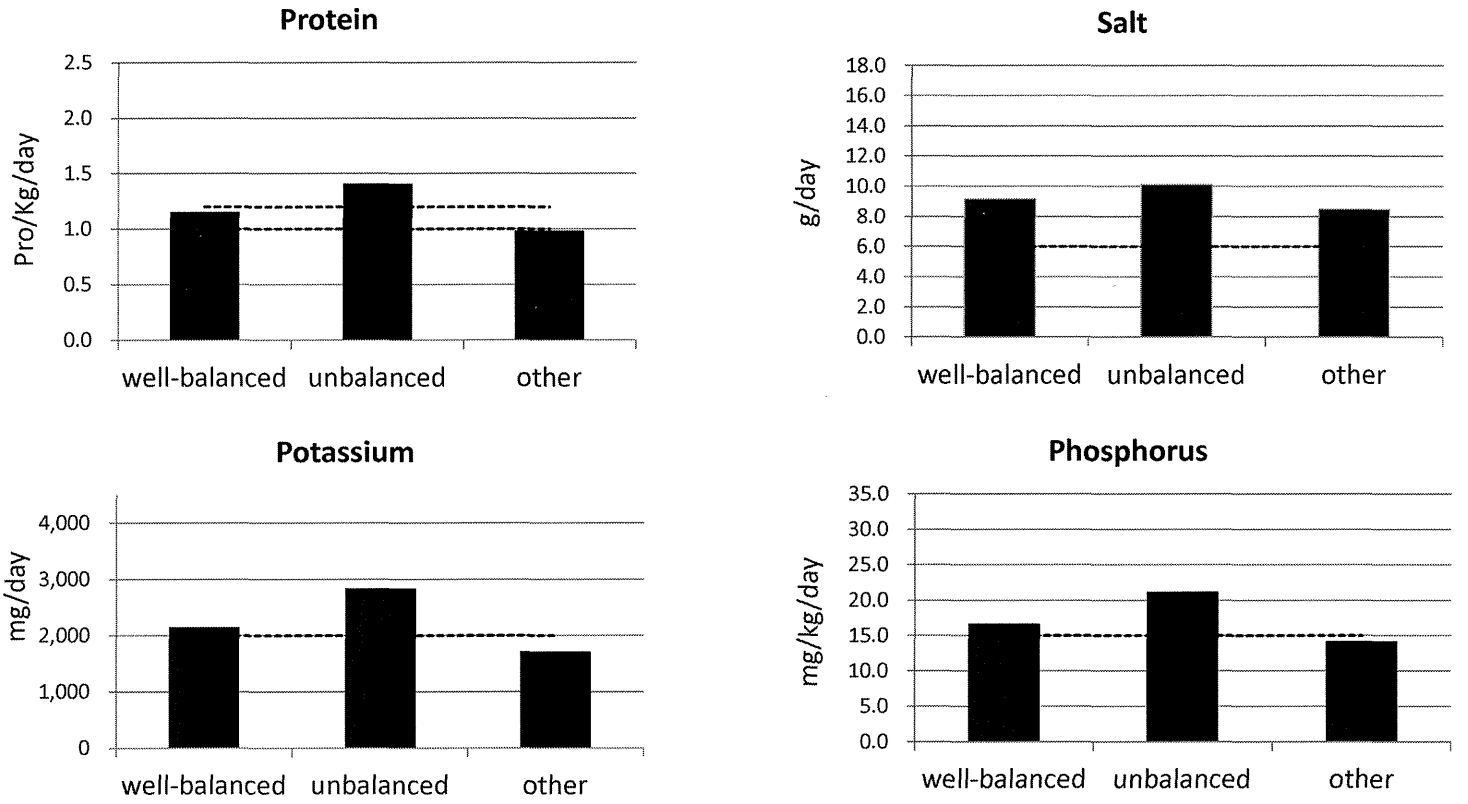

Figure 1. Micronutrient intake stratified by dietary pattern. Estimated micronutrient intake stratified by dietary pattern. Dotted lines show dietary standards according to Japan's clinical guidelines (Dietary recommendations for chronic kidney disease, 2007, Japanese Society of Nephrology).

doi:10.1371/journal.pone.0116677.g001

Discussion

Using PCA with data from a representative sample of the general population of Japan, we identified three food groups: meat, fish, and vegetables. We then found that hemodialysis patients could be said to have diets that were "well-balanced" or "unbalanced" with regard to those three food groups. (As noted previously, to identify dietary patterns based on foods or on food groups, as we did in this study, is common in nutritional epidemiology.[17–19]) The hemodialysis patients whose diet was unbalanced were more likely to have important clinical events. These findings suggest that limiting food portions, which is often recommended for hemodialysis patients to prevent severe adverse clinical outcome, is not enough. In addition to portion control, a diet that is balanced among meat, fish, and vegetables might help to prevent adverse outcomes.

Nutritional epidemiologic research in hemodialysis patients has largely focused on relationships between individual food items, micronutrients, and outcomes. For example, relationships between fish consumption, phosphate consumption, and outcomes in these patients have been reported.[7,28] However, hemodialysis patients do not eat only one specific food item, but rather various foods, and therefore dietary patterns should be determined on the basis of the combinations of foods that people actually eat. We began with PCA, from which we identified three groups of foods that are in fact eaten by people in Japan: meat, fish, and vegetables. We then used cluster analysis, from which we identified hemodialysis patients' actual patterns of food consumption with reference to those groups. Those patterns (well-balanced, unbalanced, and other) were associated with important clinical outcomes.

In hemodialysis patients, adequate protein intake (1.0 to 1.2 g/kg per day), such as can be obtained from the meat and fish groups we identified, is recommended to counteract loss of

Table 4. JDOPPS patient characteristics at baseline, by dietary pattern (n = 1,355).

	Well-balanced (49.2%)	Unbalanced (14.0%)	Other (36.9%)
Mean (SD) age, years	62.3 (11.8)	64.2 (11.9)	59.2 (11.5)
Male (%)	57.2	40.7	74.8
Mean (SD) dialysis duration, years	7.7 (7.4)	7.2 (7.2)	7.6 (7.0)
Mean (SD) BMI	21.2 (3.3)	20.2 (3.0)	21.5 (3.1)
Comorbid conditions (%)			
Diabetes	31.7	32.8	32.2
Coronary Heart Disease	44.3	41.8	37.2
Cerebrovascular Disease	12.8	12.7	9.4
Other Cardiovascular Disease	28.5	36.0	31.2
Peripheral Vascular Disease	15.6	22.2	14.0
Cancer	8.7	9.6	10.3
Mean (SD) serum albumin, g/dL	3.8 (0.4)	3.8 (0.5)	3.9 (0.4)
Mean (SD) phosphorus, mg/dL	5.6 (1.3)	5.3 (1.4)	5.6 (1.4)
Mean (SD) serum potassium, mEq/L	5.1 (0.8)	5.1 (0.8)	5.0 (0.8)
Mean (SD) energy intake, cal/Kg/day	1592 (563)	1707 (538)	1640 (656)
Mean (SD) protein intake, g/Kg/day	1.16 (0.51)	1.41 (0.59)	0.99 (0.49)
Mean (SD) salt intake, g/day	9.16 (3.32)	10.10 (3.21)	8.48 (3.60)
Mean (SD) potassium intake, g/day	2.15 (0.86)	2.84 (1.01)	1.72 (0.84)
Mean (SD) phosphorus intake, mg/day	883 (370)	1018 (376)	793 (395)

The "well-balanced diet" was characterized by approximately equal intake of the three food groups (fish, meat, and vegetables). The "unbalanced diet" was characterized by relatively large vegetable intake compared with meat and fish intake, and the "other diet" refers to other intake patterns.

doi:10.1371/journal.pone.0116677.t004

protein via the dialysate.[29] Sufficient protein intake is critical to preventing malnutrition, but excessive protein intake may lead to hyperphosphatemia, which may in turn lead to cardiovascular events. Hemodialysis patients should also avoid excessive vegetable intake to prevent hyperkalemia, which, like hyperphosphatemia, is associated with cardiovascular events. It is therefore physiologically plausible that a diet well-balanced among food groups would be associated with good clinical outcomes, as was found in this study.

The present study had a number of strengths. First, the Hisayama study and the JDOPPS used representative samples of the general population of Japan and of hemodialysis patients in Japan, respectively. Therefore the findings should be generalizable to all hemodialysis patients in Japan. To the extent that differences in dietary patterns between hemodialysis patients in Japan and those in other countries can result in differences in clinical outcomes, the present findings might be used for nutritional research and possibly also for dietary recommendations to improve the prognosis of patients in, for example, the US and Europe. Second, the use of the

Table 5. Dietary patterns and the composite outcome (JDOPPS data).

Dietary patterns	Composite outcome rate (/100 person-years)	Model 1 Hazard ratio (95% CI)	Model 2 Hazard ratio (95% CI)
Well-balanced	7.4	Reference	
Unbalanced	10.3	1.81 (1.15–2.85)	1.90 (1.19–3.04)
Other	6.1	1.23 (0.82–1.83)	1.21 (0.81–1.82)

The composite outcome included hospitalization due to cardiovascular disease, and death due to any cause. Model 1: Adjusted for age, gender, and dialysis duration. Model 2: Adjusted for age, gender, dialysis duration, serum albumin, BMI, total daily energy intake, and comorbid conditions (diabetes, coronary heart disease, cerebrovascular disease, other cardiovascular disease, and peripheral vascular disease).

doi:10.1371/journal.pone.0116677.t005

BDHQ enabled us to measure food intake in clinical settings.[21–24] Third, results of the sensitivity analyses indicated that the association of dietary pattern with the composite outcome was robust with respect to hemoglobin level, ESA dose, Kt/V, and smoking habit.

One possible limitation of this study is that food intake was self-reported. Actual food intake might have differed from that estimated from the food-frequency questionnaire.[30] In particular, social-desirability bias might have caused hemodialysis patients, who were aware of their dietary proscriptions, to report inaccurately-low levels of food intake, and the estimated intake of micronutrients might therefore have been incorrect.

In summary, eating a diet that was not balanced among meat, fish, and vegetables was associated with important adverse clinical events, which suggests that hemodialysis patients should not only limit their food intake but should also strive for a proper balance among those three food groups.

Acknowledgments

We thank the residents of Hisayama for participating in the survey and the staff of the Division of Health and Welfare of Hisayama for cooperating in this study. We also thank the patients in the JDOPPS for participating in the survey, and the staff of the dialysis facilities participating in the JDOPPS. We thank Joseph Green for advice and suggestions.

Author Contributions

Conceived and designed the experiments: KT S. Fukuma TN S. Fukuhara. Performed the experiments: MN HY S. Fujimi YK TK KU TS T. Akizawa T. Akiba AS. Analyzed the data: S. Fukuma TW. Wrote the paper: KT S. Fukuma TW TN S. Fukuhara.

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©Journal of Sports Science and Medicine (2014) 13, 590-596
http://www.jssm.org

Research article

Physical Fitness Measures As Potential Markers of Low Cognitive Function in Japanese Community-Dwelling Older Adults without Apparent Cognitive Problems

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Abstract
Detecting signs of cognitive impairment as early as possible is one of the most urgent challenges in preventive care of dementia. It has still been unclear whether physical fitness measures can serve as markers of low cognitive function, a sign of cognitive impairment, in older people free from dementia. The aim of the present study was to examine an association between each of five physical fitness measures and global cognition in Japanese community-dwelling older adults without apparent cognitive problems. The baseline research of the Sasaguri Genkimon Study was conducted from May to August 2011 in Sasaguri town, Fukuoka, Japan. Of the 2,629 baseline subjects who were aged 65 years or older and not certified as individuals requiring nursing care by the town, 1,552 participants without apparent cognitive problems (Mini-Mental State Examination score ≥24) were involved in the present study (59.0% of the baseline subjects, median age: 72 years, men: 40.1%). Global cognitive function was measured by the Japanese version of the Montreal Cognitive Assessment. Handgrip strength, leg strength, sit-to-stand rate, gait speed, and one-leg stand time were examined as physical fitness measures. In multiple linear regression analyses, each of the five physical fitness measures was positively associated with the Montreal Cognitive Assessment score after adjusting for age and sex ($p < 0.001$). These associations were preserved after additional adjustment for years of formal education, body mass index, and other confounding factors ($p < 0.001$). The present study first demonstrated the associations between multiple aspects of physical fitness and global cognitive function in Japanese community-dwelling older people without apparent cognitive problems. These results suggest that each of the physical fitness measures has a potential as a single marker of low cognitive function in older populations free from dementia and thereby can be useful in community-based preventive care of dementia.

Key words: Cognitive screening, community-based study, cross-sectional study, mild cognitive impairment, physical function, primary prevention.

Introduction

Dementia has been perceived as a burdensome public health issue in aging societies (Wimo et al., 2013; Wimo and Prince, 2011). One of the most urgent challenges in the primary care field is to detect signs of cognitive impairment as early as possible before clinical diagnosis. Earlier detection has been suggested to allow for effective medical treatments preventing or slowing the onset of dementia (Siemers, 2011; Sperling et al., 2011). Hence, there is a great need for identifying biomarkers and other lifestyle-related markers which help the detection of subtle cognitive impairment occurring in the preclinical or earlier phase of the disease.

Physical fitness has been reported to be a lifestyle-related factor predicting future incidence of dementia and cognitive impairment (Alfaro-Acha et al., 2007; Buchman et al., 2007; Sattler et al., 2011; Wang et al., 2006). In contrast, it has not been fully understood whether physical fitness measures can serve as markers of low cognitive function, a sign of cognitive impairment, in older people free from dementia. Two recent population-based studies suggested a role of gait speed as a marker of low cognitive function in the pre-dementia stage by demonstrating its association with global cognition in cognitively intact older people (Fitzpatrick et al., 2007; Mielke et al., 2013). However, the knowledge for other physical fitness measures has still been limited. The other measures not yet investigated include those often administered in community-based health checkups to evaluate different aspects of physical fitness. Because the primary detection essentially needs to cover community-dwelling older individuals having diverse physical functional status, it is worth understanding abilities of the other physical fitness measures as single markers of low cognitive function in the pre-dementia stage. Therefore, the aim of the present study was to examine if each of the physical fitness measures determined by five common tests would be associated with global cognitive function in Japanese community-dwelling older people without apparent cognitive problems.

Methods

Participants

The present study was performed as part of the baseline research of the Sasaguri Genkimon Study (SGS) conducted from May to August 2011. The design of the SGS has been described in detail elsewhere (Narazaki et al., 2013). Briefly, it is an ongoing community-based prospective cohort study in Sasaguri Town, a regional town on Kyushu Island located in the southwest part of Japan, aiming to explore modifiable lifestyle-related factors causing older people to require nursing care. Subjects of the baseline research were 2,629 town residents who were aged 65 years or older and not certified as individuals requiring nursing care by the town at the end of January 2011. Of the baseline subjects, we excluded 17 individuals with a medical history of dementia or Parkinson's

Received: 19 March 2014 / Accepted: 09 May 2014 / Published (online): 01 September 2014

disease, 526 individuals who refused or did not complete cognitive tests, 146 individuals with signs of apparent cognitive problems determined by a Mini-Mental State Examination (MMSE) score of <24, 177 individuals who refused physical fitness tests, and 211 individuals with incomplete data on other measurements (Figure 1). Accordingly, 1,552 participants were involved in the present study (59.0% of the baseline participants). Written informed consent was obtained from all the baseline subjects prior to their participation. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Institute of Health Science, Kyushu University.

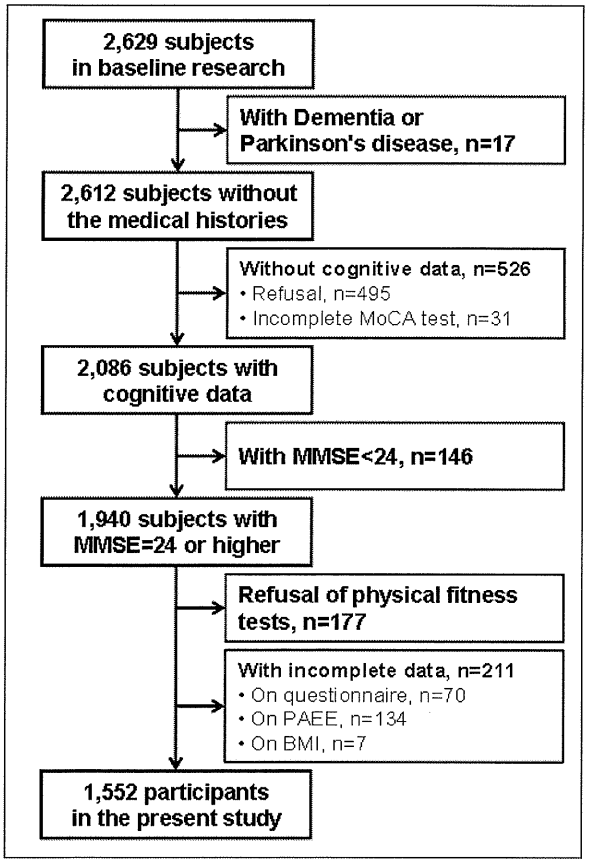

Figure 1. Flow chart of participation. This figure shows the flow of participation in the present study. MoCA, MMSE, PAEE, and BMI denote Montreal Cognitive Assessment, Mini-Mental State Examination, physical activity energy expenditure, and body mass index, respectively.

Cognitive function measures

Cognitive function was measured with the Japanese version of the Montreal Cognitive Assessment (MoCA). The details of this instrument are explained elsewhere (Fujiwara et al., 2010). Like the original one (Nasreddine et al., 2005), it is a 30-point tool for measuring global cognition in the nine domains of attention, calculations, concentration, conceptual thinking, executive functions, language, memory, orientation, and visuoconstructional ability. A higher MoCA score indicates better cognitive function. All MoCA tests were administered to the participants by trained examiners in accordance with the official instruction (Suzuki, 2010). After the measurement, MoCA scores were independently evaluated by two trained authors (K.N and T.H: 93.2% of agreement). Inconsistent evaluations between the two were judged together before final scores were determined. For the present study, MoCA scores without the 1-point correction for years of education were used. In addition to the MoCA, we performed the Japanese version of MMSE to screen apparent cognitive problems with the cut-off score of <24, as explained in the above section. This MMSE cut-off score has been widely used to detect abnormal cognitive status and to screen dementia in previous studies (Holsinger et al., 2007).

Physical fitness measures

Multiple aspects of physical fitness were objectively measured through five tests in a random manner: the handgrip test for measuring upper-extremity strength, the isometric knee extension test for lower-extremity strength, the five-times sit-to-stand test for lower-extremity agility, the 5-meter gait test for locomotive coordination, and the open-eyed one-leg stand test for postural balance. These five tests were selected because they are commonly administered in community-based regular checkups for older people in Japan. The handgrip test was performed twice for each hand using a digital grip dynamometer (TKK5401; Takei Scientific Instruments, Niigata, Japan) in a standing position. In this test, the participants were asked to grip the dynamometer as strongly as possible. The handgrip strength (HS: kg) was determined as an average of the highest scores of the left and right hands. The isometric knee extension test was also performed twice for each leg using a digital tension meter (TKK5710e; Takei Scientific Instruments, Niigata, Japan) in a seated position with the knee flexed at 90 degrees. During the test, the participants were asked to exert knee extensor force as strongly as possible against an anklet extended from the tension meter while crossing their arms on their chest. The leg strength (LS: kg) was defined in the same way as for the HS. In the five-times sit-to-stand test, the participants were requested to perform five consecutive chair stands as quickly as possible while crossing their arms on their chest, and the time (sec) spent to complete the task was recorded using a digital stopwatch. Only one trial was made for this test due to its strenuous nature. The sit-to-stand rate (SR: reps/sec) was determined by dividing 5 (reps) by the task time. The 5-meter gait test was conducted over a straight 11m lane with taped marks at the 3m and 8m points. The participants were asked to walk on the entire lane as fast as possible (but without running) in two trials, and the time (sec) for walking between the two marks was measured in each trial using a digital stopwatch. The gait speed (GS: m/sec) was calculated by dividing 5 (m) by the shortest task time in the two trials. In the open-eyed one-leg stand test, the participants tried to stand as long as possible up to 120 sec with a preferred leg while watching a taped mark on the wall 1m away from a toe line. This test was performed twice, and the time (sec) to failure of the task was measured in each trial using a digital stopwatch. The longer task time in the two trials was selected as the one-leg stand time (OT: sec). All of the five tests were admin-

istered by trained examiners with standardized procedures including standard instruction and practice. Additionally, the participants were encouraged to ask questions, if needed, throughout the procedures for better understanding and compliance. Higher values indicate better physical fitness in all of the five measures.

Other measurements

Age, sex, years of formal education, and economic status (comfortable, relatively comfortable, relatively uncomfortable, and uncomfortable) were obtained from a questionnaire. The physical activity energy expenditure (PAEE: kcal/day) was defined as average daily energy expenditure due to physical activity and objectively measured by a tri-axial accelerometer device (Active Style Pro HJA-350IT; Omron Healthcare, Kyoto, Japan) (Ohkawara et al., 2011). For this measurement, the participants were asked to wear the device all day (except for sleeping and water activities) for 7 days. The PAEE was determined only for those wearing the device for at least 10 hours a day on two or more days (mean ± standard deviation or SD of wearing period in the present participants: 6.8 ± 1.8 days). Body weight (kg) and height (m) were measured using conventional scales, and body mass index (BMI) was calculated by dividing the body weight by height squared (kg/m^2). Instrumental activities of daily living (IADL) were measured as part of the questionnaire using the five-item scale for instrumental self-maintenance of the Tokyo Metropolitan Institute of Gerontology Index of Competence (Koyano et al., 1991). The five items asked about the abilities of using public transportation, shopping for commodities, preparing meals, paying bills, and handling bank accounts in binary forms (able or unable). The participants answering "able" for all of the five items were regarded as independent in IADL and others as dependent in IADL. In the present study, the number of items answered with "able" was used as the index of IADL in regression analyses while the dependency in IADL was reported in demographic description. The psychological distress was measured by the Japanese version of the Kessler 6 psychological distress scale (K6) in the questionnaire (Sakurai et al., 2011). This is a 6-item and 24-point scale, and participants who scored 5 points or more on the scale were classified as having depressive status. In the present study, the K6 scores were used in regression analyses while the depressive status was reported in demographic description. Comorbidities of hypertension, heart disease, and diabetes and history of stroke were asked on the questionnaire in binary forms (presence or absence).

Statistical analysis

Mean ± SD or median (25th-75th percentiles) was calculated for continuous variables, appropriately, and frequency (%) for categorical variables. To confirm similarities between the present participants and the baseline subjects, the Wilcoxon rank-sum test and the chi-square test were conducted for continuous and categorical variables, respectively. To examine associations between physical fitness and cognitive function, multiple linear regression analyses were conducted for each of the five physical fitness measures in the following three models: Model 1: entered each physical fitness measure as an independent variable, MoCA as a dependent variable, and age and sex as covariates, Model 2: Model 1 plus years of formal education and BMI as covariates, Model 3: Model 2 plus economic status, PAEE, IADL, K6, comorbidities of hypertension, heart disease, and diabetes, and history of stroke as covariates. A significance level was set at two-sided $\alpha = 0.05$. All statistical analyses were performed using the SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

The median age (25th-75th percentiles) for the present participants was 72 (68-77) years and 40.1% of the participants were men (n = 623). The mean ± SD or median (25th-75th percentiles) of the MoCA and the physical fitness measures in the present participants were as follows: MoCA (n = 1,552): 22.4 ± 3.4 point, HS (n = 1,529): 27.2 ± 8.0 kg, LS (n = 1,473): 27.0 ± 10.3 kg, SR (n = 1,488): 0.60 ± 0.19 reps/sec, GS (n = 1,540): 1.72 ± 0.43 m/sec, OT (n = 1,525): 45.7 (15.1-120) sec. Table 1 shows characteristics of the present participants on these and other variables.

Results of the regression analyses were summarized in Table 2. Each of the five physical fitness scores was significantly associated with the MoCA score after adjusting for age and sex (Model 1: $p < 0.001$). After additional adjustment for years of formal education and body mass index, each physical fitness score remained associated with the MoCA score (Model 2: $p < 0.001$). After further adjustment for the other confounding factors (economic status, PAEE, IADL, K6, hypertension, heart disease, diabetes, and stroke), the association between each physical fitness score and MoCA was almost unchanged (Model 3: $p < 0.001$).

Discussion

The present study examined associations between five physical fitness measures and global cognitive function evaluated by the MoCA in Japanese community-dwelling older adults without apparent cognitive problems. The primary finding of the present study is that each of the five physical fitness measures was linearly and positively associated with the MoCA score. These associations were independent of age, sex, years of formal education, BMI, and other confounding factors.

Examining lifestyle-related markers of pre-dementia cognitive functioning is expected to be of value to promote early detection of subtle cognitive impairment in community-based settings. Despite the promise of physical fitness measures as markers of low cognitive function in the pre-dementia stage, research evidence is still limited. To our knowledge, only two studies have examined the potential role of physical fitness as a marker of low cognitive function in the stage by showing association between gait speed and global cognition in non-demented older people living in the United States (Fitzpatrick et al., 2007; Mielke et al., 2013). In one study

Table 1. Characteristics of the present participants.

Indexes	Men		Women	
	65-74 years	75+ years	65-74 years	75+ years
n	402	221	570	359
Age, years	69 (67-71)	79 (77-82)	69 (67-71)	79 (77-82)
MoCA, point	23.3 (3.1)	21.4 (3.3)	23.1 (3.2)	20.8 (3.5)
HS, kg	36.4 (5.6)	31.4 (5.5)	23.3 (3.9)	20.2 (4.1)
LS, kg	36.6 (9.9)	29.3 (8.6)	24.1 (6.9)	18.8 (6.5)
SR, reps/sec	.65 (.19)	.55 (.17)	.63 (.18)	.52 (.16)
GS, m/sec	1.93 (.44)	1.68 (.41)	1.76 (0.37)	1.44 (.36)
OT, sec	109.3 (35.0-120)	22.9 (7.2-47.6)	80.6 (24.6-120)	16.7 (5.8-43.4)
Education, years	12 (10-14)	11 (9-13)	12 (9-12)	10 (9-11)
Economy*, %	32.1	47.5	38.2	48.2
PAEE, kcal/day	557.5 (162.8)	453.6 (144.7)	536.3 (134.5)	405.6 (115.8)
BMI, kg/m^2	23.5 (2.7)	23.0 (2.8)	23.3 (3.4)	22.9 (3.3)
IADL†, %	16.9	14.0	1.4	6.7
K6‡, %	24.9	27.1	30.4	32.6
Hypertension, %	38.1	37.1	33.0	46.5
Heart disease, %	12.9	25.8	5.8	18.7
Diabetes, %	19.7	17.6	9.6	9.7
Stroke history, %	4.5	5.0	1.6	3.1

Note: continuous variables are represented as mean (SD) or median (25th-75th percentiles). * Percentage of participants answering "comfortable" and "relatively comfortable" in economic status; † percentage of participants regarded as dependent in instrumental activities of daily living (IADL score of <5); ‡ percentage of participants classified as having depressive status (K6 score of ≥5). MoCA: Montreal Cognitive Assessment; HS: handgrip strength; LS: leg strength; SR: sit-to-stand rate; GS: gait speed; OT: one-leg stand time; PAEE: physical activity energy expenditure; BMI: body mass index; IADL: instrumental activities of daily living; K6: Kessler 6 psychological distress scale.

from the Ginkgo Evaluation of Memory study group, the investigators used the Modified MMSE (3MSE) as a global cognitive test and excluded individuals with the test score of <80 from the study participants (Fitzpatrick et al., 2007). They found that the risk of low cognition defined as the 3MSE score of 80 to 85 was almost twice for participants in the slowest quartile of maximal walking speed compared with that for participants in the fastest quartile after adjusting for demographic and comorbid factors. Another study from the Mayo Clinic group also showed an association between usual walking speed and global cognitive function measured as a standard score of a neuropsychological battery covering four cognitive domains in older adults without diagnosed mild cognitive impairment and dementia (Mielke et al., 2013). These associations reasonably support the ability of gait speed as a marker of low cognitive function in the United States older people free from dementia, but comparable observations had not been made in other ethnicities including Japanese. The present study first demonstrated a similar association between gait speed and global cognition in a Japanese older population without apparent cognitive problems. Moreover, the present study further demonstrated novel association between each of the other four physical fitness measures and global cognition in the Japanese population (Table 2). A notable aspect of the present study is the use of the MoCA as a reasonable neuropsychological instrument to measure global cognitive function among the participants who were free from apparent cognitive problems. MoCA is a relatively new instrument devised to detect early cognitive changes with multiple domains for screening mild cognitive impairment (Nasreddine et al., 2005). This instrument has been reported to have higher sensitivity to subtle cognitive alterations than MMSE and other conventional tools (Nasreddine et al., 2005; Pendlebury et al., 2010), and has been used as a scale of global cognitive status in population-based studies (Donoghue et al., 2012; King et al., 2013).

One possible mechanism underlying the observed

Table 2. Associations between each physical fitness measure and MoCA.

Independent variables	n	Model 1		Model 2		Model 3	
		Regression coefficient (95% CI)	p	Regression coefficient (95% CI)	p	Regression coefficient (95% CI)	p
HS, kg	1,529	.10 (.07-.14)	<.001	.09 (.06-.13)	<.001	.08 (.05-.12)	<.001
LS, kg	1,473	.07 (.05-.09)	<.001	.06 (.04-.08)	<.001	.06 (.04-.08)	<.001
SR, reps/sec	1,488	2.34 (1.42-3.26)	<.001	1.95 (1.05-2.85)	<.001	1.55 (.64-2.45)	<.001
GS, m/sec	1,540	1.38 (.96-1.80)	<.001	1.17 (.76-1.57)	<.001	1.01 (.59-1.43)	<.001
OT, sec	1,525	.02 (.01-.02)	<.001	.01 (.01-.02)	<.001	.01 (.01-.02)	<.001

Note: Model 1: association between each physical fitness measure as an independent variable and MoCA as a dependent variable, with age and sex as covariates; Model 2: Model 1 plus years of education and BMI as covariates; Model 3: Model 2 plus other confounding factors (economic status, PAEE, IADL, K6, hypertension, heart disease, diabetes, and stroke) as covariates. MoCA: Montreal Cognitive Assessment; HS: handgrip strength; LS: leg strength; SR: sit-to-stand rate; GS: gait speed; OT: one-leg stand time; BMI: body mass index; PAEE: physical activity energy expenditure; IADL: instrumental activities of daily living; K6: Kessler 6 psychological distress scale. 95% CI denotes 95% confidential interval of regression coefficient. Coefficients of determination (adjusted R-squared) in the regression analyses are as follows: Model 1: 0.145, 0.138, 0.132, 0.148, 0.157; Model 2: 0.197, 0.192, 0.183, 0.198, 0.205; Model 3: 0.210, 0.206, 0.196, 0.209, 0.217 (for HS, LS, SR, GS, and OT, in respective models).