ORIGINAL REPORT

Association of Occlusal Force with Cognition in Independent Older Japanese People

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Abstract: *Recent longitudinal studies have shown the influence of multiple tooth loss on cognitive impairment, and earlier studies suggested that periodontal disease was related to cognitive decline. Tooth loss is associated with reduced masticatory function, which may affect stimulation of the central nervous system and dietary intake. Although some studies have reported a relationship between chewing ability and cognitive function, no studies have examined this area in terms of objective oral function. The aim of this study was to examine the association of occlusal force with cognitive decline in the preclinical stage among older people with higher-level functional capacity. This cross-sectional study for community-dwelling older people living in urban and rural areas in Japan examined 994 persons in the 70-y group (age range, 69–71 y) and 968 persons in the 80-y group (age range, 79–81 y). Retention of higher-level competence was defined according to the Tokyo Metropolitan Institute of Gerontology Index of Competence. Cognitive function was measured with the Japanese version of the Montreal Cognitive Assessment (MoCA-J). Oral status and function were assessed by the number of remaining teeth, periodontal pocket depth, and maximal occlusal force. Associations between the MoCA-J score and occlusal force were examined by bivariate and multivariate analysis. Approximately one-half of the participants retained higher-level functional capacity and were included in the analysis. Multiple regression analysis showed that occlusal force was significantly related to cognitive function after controlling for possible predictors including age, sex, socioeconomic status, medical condition, and handgrip strength. The number of remaining teeth and periodontal pocket depth were not significantly associated with cognitive function. Among community-dwelling older people with retained competence, maximal occlusal force was positively associated with their cognitive function. These results suggest that oral function might be a predictor for preclinical cognitive decline.*

Knowledge Transfer Statement: *Multiple regression analysis showed that occlusal force was significantly related to cognition after controlling for possible predictors including handgrip strength as an indicator of general muscle strength, suggesting the independence of oral function. The number of remaining teeth did not have this association. The majority of older people have lost teeth and have received prosthodontic treatment, and their occlusal force is determined not only by the number of remaining teeth but also by prosthetic rehabilitation. These results can be used by clinicians focusing on prevention of tooth loss among the entire population, as well as to encourage partially edentulous and fully edentulous patients to restore their oral function with prostheses in order to eliminate a possible risk factor for cognitive impairment.*

Keywords: geriatric dentistry, oral function, cognitive function, Montreal Cognitive Assessment, multiple regression analysis, community-dwelling older people

Introduction

Cognitive impairment is an early symptom of dementia. Risk factors for dementia include genetic factors,

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aging, lower education level, lower socioeconomic status, cardiovascular risk factors (e.g., overweight, smoking, hypertension, and diabetes mellitus), unhealthy diet, lack of exercise, and social inactivity (Weijenberg et al. 2011). These risk factors are mutually related, resulting in a complex etiological model (Lexomboon et al. 2012).

Several studies have reported associations between oral health and cognitive decline. Recent longitudinal studies showed the influence of multiple tooth loss on cognitive impairment (Okamoto et al. 2015; Stewart et al. 2015; Tsakos et al. 2015). Previously, periodontal disease was mainly discussed in relation to cognitive decline (Gaur and Agnihotri 2015). It has been hypothesized that the biological basis for the association may include inflammatory molecules, bacteria, and bacterial products that enhance neuroinflammation (Noble et al. 2013). Tooth loss was considered to be the ultimate outcome of periodontal disease, rather than a risk factor in itself.

Tooth loss is associated with reduced masticatory function, which may affect stimulation of the central nervous system and dietary intake and, as a consequence, may lead to cognitive decline. Various animal studies have shown a relationship between mastication and cognitive impairment. Several mechanisms underlying this relationship have been proposed. For example, poor masticatory function causes reduced neurogenesis in the part of the brain associated with cognition, and an impairment of the cholinergic neurotransmitter system is associated with learning ability and spatial memory (Weijenberg et al. 2011). In humans, an experimental study showed that chewing and clenching increase cerebral blood flow (Hasegawa et al. 2007). People with poor masticatory efficiency may avoid eating food high in fiber and essential micronutrients (Sheiham and Steele 2001), and these individuals tend to eat food high in saturated fats and cholesterol (Walls et al. 2000). Women with fewer teeth have unhealthier diets such as decreased intake of fruits and vegetables, which could increase cardiovascular risk. Diet may partially explain associations between oral health and cardiovascular disease (Hung et al. 2005). Dietary changes that are adaptive to low masticatory efficiency could potentially increase the risk of stroke and dementia. However, there is insufficient epidemiological evidence to demonstrate this pathway in humans.

Some human studies have shown an association between chewing difficulty or denture wearing and cognitive decline (Lexomboon et al. 2012; Paganini-Hill et al. 2012). However, because older people are likely to have missing teeth and to have received prosthodontic treatment (e.g., removable dentures, bridges, and dental implants), it is impossible to evaluate masticatory function only by the number of teeth or the presence or absence of dentures. Generally, objective measurement of masticatory function is more complicated than self-assessed chewing ability. To our knowledge, no study has been conducted on the relationship between objectively measured masticatory function and cognitive decline in a large older population. The validity of self-assessed chewing ability is questionable because it is not always significantly related to objective function (Ikebe et al. 2007) and is influenced by an individual's personality (Takeshita et al. 2015). In addition, we previously showed that occlusal force is easier to measure with scientific validity than masticatory performance and can therefore be used as a surrogate marker of masticatory performance (Ikebe et al. 2011).

We hypothesized that a decline in masticatory function is associated with the early stages of cognitive decline in an older population. To test this hypothesis, we analyzed a group of older people who retained higher-level functional capacity as defined by the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC), which evaluates intellectual activity and social role, to exclude the influence of functional decline on oral health. Because advanced cognitive decline leads to muscle weakness (Taekema et al. 2012) and impairment of competence in the activities of daily living (Dodge et al. 2005), it also consequently affects oral hygiene (Naorungroj et al. 2013).

The aim of this cross-sectional study was to show the association of reduced occlusal force with mild cognitive decline independent of the number of teeth lost, periodontal status, and general muscle strength among community-dwelling older people with higher-level functional capacity.

Methods

The study protocol was approved by the Institutional Review Board of Osaka University Graduate School of Dentistry (approval number H22-E9). This article was prepared in accordance with the STROBE statement.

Study Population and Procedure

This was a cross-sectional examination of data collected during the baseline assessment of a prospective study of health and longevity called Septuagenarians, Octogenarians, Nonagenarians Investigation with Centenarians (Inomata et al. 2014; Okada et al. 2014). The research data were collected from the 2 main regions of eastern and western Japan (Tokyo metropolitan area and Hyogo Prefecture, respectively) from 2010 to 2012. The participants were older adults living in private residences and consisted of a 70-y group (age range, 69–71 y, $n = 994$) and an 80-y group (age range, 79–81 y, $n = 968$). The recruiting procedure is detailed elsewhere (Inomata et al. 2014).

Participants with no occlusal contact between their own teeth or prostheses were excluded because measurement of their occlusal force was impossible. This was the only exclusion criterion. All participants in this study gave written informed consent to participate.

Number of Remaining Teeth, Denture Use, Periodontal Pocket Depth, and Maximal Occlusal Force

Registered dentists performed all dental examinations with a dental mirror and an

explorer without X-rays. The periodontal pocket depth (PPD) of each tooth was measured with a color-coded probe (CP-12; Hu-Friedy, Chicago, IL, USA). PPD was assessed at 6 sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) for all teeth present. Mean PPD and maximum PPD were used as indicators of periodontal status (Takeuchi and Yamamoto 2008).

Evaluation of masticatory performance involves a comprehensive evaluation of oral function with respect to eating ability. However, such an evaluation is impractical in a large number of older people because it is very time-intensive. Therefore, maximal occlusal force was used as a proxy measurement because it is strongly correlated with objectively measured masticatory performance (Ikebe et al. 2006, 2011, 2012) and can be tested in just a few seconds. The bilateral maximal occlusal force was measured with 97-μm–thick pressure-sensitive sheets (Dental Prescale 50H R type; Fuji Film Co., Tokyo, Japan). The participants performed maximal clenching in the intercuspal position for 3 s with the pressure-sensitive film placed between the maxillary and mandibular dental arches. Participants with removable partial dentures kept their dentures in place during the measurement. Occlusal force was measured 1 time for each participant. The interexaminer and intraexaminer reliability of this type of measurement was described in an earlier article (Inomata et al. 2014).

Higher-Level Functional Capacity

Higher-level functional capacity (intellectual activity and social role) was evaluated using the TMIG-IC (Koyano et al. 1991; Ishizaki et al. 2000). Disability in instrumental self-maintenance (5 questions), intellectual activity (4 questions), and social role (4 questions) are subscales of the index. The subjects were requested to choose a response option of "yes/can" (1 point) or "no/cannot" (0 points) for each question. Lower scores indicate reduced higher-level functional capacity. In a previous study, a person recording a score of ≥1 below the respective full score was defined as having a disability (Fujiwara et al. 2003). Following this, in our study, participants who scored 13 points (full score) were defined as the high-level group and were included in the analysis.

Cognitive Function

We used the Japanese version of the Montreal Cognitive Assessment (MoCA-J) (Fujiwara et al. 2010) as a general index of cognitive status (Nasreddine et al. 2005). The MoCA is a brief cognitive screening tool for detecting mild cognitive impairment in older people (Petersen et al. 1999). The MoCA assesses the following domains of cognition: visuospatial ability (4 points), executive function (4 points), attention (6 points), linguistic cognition (5 points), delayed recall (5 points), and orientation (6 points). The MoCA-J has shown good reliability and validity for predicting early cognitive decline compared with conventional cognitive tests (Fujiwara et al. 2010). The MoCA-J total score (0–30 points) was therefore used as a measure of cognitive function. A higher score reflects higher cognitive function.

Handgrip Strength

Handgrip strength was recorded as a proxy for upper-extremity muscle strength. Isometric grip strength was measured using a Smedley handgrip dynamometer (model YD-100; Yagami Ltd., Tokyo, Japan). The details were described in an earlier article (Okada et al. 2015).

Grip strength (in kilogram force) was recorded as the average of the highest scores for the left and right hands. Grip strength has been reported to be positively related to cognitive function (Taekema et al. 2010; Narazaki et al. 2014) and also to occlusal force (Okada et al. 2014).

Other Recorded Variables

Participants were interviewed to record their education level (junior high school, high school, or college or higher), self-rated financial status (good, fairly good, or poor), current drinking habits (yes or no), smoking habits (yes or no), chronic medical conditions (diabetes mellitus and hypertension), and body mass index (BMI). Participants with a BMI of ≥25 were classified as overweight.

Statistical Analysis

In the overarching study from which our data were drawn, dental, medical, and psychological professionals undertook a comprehensive evaluation of health in the same cohort of individuals. The numerous measurements and statistical methods used needed a large sample size to produce adequate statistical power. The total sample size was estimated as approximately 850 by G*Power when the defined effect size was 0.02, α error was 0.05, power equal to $1 - \beta$ error was 0.80, and number of predictors was 11 in a multiple linear regression. Therefore, the number of study participants was sufficient for our study.

The Mann–Whitney *U* test, Kruskal–Wallis test, and Spearman's rank-order correlation coefficient (*rs*) test were performed to evaluate the association between cognitive function and other variables. Finally, multiple linear regression analysis was conducted. The dependent variable was the MoCA-J score, and independent variables were the number of teeth, maximal occlusal force, PPD, sex, age group, educational level, economic status, smoking and drinking habits, medical history, and grip strength. Analyses with a forced-entry method using seemingly relevant explanatory variables were performed. The first model included only the dental status. In the second and third models, we added sociodemographic status, habits, medical status, and occlusal force. In the fourth model, we further adjusted for hand grip strength. If any of the variables on the model was missing, the participants were excluded only from the analysis. $P < 0.05$ was considered to denote a statistically significant difference.

Results

A total of 949 participants scored 13 points (full score) on the TMIG-IC (48.4% of the total study population; 57.6% of

Table 1.
Comparison of MoCA-J Score by Possible Related Variables.

Variables	Percent of Study Population	MoCA-J Score Mean (95% CI)	*P* Value
Total (n = 949)		22.5 (22.3–22.7)	
Age group[a]			
70 y	57.6	23.1 (22.8–23.4)	<0.001
80 y	42.4	21.7 (21.4–22.1)	
Sex[a]			
Men	39.9	22.1 (21.8–22.4)	0.002
Women	60.1	22.8 (22.5–23.1)	
Education level[b]			
Junior high school	26.0	21.1 (20.7–21.5)	<0.001
High school	43.6	22.8 (22.5–23.1)	
College or higher	30.4	23.3 (22.9–23.6)	
Self-rated financial status[b]			
Poor	18.1	21.7 (21.2–22.3)	<0.001
Fairly good	54.8	22.5 (22.2–22.8)	
Good	27.1	23.1 (22.7–23.5)	
Smoking habit[a]			
Yes	30.5	22.2 (21.8–22.6)	0.067
No	69.5	22.7 (22.4–22.9)	
Drinking habit[a]			
Yes	20.9	22.0 (21.5–22.5)	0.023
No	79.1	22.7 (22.4–22.9)	
Hypertension[a]			
Yes	53.1	22.7 (22.4–23.0)	0.170
No	46.9	22.4 (22.0–22.7)	
Diabetes[a]			
Yes	15.4	22.5 (22.2–22.7)	0.551
No	84.6	22.7 (22.2–23.2)	
Overweight[a]			
Yes	19.8	22.2 (21.7–22.7)	0.166
No	80.2	22.6 (22.3–22.8)	0.166

95% CI, 95% confidence interval; MoCA-J, Japanese version of the Montreal Cognitive Assessment.
[a]Mann–Whitney *U* test.
[b]Kruskal–Wallis test.

the 70-y group and 42.4% of the 80-y group) and were categorized as having higher-level functional capacity.

The correlation matrix with MoCA-J scores and other variables is shown in the Appendix. Table 1 shows a comparison of MoCA-J scores according to possible related variables. Significantly higher MoCA-J scores were seen for women, the 70-y group, and participants with a higher education level, better financial status, and no drinking habit. However, there were no significant differences in the MoCA-J scores related to smoking habit, medical history, and obesity. Table 2 shows the correlations between MoCA-J scores and possible related variables. The number of teeth (rs = 0.178), maximal occlusal force (rs = 0.216), and mean PPD (rs = –0.083) were significantly correlated with the MoCA-J score.

Table 3 shows the results of multiple linear regression analysis for associations with MoCA-J score (the dependent variable). Models 1-1 and 1-2 indicated that occlusal force was significantly associated with the MoCA-J score (P < 0.001); however, the number of remaining teeth (P = 0.078, model 1-1) and mean PPD (P = 0.170, model 1-2) were not significantly associated with the MoCA-J score after controlling for occlusal force. Model 2 without occlusal force showed that age, sex, education level, and self-rated financial status were significantly associated with the MoCA-J score. After controlling for these variables without occlusal force, the number of teeth had a significant association with the MoCA-J score (P = 0.003). However, smoking and drinking habits, hypertension, diabetes mellitus, and overweight were not significantly related to the MoCA-J score. Model 3, which included occlusal force, showed that the number of teeth was not significant for the MoCA-J score (P = 0.707), but occlusal force had a significant association (P = 0.003) after controlling for the number of teeth and those possible variables. In model 4, further adjusted for grip strength (P < 0.001), occlusal force remained a significant variable for the MoCA-J score (P = 0.017).

Discussion

This study intended to investigate the effect of oral function on cognitive function among community-dwelling older Japanese people with higher-level functional capacity. Our findings demonstrate that occlusal force, rather than the number of teeth or periodontal status, was significantly associated with cognitive function after adjustment for possible variables. Reduced occlusal force could explain the association between tooth loss and cognitive decline, independent of general muscle strength.

People with impaired cognition are likely to have little interest in oral hygiene, lack manual skills, and may have restricted access to a dentist for routine oral care, resulting in multiple carious lesions, periodontitis, and finally tooth loss. The opposite perspective, that oral health may influence cognitive decline, is less common. To establish our hypothesis, we excluded the possible effects on oral health of any decline in daily functioning by selecting only the older people who maintained higher-level functional competence. Among the participants, about 60% of the 70-y group and 40% of the 80-y group had retained complete functional capacity and were included in the further analysis.

To assess cognitive function, the MoCA-J (Fujiwara et al. 2010) was employed, although the Mini-Mental State Examination (MMSE) has been most widely used in previous studies. The MoCA is a brief screening tool with high sensitivity and specificity for detecting mild cognitive decline in older adults in the normal range on the MMSE (Gluhm et al. 2013; Kenny et al. 2013), and the MoCA-J total score was therefore considered to be a valid measure of cognitive function.

In the bivariate analysis, participants who were male, older, and less educated recorded lower cognitive function, and these results are consistent with most previous reports (Kenny et al. 2013). Number of teeth, PPD, and occlusal force were significantly associated with cognitive function, suggesting that oral status may contribute to cognitive function. In the multivariate analysis, occlusal force was positively related to cognitive function; however, the relationship of the number of teeth and PPD to cognitive function became statistically insignificant after adjustment for occlusal force. The theoretical reason for this finding may be that both the number of teeth and PPD had significant correlations (rs = 0.681 and −0.285, respectively) with occlusal force, and their association with cognitive function might have been confounded by occlusal force.

In earlier studies, periodontal disease by itself, and tooth loss as an end point of periodontal disease, has been reported to be associated with cognitive decline (Desvarieux et al. 2003; Humphrey et al. 2008; Grabe et al. 2009; Kaye et al. 2010; Noble et al. 2013) in relation to periodontal disease–derived inflammation (Watts et al. 2008). However, none of these studies included oral function in addition to tooth loss or periodontal disease in the regression models for cognitive function. To our knowledge, our study is the first to show that occlusal force is more important than current periodontal status or the number of teeth lost. It is possible that the significant relationship identified between cognitive function and the number of teeth in previous studies may be linked by extension to masticatory function, and masticatory function may therefore be important in the early stages of cognitive decline.

Lexomboon et al. (2012) showed that multiple tooth loss became insignificant in terms of cognitive impairment when sex, age, and educational level were included in the regression analysis, whereas chewing difficulty remained significant. However, their evaluation of tooth loss and chewing ability were self-rated and dichotomized (multiple tooth loss or having natural teeth; having chewing difficulty or not). Our findings have the advantage of using an objectively measured value, therefore providing more conclusive evidence for an association between oral function and cognitive ability.

General muscle strength is a considerable factor in the relationship between occlusal force and cognitive function. A number of researchers have speculated that reduced occlusal force is a reflection of weakened general muscle strength. Previous studies have used grip strength as a predictor of general muscle strength and have related it not only to occlusal force (Iinuma et al. 2012; Okada et al. 2014) but also to cognitive function (Taekema et al. 2010). However, our multivariate model (model 4) indicates that occlusal force is positively associated with cognitive function even after controlling for grip strength. Our findings suggest that masticatory function is uniquely independent of general muscle strength (Weijenberg et al. 2011).

A model excluding number of teeth from model 4 was run as a sensitivity analysis. Although the standardized partial regression coefficient (beta) of occlusal force slightly increased, the coefficient of determination and beta of the other variables showed little change from model 4. Therefore, it suggested that the effect of the number of teeth on cognitive function is quite small in this study population.

The majority of older people have lost teeth, and almost all have received prosthodontic treatment (e.g., removable partial dentures, fixed partial dentures, or dental implants). Oral function in older people with missing teeth is determined not only by the number of remaining teeth but also by prosthetic rehabilitation. In our study, 70.1% of participants used some sort of dental prosthesis, and this may reflect on the results of this study. Although tooth loss and periodontal disease may be preventable risk factors, oral function can be restored by treatment with dental prostheses. A significant point to be emphasized is that although dentists cannot regenerate lost

Table 2.
Correlation between MoCA-J Score and Possible Related Variables.

Variables	*rs*	*P* Value
Number of teeth	0.178	<0.001
Occlusal force	0.216	<0.001
Maximal PPD	−0.032	0.348
Mean PPD	−0.083	0.016
Grip strength	0.059	0.069

rs are Spearman rank-order correlation coefficients to MoCA-J.
MoCA-J, Japanese version of the Montreal Cognitive Assessment; PPD, periodontal pocket depth.

Table 3.
Linear Regression Models for Occlusal Force and MoCA-J Score Adjusted for Significant Independent Variables.

Models	Significant Independent Variables	Beta	*P* Value	Adjusted R^2
Model 1-1	Occlusal force	0.166	<0.001	0.045
Adjusted for number of teeth	Number of teeth[b]	0.072	0.078	
Model 1-2[a]	Occlusal force	0.185	<0.001	0.039
Adjusted for periodontal pocket depth	Periodontal pocket depth[b]	−0.048	0.170	
Model 2 without occlusal force	Number of teeth	0.107	0.003	0.122
Adjusted for other significant variables	Age group	−0.166	<0.001	
	Women	0.102	0.034	
	Education level	0.230	<0.001	
	Financial status	0.101	0.004	
Model 3	Occlusal force	0.133	0.003	0.131
Adjusted for number of teeth and other significant variables	Number of teeth[b]	0.017	0.707	
	Age group	−0.154	<0.001	
	Women	0.126	0.009	
	Education level	0.223	<0.001	
	Financial status	0.096	0.006	
Model 4	Occlusal force	0.109	0.017	0.144
Adjusted for model 3 plus grip strength	Number of teeth[b]	0.025	0.578	
	Age group	−0.119	0.002	
	Women	0.269	<0.001	
	Education level	0.213	<0.001	
	Financial status	0.091	0.009	
	Grip strength	0.192	<0.001	

Beta values are the standardized partial regression coefficients. Model 1-1 was adjusted for occlusal force and number of teeth. Model 1-2 was adjusted for occlusal force and mean periodontal pocket depth. Model 2, without occlusal force, was adjusted for number of teeth, age, sex (male or female), self-rated financial status (poor, fairly good, or good), education level (junior high school, high school, or college or higher), smoking and drinking habit (yes or no), hypertension (yes or no), diabetes mellitus (yes or no), and overweight (BMI <25 kg/m^2 or ≥25 kg/m^2). Model 3 was adjusted for the variables in model 2 as well as occlusal force. Model 4 was adjusted for the variables in model 3 as well as hand grip strength.
MoCA-J, Japanese version of the Montreal Cognitive Assessment.
[a]Excluded edentulous participants.
[b]No significance.

teeth, they can improve mastication by treatment with appropriate prostheses.

Indeed, smoking, obesity, hypertension, and diabetes have been reported as risk factors for dementia. However, in the case of mild cognitive decline, its risk factors are much disputed. In addition, in past studies investigating the relationship between cognitive decline and oral condition among older people, diabetes and smoking habit were not significantly associated (Stewart et al. 2015). One possible reason is that their lifestyle influenced both oral status and chronic conditions. Medications for these risk factors, such as antihypertensive drugs or statins, may influence the results.

Among the study's strengths, the data collection was designed by specialists in dentistry, medicine, and psychology for the purposes of their own research in health and longevity. This allowed our study to cover the main variables (occlusal force and cognitive function) and a number of important confounding factors (e.g., sociodemographic characteristics, medical history, BMI, and grip strength) at a high quality level.

Several aspects of our study design limited our conclusions. The first point of concern is the narrow range of our study population, which included only nonclinical, noninstitutionalized community-dwelling Japanese people in their 70s and 80s, despite the sample being drawn from a complete enumeration of the resident record. Consequently, our results cannot be generalized to younger, older, or less healthy people.

Another limitation of the present study is the impossibility of uncovering causal relationships between oral function and cognitive impairment. Because this study used an epidemiological approach and

a cross-sectional design, the findings cannot meet the most important of Bradford Hill's causality criteria (i.e., temporality, demonstrating that exposure to one preceded development of the other). Similarly, the strength of the association and the biological gradient were convincing. However, we are following up on the participants every 3 y in an effort to establish causal connections between oral function and cognitive decline. Our suppositions can be validated only by a longitudinal clinical study measuring occlusal force and cognitive function of the same patients before and after prosthetic rehabilitation.

Most of the observed correlation coefficients and the coefficient of determination are small but statistically significant, suggesting that there must be other risk factors for cognitive function, such as diet, sleep, depression, cognitive activity, physical activity, and genetic factors. In epidemiological studies dealing with cognitive function in older populations, such correlation coefficients are frequently small. Considerable parts of the etiology of cognitive impairment are still unknown. Thus, the present study warrants further studies adjusting those risk factors.

Conclusion

Among community-dwelling older people with retained higher-level functional competence, maximal occlusal force was positively associated with cognitive function. However, the number of remaining teeth and periodontal status were not found to be associated with cognitive function, indicating that oral function, including prosthodontic reconstruction, is more important than the actual number of teeth. These results suggest that oral function may be related to the early stages of cognitive decline.

Author Contributions

H. Takeshita, contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript; K. Ikebe, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; H. Inagaki, K. Matsuda, contributed to data acquisition and interpretation, critically revised the manuscript; Y. Gondo, Y. Masui, K. Kamide, R. Takahashi, Y. Arai, Y. Maeda, contributed to conception, data acquisition, and interpretation, critically revised the manuscript; C. Inomata, Y. Mihara, M. Uota, contributed to data acquisition, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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Significance of Oral Function for Dietary Intakes in Old People

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Summary There is growing interest in the connection between oral health and systemic health. In recent years, oral health in particular is considered a predictor of circulatory mortality. Two major pathways may mediate this relationship, namely (1) the inflammatory effects of chronic periodontal infection on the circulatory system and (2) the effects of masticatory dysfunction on dietary behavior, nutrition and systemic diseases.
Previous studies have shown that adults who are edentulous, or have fewer natural teeth are less likely to eat fruits, vegetables and meats. Because it can be easily assessed, the number of teeth has frequently been used as an indicator of oral health in investigations of food intake. However, the number of teeth alone presents a misleading picture. The role of prosthetic rehabilitation (i.e., dentures) on oral function must be taken into account as well. We investigated the association of occlusal force with food and nutrient intakes after adjusting for the number of teeth in independently living 70-y-old Japanese. After adjusting for socioeconomic status and the number of remaining teeth, decline of occlusal force was significantly associated with lower intakes of vegetables, vitamins A, C, and B6, folate, and dietary fiber (p for trend<0.05). It is concluded that occlusal force as a representative of oral function was significantly associated with intakes of vitamins and dietary fiber rather than number of remaining teeth.

Key Words occlusal force, dietary intake, vegetable, elderly, multivariate analysis

There is growing interest in the connection between oral health and systemic health. In recent years, oral health in particular has been considered a predictor of circulatory mortality. Two major pathways may mediate this relationship (*1*), namely (1) the inflammatory effects of chronic periodontal infection on the circulatory system and (2) the effects of masticatory dysfunction on dietary behavior, nutrition and systemic diseases. Consistent unbalanced food selection could result in poor diets high in calories but low in fiber, vitamins, and protein, possibly resulting in certain diseases, frailty or death in older persons (*2*). Several studies have clearly demonstrated the inverse association between vegetable intake and the development of cardiovascular disease and risk of stroke.

The significant role of nutrition is recognized as a factor that bridges oral and systemic disease. In general, ageing is a risk factor for sensory and motor deterioration, with the rate of decline varying among individuals. Concerning masticatory function, missing teeth along with oral disease seem to accelerate the dysfunction. Declines in occlusal contact, occlusal force and salivary flow appear to be associated with reduction of masticatory performance in older adults (*3*, *4*). Chewing disorder can lead to avoidance of foods considered difficult to chew and a preference for soft, easily chewable foods.

Literature Reviews

Hung et al. (*5*) evaluated the associations between the number of natural teeth and the self-reported consumption of fruits and vegetables and selected CVD-related nutrients in more than eighty thousand US women. After adjusting for age, total calorie intake, smoking and physical activity, edentulous women appeared to have dietary intakes associated with increased risk for CVD, including significantly higher intakes of saturated fat and cholesterol, and lower intakes of polyunsaturated fat, fiber, carotene, vitamin C, vitamin E, vegetables, and fruits, compared with women with 25–32 teeth.

Nowjack-Raymer et al. (*6*) assessed the relationship between numbers of teeth and diet and nutritional status in seven thousand US adults without prostheses. Multiple linear regression models adjusted for socioeconomics and demographics covariates showed that people with fewer than 28 teeth had significantly lower intakes of carrots, tossed salads, and dietary fiber than did fully dentate people, and lower serum levels for beta carotene, folate, and vitamin C.

Ervin and Dye (*7*) examined the relationship between the number and type of teeth and nutrient intakes in adults, aged 60 y and above. They ran both unadjusted and adjusted models, controlling for age, race/ethnicity, education and smoking status. They reported significant associations between dental status and all the examined nutrients for men and for the carotenes and folate for women in the unadjusted model. Only caloric and vitamin C intakes were significant for men, and beta-carotene was significant for women in the adjusted model. It is concluded that demographic and behavioural variables, such as age, race/ethnicity, education and smok-

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ing status explained many of the differences seen in nutrient intakes. After controlling for these variables, the number and type of teeth present affected some nutrient intakes.

Wakai et al. (*8*) reported, in twenty thousand Japanese dentists (mean age: 52.2 y), adjusting for age, sex, smoking, physical activity, and history of diabetes, the mean intakes of some key nutrients and food groups, such as carotene, vitamins A and C, and vegetables including green-yellow vegetables, decreased with the increasing number of teeth lost (p for trend <0.05). On the other hand, mean intakes of carbohydrate, rice, and confectioneries increased among those with fewer teeth (p for trend <0.05).

As stated above, previous studies have shown that adults who are edentulous, or have fewer natural teeth, are less likely to eat fruits, vegetables and meats. However, past studies have many limitations, such as using a self-reported tooth number, or including too wide an age range, thereby producing a generation gap. Both dietary habits and attitudes regarding oral health strongly reflect one's social situation and the era in which one has grown up, neither of which can be adjusted by statistical procedures. For example, whereas 24.1% of 80-y-old Japanese people had 20 or more teeth in 2005, 38.3% of the same age group did only 6 y later in 2011.

Additionally, because it can be easily assessed, the number of teeth has frequently been used as an indicator of oral health in investigations of food intake (*9*). However, the number of teeth alone presents a misleading picture. The role of prosthetic rehabilitation (i.e., dentures) on oral function must be taken into account as well. Optimal masticatory performance is a better indicator of the recommended food intake than the number of teeth. Masticatory performance diminishes as a result of reduced occlusal force, which is a simpler measure (*3*, *10*).

Our Study

We hypothesized that masticatory performance has a greater impact on food intake than does the number of remaining teeth. We investigated the association of occlusal force with food and nutrient intakes after adjusting for the number of teeth in independently living 70-y-old Japanese (*11*).

The study population was 757 community-dwelling people aged 69 to 71 y old. Bilateral maximal occlusal force in the intercuspal position was measured with pressure-sensitive sheets. Removable denture wearers kept their dentures in place during the measurements. Dietary habits during the preceding month were assessed using a brief self-administered diet history questionnaire that measures consumption frequencies of selected foods commonly consumed and calculates energy-adjusted dietary intakes. Linear trends of food and nutrient intakes with decreasing occlusal force were tested after adjusting for gender and socioeconomic factors.

After adjusting for socioeconomic status and the number of remaining teeth, decline of occlusal force was significantly associated with lower intakes of vegetables, vitamins A, C, and B6, folate, and dietary fiber (p for trend<0.05). In contrast, the number of teeth was significantly associated only with calcium and zinc, after controlling for occlusal force.

It is concluded that occlusal force as a representative of oral function was significantly associated with intakes of vitamins and dietary fiber rather than number of remaining teeth in independently living older Japanese.

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ORIGINAL ARTICLE

Association Analysis of *FOXO3* Longevity Variants With Blood Pressure and Essential Hypertension

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BACKGROUND
The minor alleles of 3 *FOXO3* single nucleotide polymorphisms (SNPs)—*rs2802292*, *rs2253310*, and *rs2802288*—are associated with human longevity. The aim of the present study was to test these SNPs for association with blood pressure (BP) and essential hypertension (EHT).

METHODS
In a primary study involving Americans of Japanese ancestry drawn from the Family Blood Pressure Program II we genotyped 411 female and 432 male subjects aged 40–79 years and tested for statistical association by contingency table analysis and generalized linear models that included logistic regression adjusting for sibling correlation in the data set. Replication of *rs2802292* with EHT was attempted in Japanese SONIC study subjects and of each SNP in a meta-analysis of genome-wide association studies of BP in individuals of European ancestry.

RESULTS
In Americans of Japanese ancestry, women homozygous for the longevity-associated (minor) allele of each *FOXO3* SNP had 6 mm Hg lower systolic BP and 3 mm Hg lower diastolic BP compared with major allele homozygotes (Bonferroni corrected $P < 0.05$ and >0.05, respectively). Frequencies of minor allele homozygotes were 3.3–3.9% in women with EHT compared with 9.5–9.6% in normotensive women ($P = 0.03$–0.04; haplotype analysis $P = 0.0002$). No association with BP or EHT was evident in males. An association with EHT was seen for the minor allele of *rs2802292* in the Japanese SONIC cohort ($P = 0.03$), while in European subjects the minor allele of each SNP was associated with higher systolic and diastolic BP.

CONCLUSION
Longevity-associated *FOXO3* variants may be associated with lower BP and EHT in Japanese women.

Keywords: blood pressure; essential hypertension; FoxO3 gene (*FOXO3*); genetic association analysis; hypertension; longevity gene.

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The forkhead box-O3 (FoxO3) gene (*FOXO3*) codes for an evolutionarily conserved transcription factor in the insulin signaling pathway. FoxO3 is recognized in model organisms for its ability to protect against environmental and other biological stressors and thereby enhance lifespan.[1] FoxO3 controls expression of multiple downstream genes whose products affect diverse processes involved in aging and age-related diseases (e.g., energy metabolism, oxidative stress, inflammation, apoptosis, and the cell cycle).[2–4]

In a study of American men of Japanese ancestry, we were the first to report an association of 3 single nucleotide polymorphisms (SNPs) in *FOXO3* (chromosome 6q21) with longevity, the strongest being *rs2802292*.[5] Eleven independent studies of populations of diverse ancestry then confirmed and extended these findings. A meta-analysis confirmed the minor (*G*) allele of *rs2802292* as exhibiting the strongest association with longevity in men (odds ratio, 1.54; 95% confidence intervals, 1.33–1.67).[6] A meta-analysis of genome-wide association studies of this SNP yielded an odds ratio of 1.17 ($P = 1.9 \times 10^{-10}$).[7] The *rs2802292 G* allele has a stronger effect on longevity in Asians than Europeans.[8]

The longevity-associated SNPs were located in or near intron 2 of *FOXO3*.[9–11] Our extensive sequencing analyses have ruled out involvement of coding region variation,[11] suggesting that the causative variant(s) may affect *FOXO3* expression. Understanding the disease-specific protection that enhances lifespan may reveal biological targets for future therapies that could help ameliorate aging-related diseases and thereby confer healthier aging.

FoxO3 and the sirtuin family of NAD^+-dependent deacetylases have been implicated in protection against chronic conditions of aging.[3,5,12–15] This includes cardiovascular disease, with FoxO proteins being able to regulate a number of specific intracellular pathways in the vascular wall and heart.[16] These pathways have implications for hypertension.

We hypothesized that the protective effect of longevity-associated alleles of *FOXO3* is mediated in part by protection against blood pressure (BP) elevation and thus the prevalence of essential hypertension (EHT). Since EHT is a major age-associated risk factor for cardiovascular death,[17] such an effect might explain, at least in part, the association of the *FOXO3* variants with attainment of extreme old age. The aim of the present study was to determine whether there is a genetic association of longevity-associated alleles of our *FOXO3* SNPs with lower BP and EHT.

METHODS

Study population

The primary study involved American subjects of Japanese ancestry living on Oahu and other islands of Hawaii. This cohort was part of the multiracial Stanford Asia and Pacific Program for Hypertension and Insulin Resistance (SAPPHIRe) cohort within the Family Blood Pressure Program (FBPP II[18]; http://clinicaltrials.gov/ct2/show/record/NCT00005270?term=FBPP&rank=3). Unlike other cohorts, the particular population used was relatively homogeneous with regard to geographical finding of genetic associations, should any exist. The characteristics of these subjects have been described in a previous molecular genetic study of EHT.[19] The present study involved 843 subjects after exclusion of 1 of 844 subjects genotyped but lacking BP data. All subjects were offspring of participants of the Honolulu Heart Program, which consisted of unselected American men who self-reported as having Japanese ancestry and who were recruited in 1965–1968.[20] All 4 grandparents of each of the FBPP subjects were therefore of Japanese ancestry. The participants were from 461 families in which 233 families had a single participant, 122 families had two participants, 68 three, 32 four, 4 five, 1 six, and 1 eight. Logistic models with generalized estimating equations were used to adjust for age and sibling correlation to reduce any cluster effect arising from familial relationships. Exclusion criteria for the current study were: treatment for cancer by chemotherapy or radiation within the past 6 months, clinical diagnosis of other chronic disease, pregnancy and having given birth within the past 6 months. We analyzed 432 male subjects aged 56.7 ± 6.6 SD (range 40–75) years and 411 female subjects aged 56.6 ± 6.5 (range 39–77) years.

Procedures performed were in accord with institutional guidelines and were approved by the Institutional Review Board of Kuakini Medical Center. Written informed consent was obtained from all study participants or from family representatives, if participants could not provide consent.

Blood pressure measurement

BP was measured using the Dinamap automated BP reading device. Three separate readings were taken over a period spanning 1 minute and the means of the readings for systolic and diastolic BP were computed for each subject. Subjects were considered to have EHT if their systolic/diastolic BP was ≥140/≥90 mm Hg, or they were receiving antihypertensive medication subsequent to an initial diagnosis of EHT. The normotensive group of subjects had a systolic/diastolic BP reading of <140/<90 mm Hg and were not taking antihypertensive medication.

Genotyping

DNA was isolated from the buffy coat of peripheral blood using the PureGene system (Gentra Systems, Minneapolis, MN) and quantified using PicoGreen staining (Molecular Probes, Eugene, OR). We selected 3 SNPs (*rs2802288*, *rs2253310*, and *rs2802292*; minor allele frequencies in Japanese HapMap samples (MAF) = 0.280, 0.244, and 0.239, respectively) that represented the strongest associations with longevity within intron 2 of *FOXO3* we found previously.[5] Genotyping involved an allelic discrimination assay, TaqMan (Applied Biosystems, Foster City, CA), as performed on a Life Technologies (Carlsbad, CA) QuantStudio 12K Flex OpenArray system. All positive controls on each genotyping plate were evaluated for consistency. We have found that these methods are exceptionally robust, have an accuracy of >99.9% on retesting, and a success rate of 99.6% (data not shown).

Statistical analyses

The study had >80% power to detect genotypic association at the $P = 0.05$ level. Pairwise linkage disequilibrium

Table 1. Association of each genotype of *FOXO3* SNP *rs2802292* with demographic parameters in 411 women and 432 men (adjusted for age and sibling correlation)

Variable	Genotype			P[b]
	TT[a]	*TG*	*GG*	
(a) Women				
n	207	176	28	
Age (years)	56.8±6.7	56.0±6.4	55.0±6.2	0.15
BMI (kg/m^2)	25.6±4.6	25.4±4.7	25.4±4.7	0.87
Cholesterol (mg/ml)	210±39	212±38	215±36	0.49
HDL (mg/ml)	59.4±14.3	59.5±14.4	57.3±14.1	0.46
Triglycerides (mg/ml)	163±113	155±112	132±71	0.09
Glucose (mg/ml)	112±28	111±24	112±31	0.95
Insulin (mU/ml)	7.4±5.5	7.0±5.5	6.6±3.6	0.34
Glucose at 1 h (mg/ml)	188±50	191±54	196±48	0.35
Insulin at 1 h (mg/ml)	62.5±38.7	61.1±43.3	54.8±29.9	0.27
Glucose at 2 h (mg/ml)	160±53	163±54	163±51	0.78
Insulin at 2 h (mU/ml)	66.7±58.5	64.1±56.6	48.1±26.3	**0.009**
Systolic BP (mm Hg)	127.5±19.2	124.8±20.3	118.4±21.2	**0.030**
Diastolic BP (mm Hg)	73.5±10.6	72.5±11.4	69.2±10.8	**0.046**
Essential hypertension (%)	46.6	46.6	25.5	**0.013**
BP medication, ever (%)	33.6	32.2	21.8	0.13
Myocardial infarct, ever (%)	0.9	1.2	0.2	0.26
Bypass, ever (%)	1.0	0.0	3.6	0.45
Stroke, ever (%)	1.9	1.2	3.8	0.60
Cancer ever (%)	3.7	7.4	11.3	0.22
Diabetes, current (%)	2.9	2.4	10.9	0.18
Smoking, ever (%)	4.3	1.6	3.1	0.77
Alcohol ever (%)	52.9	52.5	52.9	1.00
(b) Men				
n	220	173	39	
Age (years)	56.7±6.5	56.7±7.0	55.8±5.6	0.41
BMI (kg/m^2)	27.3±4.3	26.9±4.1	27.1±4.0	0.68
Cholesterol (mg/ml)	197 ± 33	198 ± 34	198 ± 39	0.95
HDL (mg/dl)	49.7±10.9	52.5±13.4	47.3±10.1	0.14
Triglycerides (mg/ml)	175±129	148±97	201±199	0.40
Glucose (mg/ml)	120±30	120±32	121±30	0.87
Insulin (mU/ml)	7.5±4.8	8.8±7.5	9.3±8.2	0.18
Glucose at 1 h (mg/ml)	197±54	100±47	188±48	0.34
Insulin at 1 h (mg/ml)	59.9±36.9	66.3±48.1	79.0±88.2	0.21
Glucose at 2 h (mg/ml)	153±52	148±51	138±41	0.068
Insulin at 2 h (mU/ml)	54.0±41.5	58.6±55.5	73.7±123.0	0.36
Systolic BP (mm Hg)	128.2±17.5	131.7±17.5	132.1±16.4	0.19
Diastolic BP (mm Hg)	79.9±10.4	81.0±10.1	82.4±10.2	0.18
Essential hypertension (%)	49.8	55.4	62.7	0.14
BP medication, ever (%)	39.0	44.0	48.1	0.30
Myocardial infarct, ever (%)	4.3	5.1	2.9	0.59

Table 1. Continued

Variable	Genotype			
	TT[a]	*TG*	*GG*	*P*[b]
Bypass, ever (%)	8.6	5.7	5.7	0.44
Stroke, ever (%)	0.4	1.7	0.1	0.46
Cancer ever (%)	4.5	3.9	5.6	0.77
Diabetes, current (%)	4.8	2.2	0.3	0.003
Smoking, ever (%)	12.3	7.6	5.1	0.082
Alcohol ever (%)	79.3	71.5	65.4	0.069

Glucose at 1 h = blood glucose 1 hour after ingestion of oral Glucola glucose load. Insulin at 1 h = insulin hour after oral Glucola glucose load. Glucose at 2 h = blood glucose 2 hours after ingestion of oral Glucola glucose load. Insulin at 2 h = insulin 1 hour after oral Glucola glucose load. Significant *P* values are shown in bold.

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; BP, blood pressure.

[a]*T* = major allele and *G* = minor allele of *rs2802292*. [b]After correction for multiple comparisons by the Bonferroni method all $P < 0.05$ values became nonsignificant ($P > 0.05$).

Table 2. Contingency table analysis of *FOXO3* genotype frequencies in subjects with essential hypertension (EHT) compared with those who were normotensive (NT)

SNP		Women					Men				
rs2802292		***TT***	***TG***	***GG***	χ^2	***P***	***TT***	***TG***	***GG***	χ^2	***P***
	NT	111	97	22	6.4	**0.042**	110	77	15	2.4	0.31
	EHT	96	80	6			110	96	24		
rs2253310		***GG***	***GC***	***CC***	χ^2	***P***	***GG***	***GC***	***CC***	χ^2	***P***
	NT	109	98	22	5.2	0.074	111	76	16	2.3	0.32
	EHT	95	78	7			110	96	24		
rs2802288		***GG***	***GA***	***AA***	χ^2	***P***	***GG***	***GA***	***AA***	χ^2	***P***
	NT	111	98	22	6.2	**0.046**	111	77	16	2.4	0.31
	EHT	94	80	6			109	97	24		

Significant *P* values are shown in bold.

was measured as the correlation (r^2 value) between the 3 *FOXO3* SNPs using the HapMap JPT + CHB populations (a combined panel of Japanese in Tokyo, Japan and Han Chinese in Beijing, China; HapMap phase 2 release 22). Linkage disequilibrium was calculated using SNAP (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php).[21] Genotypes were evaluated for deviation from Hardy–Weinberg equilibrium. The association of *FOXO3* genotype with each demographic parameter shown in the Results section was assessed using generalized linear models including logistic regression, adjusting for sibling correlation in the data sample. Association of *FOXO3* genotype with EHT was determined by contingency table analysis. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC). Correction for multiple testing was performed by the Bonferroni method.

Replication studies

Association of *rs2802292* genotypes with hypertension in the SONIC (Septuagenarians, Octogenarians, Nonagenarians Investigation with Centenarians) study[22] of Japanese subjects aged 80 ± 1 years from Hyogo and Tokyo was tested. In this study genotyping involved the TaqMan method.[23] In addition, data for each of the 3 *FOXO3* SNP obtained from a meta-analysis of genome-wide association studies of systolic and diastolic BP in subjects of European descent were examined.[24]

RESULTS

Demographic parameters for each *FOXO3* genotype

The 3 SNPs were in strong linkage disequilibrium (Supplementary Table S1). As a result, demographic parameters were similar for genotypes of each SNP. Table 1 shows physiological and clinical phenotypes for *rs2802292* genotypes in female and male subjects adjusting for sibling relatedness and age and Supplementary Tables S2 and S3 show these results for *rs2253310* and *rs2802288*. In women, lower systolic and diastolic BP, as well as EHT prevalence, were associated with the minor allele of each SNP. No such association was seen in men or all subjects combined.

Association of genotype with essential hypertension

No significant association of *FOXO3* genotype with EHT was seen for all subjects or for men, but for women, χ^2 analysis showed a significant association of *FOXO3* genotype with EHT (Table 2). The frequency of minor allele homozygotes in EHT vs. normotensive female subjects was 3.3% vs. 9.6%, 3.9% vs. 9.6% and 3.3% vs. 9.5% for *rs2802292*, *rs2253310*, and *rs2802288*, respectively. Haplotype χ^2 analysis of data for the 3 SNPs gave $P = 0.0002$ for women (which remained significant after correction for multiple testing), $P = 0.031$ for all subjects and $P = 0.29$ for men. The minor allele was protective against EHT after adjusting for age, and using logistic models with generalized estimating equations to adjust for any cluster effect arising from the inclusion of subjects who were siblings (Table 3). No difference was found between pre- and postmenopausal women.

Association of genotype with BP in hypertensive and normotensive subjects

Since BP was affected by antihypertensive medication in EHT subjects, data for these are not shown. For normotensive subjects the longevity-associated minor allele of each *FOXO3* SNP was associated with lower systolic and diastolic BP in women but not in men or all subjects combined using a stratified analysis (Table 4). This was statistically significant only for systolic BP. After Bonferroni correction the association remained statistically significant. In women, whereas systolic BP increased with age for heterozygotes and major allele homozygotes of each SNP, this was not seen for the minor allele (longevity-associated) homozygotes (Supplementary Figure S1), consistent with a protective effect of this genotype against the usual rise in systolic BP with age.

Replication studies

The *G* allele of *rs2802292* was associated with lower prevalence of EHT in Japanese subjects from the SONIC study (Table 5). Subdivision by sex was not significant, possibly because of the lower *n* value of the SONIC cohort. Combining SONIC data with FBPP data was precluded by the older age of the former (80 ± 1 vs. 56.6 ± 6.5 years).

In the genome-wide association studies meta-analysis of BP in European subjects,[24] *T* allele carriage of *rs2802292*, *G* allele carriage of *rs2253310*, and *G* allele carriage of *rs2802288* tracked with significantly lower systolic BP ($P = 0.0011$, 0.0009, and 0.0010, respectively) and diastolic BP ($P = 0.42$, 0.043, and 0.044), respectively, not stratified for sex. The *P* values did not reach genome-wide significance.

Allele frequencies for *rs2253310*, *rs2802288*, and *rs2802292* were *C/G* 0.425/0.575, *A/G* 0.425/0.575, and *G/T* 0.424/0.576, respectively.

DISCUSSION

The longevity-associated allele of 3 *FOXO3* SNPs was associated with lower systolic and diastolic BP and EHT in Japanese American women, but not in men. The reason for the sex difference is not apparent. Since EHT is a major risk factor for myocardial infarction and stroke, these findings suggest a possible protective mechanism by which genetic variation in *FOXO3* might assist in lifespan extension in women.

Although the level of statistical significance obtained was modest, the direction of the association seen was consistent for each of systolic BP, diastolic BP, and EHT in Japanese subjects. The study cohort was not selected based on a subgroup of the Honolulu Heart Program that was long-lived. Rather, offspring of all Honolulu Heart Program participants were contacted. Since related individuals were included, all data were adjusted for effect of familial clustering and only adjusted data are presented. Adjusted data nevertheless yielded results similar to unadjusted data. Confirmation in larger cohorts is required.

Replication in Japanese subjects from Japan supports the findings. But in White subjects of European descent genotypic association with systolic and diastolic BP was opposite.

Table 3. Effects of genotype on essential hypertension

		Women			Men		
SNP		OR	95% CI	*P*	OR	95% CI	*P*
rs2802292	*TT*	—[a]			—		
	TG	1.00	0.66–1.51	1.00	1.26	0.85–1.88	0.26
	GG	0.36	0.14–0.92	**0.034**	1.71	0.81–3.61	0.16
rs2802288	*GG*	—			—		
	GA	1.02	0.67–1.54	0.94	1.30	0.87–1.95	0.20
	AA	0.37	0.15–0.95	**0.038**	1.66	0.80–3.46	0.17
rs2253310	*GG*	—			—		
	GC	0.96	0.63–1.45	0.84	1.30	0.86–1.94	0.21
	CC	0.40	0.17–0.96	**0.041**	1.58	0.76–3.29	0.22

Shown are results from use of logistic models adjusting for age, smoking, alcohol consumption, and sibship correlation. Significant *P* values are shown in bold.

Abbreviations: OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism.

[a]Major allele homozygotes for each SNP were used as the reference group for calculation of OR.

Table 4. The effect[a] of *FOXO3* genotype on systolic and diastolic BP in normotensive women and men

Sex	BP	Genotype	Change in BP (mm Hg)[b]	95% CI	*P*	*P*[c]
rs2802292						
Women	SBP	*TG*	−3.7	(−7 to −0.3)	**0.033**	0.13
n = 229	SBP	*GG*	−6.2	(−10.7 to −1.7)	**0.0071**	**0.028**
	DBP	*TG*	−2.2	(−4.6 to 0.2)	0.070	—
	DBP	*GG*	−3.4	(−6.7 to −0.1)	**0.043**	0.17
Men	SBP	*TG*	−0.1	(−3.5 to 3.2)	0.94	—
n = 202	SBP	*GG*	3.8	(−1.1 to 8.7)	0.13	—
	DBP	*TG*	−2.1	(−4.5 to 0.4)	0.096	—
	DBP	*GG*	1.3	(−2.8 to 5.4)	0.54	—
rs2802288						
Women	SBP	*GC*	−3.6	(−6.9 to −0.3)	**0.035**	0.14
n = 230	SBP	*CC*	−5.9	(−10.4 to −1.4)	**0.0095**	**0.038**
	DBP	*GC*	−2.2	(−4.6 to 0.2)	0.072	—
	DBP	*CC*	−3.3	(−6.6 to 0.1)	0.054	—
Men	SBP	*GC*	−0.1	(−3.4 to 3.3)	0.96	—
n = 204	SBP	*CC*	3.4	(−1.2 to 8.1)	0.15	—
	DBP	*GC*	−2.1	(−4.6 to 0.3)	0.092	—
	DBP	*CC*	0.9	(−3.1 to 4.8)	0.66	—
rs2253310						
Women	SBP	*GC*	−3.6	(−7.0 to −0.2)	**0.036**	0.14
n = 228	SBP	*CC*	−5.9	(−10.4 to −1.4)	**0.0099**	**0.040**
	DBP	*GC*	−2.1	(−4.5 to 0.3)	0.082	—
	DBP	*CC*	−3.2	(−6.6 to 0.1)	0.059	—
Men	SBP	*GC*	−0.3	(−3.7 to 3.1)	0.86	—
n = 203	SBP	*CC*	4.5	(−0.2 to 9.3)	0.063	—
	DBP	*GC*	−2.3	(−4.8 to 0.1)	0.062	—
	DBP	*CC*	1.8	(−2.3 to 5.9)	0.39	—

Significant P values are shown in bold.
Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.
[a]Effects and 95% CI are adjusted for age, smoking, alcohol drinking and sibship correlation using generalized linear models. [b]Effect is compared to the major allele homozygotes of each SNP. [c]Values for significant *P* values after Bonferroni correction for multiple comparisons.

Table 5. Association analysis of *rs2802292* genotypes with hypertension in Japanese subjects in the SONIC study from Hyogo and Tokyo

	rs2802292		
	TT	*TG/GG*	*P*[a]
EHT	343	272	0.039
NT	61	72	

[a]*P* value from multiple regression adjusting for BMI, diabetes, and dyslipidemia.

The reason for this is unclear, although correction for multiple testing would render the results nonsignificant. It is, however, of possible interest that although *FOXO3 rs2802288* genotype is strongly associated with longevity in both Asians and Europeans, for *rs2802292* the association in Europeans is weaker than in Asians.[8]

The various cardiovascular actions of FoxO3 support the possibility of protection against EHT. In a twin study, the *FOXO3 rs2800292 G* allele was associated with more favorable insulin sensitivity and increased *FOXO3* expression.[25] Mice lacking *FoxO1*, *FoxO3*, and *FoxO4* in myeloid cells had a pro-atherogenesic phenotype.[26] By inhibiting vascular smooth muscle cell proliferation and neointimal hyperplasia,[27] FoxO3 reduces peripheral vascular resistance and BP. FoxO3 reduces cardiomyocyte proliferation and size,[28–31] so protecting against cardiac hypertrophy seen commonly in EHT.[32] It also decreases oxidative stress in cardiac fibroblasts.[33] FoxO3 protects the kidney against aging-related damage[34] by reducing reactive oxygen species and inflammation that increase EHT risk.[35,36]

FoxO3 reduces production of inflammatory cytokines[37–39] and *FOXO3* SNP *rs12212067* (in linkage disequilibrium with *rs2802292*, *rs2253310*, and *rs2802292*) was associated with inflammation suppression.[40] Vascular aging is, in part, an inflammatory process,[41] leading to remodeling of resistance vessels.[42] Age-related loss of microvascular density and plasticity is associated with adult stem cell dysfunction in vascular niches driven, in part, by inflammatory cytokines inhibited by FoxO3.[40]

FoxO3 is a primary driver of a protective autophagy program and quiescence[43] in adult cardiac stem cells,[44] helps maintain the hemopoietic stem cell pool,[45,46] and promotes the quiescent state during stem cell self-renewal in adult muscle regeneration.[43] Regenerative stem cell survival could profoundly benefit myocardial remodeling with age.

In conclusion, longevity-associated genetic variants in *FOXO3* might reduce BP and EHT in American women of Japanese ancestry. Dietary modulators of FoxO3 lower BP[47] and mitigate hypertension, reduce renal inflammation and oxidative stress, restore antioxidant capacity in the spontaneously hypertensive rats,[48] and lower systolic, but not diastolic, BP[49,50] in type 2 diabetes patients.[49,50] Such effects involve sirtuins, which, by modulating FoxO3 activity, regulate its transcription of genes responsible for attenuation of reactive oxygen species,[4] so ameliorating oxidative damage to tissues involved in cardiovascular control. Sirtuin activating compounds could serve as novel antihypertensive agents, with FoxO3 activation a possible mediator of part of their effect on BP.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

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Factors associated with risk for assisted living among community-dwelling older Japanese

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ABSTRACT

Objectives: To clarify the factors associated with risk for assisted living among community-dwelling older people, we conducted a large-scale survey in an urban city in Japan.
Design: Population-based cross-sectional study.
Setting: A mid-sized urban city in western Japan with a population of approximately 410,000.
Participants: Nondisabled and nondemented community-dwelling older people (≥65 years).
Measurements: A self-administered postal questionnaire, including a health checklist for the screening of older people at high risk for assisted living, as well as demographic/sociodemographic questions on sex, age, present illness, living alone, duration of residence within the current city, community participation, and employment status, was distributed.
Results: There were 41,796 returned questionnaires (response rate: 73.8%, average age: 72.0). Participants who were at high risk for assisted living accounted for 25.2%. The independent factors associated with risk for men and women were higher age, present illness, lack of community participation, unemployment, and <20 years of residence. Living alone was a significant factor for men, whereas it was insignificant among women. The types of illnesses among people at risk were different between men and women.
Conclusion: Higher age, present illness, and several social factors were independently associated with high-risk status for assisted living in the large-scale whole community survey, and there was a sex difference. Our results may provide basic information for the further application of effective preventive intervention in the community.

1. Introduction

Japan has been experiencing a rapidly aging society that is unmatched in the world. The proportion of people aged ≥65 years in Japan is the highest in the world (25.9% in 2014); the life expectancy at birth has lengthened and is highest among Japanese women in the world (women: 87, men: 80) (World Health Organization, 2015; Cabinet Office, Government of Japan, 2015). However, extended survival in older people is not always accompanied by good health; it has been inevitably associated with greater numbers of disabled older people who need additional support in daily life (Ho, Woo, Yuen, Sham, & Chan, 1997). The percentage of the old-old population (aged ≥75 years), which contains more frail or lower functioning people than the young-old population (aged 65–74 years), is growing rapidly and exceeded 12.3% of the Japanese national population in 2014 (Japanese National Institute of Population and Social Security Research, 2015). Frailty or lower function results in increasing dependency and an overall reduction in the quality of life. Therefore, preventive health care should include the prevention of not only disease but also its earlier phases, such as decline in function and frailty, in order to prevent older people from requiring assisted living and to establish a healthier aged society in which all people can spend healthy, satisfying lives (Ho et al., 1997; Kabayama, Kamide, Sakakibara, & Hayakawa, 2014). Given this background, the Japanese Ministry of Health, Labour and Welfare (MHLW) has started to emphasize preventive care for the older people at high risk for assisted living (Arai et al., 2012). The MHLW appointed local governments to manage preventive care for functional decline in older people at high risk for assisted living by focusing on the improvement of physical function, cognitive function, and malnutrition status. To identify community-dwelling older high-risk persons, many local governments have used a basic screening health checklist called "Kihon Checklist" (KCL) (Fig. 1).

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