Table 5. Correlations between the SRPO factors and other indices

	SRPO Total	Factor I Family and environment support	Factor II Social interaction	Factor III Social adjustment
Total ($n = 66$)				
Decisional Balance for Exercise Scale ($n = 65$)	0.38**	0.32*	0.27*	0.18
Pros ($n = 65$)	0.12	0.06	0.12	0.02
Cons ($n = 65$)	−0.48**	−0.42**	−0.34**	−0.22
Motivation for exercise ($n = 63$)	0.47**	0.28*	0.35**	0.45**
Marital status ($n = 65$)	0.18	0.34*	0.02	−0.24
Tele-care group ($n = 36$)				
Decisional Balance for Exercise Scale ($n = 35$)	0.27	0.26	0.20	0.05
Pros ($n = 35$)	0.14	0.09	0.13	−0.07
Cons ($n = 35$)	−0.31	−0.34*	−0.22	−0.15
Motivation for exercise ($n = 33$)	0.49**	0.31	0.41*	0.46**
Marital status ($n = 36$)	0.18	0.28	0.03	−0.15
Self-help group ($n = 30$)				
Decisional Balance for Exercise Scale ($n = 30$)	0.47**	0.38*	0.31	0.33
Pros ($n = 30$)	0.09	0.00	0.08	0.13
Cons ($n = 30$)	−0.62*	−0.55**	−0.42*	−0.34
Motivation for exercise ($n = 29$)	0.40*	0.13	0.33	0.38*
Marital status ($n = 28$)	0.16	0.41**	−0.02	−0.33

Pros/Cons: perceived advantages/disadvantages of exercise.
Values are scores at baseline.
*$p < 0.05$; **$p < 0.01$.

months, 3: intending to exercise next month, 4: sustaining exercise for 6 months or less, 5: sustaining exercise for over 6 months) (Marcus *et al.*, 1992). These levels express the degree to which one is prepared for lifestyle modifications related to exercise. The level of motivation for exercise is strongly correlated with the level of social support (Prochaska *et al.*, 1994). Thus, these two variables – marital status and improvements in the level of motivation for exercise – could be used to represent social-relationship elements that prevent obesity by promoting exercise (Ruggiero & Prochaska, 1993; Glanz *et al.*, 1994; Vallis *et al.*, 2003). First, the model was constructed with already collected data, to estimate whatever outcomes possible considering the flexibility of these data. Then, to evaluate the model's validity, split sample validation was performed. Although split sample validation is an accepted method, researchers should ideally collect new data to confirm model fit (Katz, 1999). In split sample validation, the full sample is split into two groups of approximately equal size and results of each group are compared. Therefore, in the current study, to confirm the validity of the scale and suitability of the analytical methods used, the sample was split into randomly selected groups and whether the previously obtained findings were robust verified. For further confirmation, analyses were conducted with randomly selected subgroups of 33 participants each.

A high degree of association was found between the SRPO and scores obtained on the Decisional Balance for Exercise Scale (Table 5). This instrument measures what people think the pros and cons of exercise are. The SRPO score was significantly and

Table 6. Descriptive statistics: dietary habits of the respondents

Variable	*n*	%
How many meals do you have in a day?	($n = 63$)	
Two	4	(6.3)
Three	58	(92.1)
Four	1	(1.6)
Do you have breakfast daily?	($n = 64$)	
Yes	60	(93.8)
No	4	(6.2)
Do you have regular meal times?	($n = 63$)	
Yes	51	(81.0)
No	12	(19.0)
How many hours do you usually keep between your last meal of the day and bedtime?	($n = 63$)	
1 hour	4	(6.3)
1–2 hours	7	(11.1)
Over 2 hours	52	(82.6)
How many times a day do you have a snack between meals?	($n = 60$)	
Never	11	(18.3)
Every few days	1	(1.7)
Once	30	(50.0)
Twice	14	(23.3)
Three times	4	(6.7)
How many alcoholic drinks do you have in a week?	($n = 64$)	
None	31	(48.4)
One drink a week/a few drinks a month	19	(29.7)
One almost every day	14	(21.9)
How many times in a month do you eat out at a restaurant or suchlike?	($n = 61$)	
Never	31	(50.8)
Fewer than 4 times	14	(23.0)
8 times or less	2	(3.2)
9 times or more	14	(23.0)

negatively correlated with the scores for the 'Cons' subscale of this instrument, which measures perceived disadvantages of exercise, and Factor I of the SRPO positively associated with the marital status. The SRPO score was also found to be positively and significantly correlated with the level of motivation for exercise.

The correlation between the scores on the Decisional Balance for Exercise Scale and the SRPO confirm the SRPO's validity. Although Factor III by itself had little correlation with the score for the Decisional Balance for Exercise Scale, a higher total score on the SRPO, including Factor III, was found to correlate with a significantly higher score on the Decisional Balance for Exercise Scale (Table 5). Moreover, the level of motivation for exercise was significantly correlated with family and environmental support, social interaction and adjustment (Factors I, II and III) and with the SRPO total score. Further analysis revealed that the tele-care and self-help groups also had similar correlations as with the full sample. Another randomly selected group indicated that the reliability was similar to that reported above (Group 1 $\alpha = 0.81$, Group

Table 7. Significant correlations between eating habits and SRPO items

Factors and items	Number of meals	Breakfast	Snacks	Drinks	Eating out	Meal time[a]	Hours[b]
Factor I: Family and environmental support							
1. My family or friends exercised with me and gave me helpful reminders to exercise	−0.05	−0.06	0.11	−0.17	0.07	0.09	0.09
2. My family or friends helped plan activities around my exercise	0.02	−0.08	0.21	−0.24	0.07	0.02	0.09
3. My family or friends reminded me not to eat high-fat, high-salt foods	−0.17	0.03	0.30*	0.25*	0.06	−0.08	0.07
4. My family or friends discussed my eating habit changes with me	−0.19	0.00	0.37**	0.33**	−0.03	−0.12	0.22
Factor II: Social interaction							
5. How many volunteer groups or organizations do you belong to (e.g. church, temple, shrine groups, clubs in the community or parent groups)?	−0.25*	0.12	−0.05	0.06	−0.27*	−0.04	−0.03
6. How active are you in the affairs of the groups or clubs to which you belongs?	−0.24	0.10	−0.08	−0.02	−0.23	−0.04	−0.11
7. [How often do you have] someone to get together with for relaxation?	−0.24	0.09	0.08	−0.12	−0.25	−0.00	−0.06
8. [How often do you have] someone to prepare your meals if you were unable to make them yourself?	−0.16	−0.11	0.36**	−0.13	−0.01	−0.09	−0.03
Factor III: Social adjustment							
9. How many times in the last two weeks have you gone out socially (visited friends, gone to movies, churches, restaurants, etc.)?	0.26*	−0.19	−0.04	−0.02	−0.07	0.19	−0.12
10. How much time have you spent on hobbies or items of interests during the last two weeks?	0.19	−0.27*	0.26*	−0.10	−0.05	0.11	−0.17

[a] Meal time: regular meal times.
[b] Hours: the hours between the last meal of the day and bedtime.
*$p < 0.05$; **$p < 0.01$.

2 $\alpha = 0.67$; results not shown in Table 5). Thus, split-sample validation suggests that the scale is well calibrated (Table 5). Thus, the SRPO's construct validity is substantiated by the high correlations between the SRPO and the level of motivation for exercise and between the SRPO and the Decisional Balance for Exercise Scale (Yata *et al.*, 2003).

Concurrent criterion-related validity. To determine concurrent criterion-related validity, using two-tailed Pearson correlations, correlations between the SRPO and data obtained from the questions about eating habits were examined: (1) number of meals, breakfast daily, regular meal times, the hours between the last meal of the day and bedtime, (2) the number of snacks and alcoholic drinks consumed, and (3) instances of eating out. The responses to the questions pertaining to eating habits are shown in Table 6, and the correlations between eating habits and SRPO items are shown in Table 7. Items 3 and 4 of the SRPO, both of which concern eating habits, were significantly correlated

with the number of times snacks and alcohol was consumed. Item 8 and 10 also were significantly correlated with consumption of snacks. Items 5 and 9 were significantly correlated with the number of meals. Item 5 was significantly correlated with instances of eating out. Item 10 was significantly correlated with consumption of breakfast and snacks. No items significantly correlated with regular meal time and the hours between the last meal of the day and bedtime.

The association between eating habits and the SRPO is proved by the following correlations: (1) The presence of cordial relations with family members was inversely related to drinking and positively correlated to eating snacks. (2) The number of social activities and participation in social affairs was inversely related to the number of meals and instances of eating out and directly related to eating snacks. (3) The time spent on hobbies was directly related to the number of meals, consumption of snacks and inadequate breakfasts. The correlations between items for social adjustment or communication in the SRPO and items on dietary habits suggest that the quality and quantity of a person's food intake may be affected by the person's relationships with his or her supporters. Moreover, the response to each question on dietary habits was significantly correlated with Factors I, II and III of the SRPO (Table 7).

Discussion

Advantages of the SRPO scale

Many instruments have been developed in studies on weight control, but these contain a bewildering number of items on many different aspects, including the physical and psychosocial (Stunkard & Messick, 1985; McDowell & Newell, 1996). Because the SRPO contains fewer items with high factor-loading values, validity and reliability, it is a more useful and convenient instrument for study participants and researchers than previous scales. The response rate in the Takada *et al.* (2011) study (55.9%) shows that the participants faced no inconvenience in providing responses, except for the excluded questions described above. Health information and knowledge helps people to choose a healthier lifestyle by improving their understanding of the relationships between health behaviour and health outcomes (Kenkel, 1991). The protective health effects of social relationships may be as important as the negative effects of established risk factors, such as smoking, obesity and high blood pressure (House *et al.*, 1988; Boothroyd & Fisher, 2010). Each item in the SRPO provides information about key health behaviours in social relationships. To convince the clinical practitioner in weight control of the SRPO's usefulness, it would be helpful to show that the SRPO directly correlates with the decisional balance for exercise in the Transtheoretical Model.

This study finds that the quantity and quality of social relationships are positively related to marital status. It seems that the SRPO score might be influenced, in implicit or explicit ways, by a spouse, family members or other important people outside the family through a peer effect (Wallston *et al.*, 1978). In terms of LOC, it was assumed that a higher SRPO score would indicate greater environmental influence or the presence of a large number of high-quality social relationships. This effect could result in a dependency-related tendency, perhaps caused by the influence of strong relationships with others, such as a spouse, siblings, family members, colleagues, co-workers and

neighbours, and the effect of the shared environment (Wallston *et al.*, 1978; Macgregor *et al.*, 1997; Renna *et al.*, 2008; Fujita & Noguchi, 2009; Fortin & Yazbeck, 2011; Yakusheva *et al.*, 2011). Although Nir and Neumann (1995) reported no significant differences in weight loss between those with internal and external LOC during the post-intervention period of their study, the internal group gained less weight than the external group did. This evidence leads to the conclusion that internal LOC has a long-term effect and that modification of an external LOC is required to bring about meaningful change.

The importance of peer support has policy significance. Group-level interventions might be more cost-effective, successful and open to variation than individual interventions (Christakis & Fowler, 2007; Fowler & Christakis, 2008; Renna *et al.*, 2008; Trogdon *et al.*, 2008; Bahr *et al.*, 2009; Cobb *et al.*, 2011; Fortin & Yazbeck, 2011; Yakusheva *et al.*, 2011). Several studies have examined policy interventions targeted at altering the environment in such a way as to increase people's physical activity levels (Sallis & Owen, 1998; Ståhl *et al.*, 2001; Pratt *et al.*, 2004; Roux *et al.*, 2008; Li *et al.*, 2009; Cobb *et al.*, 2011; Montes *et al.*, 2012). Results from the current study suggest that individuals who have high SRPO scores at baseline are more influenced by their spouses, family members and friends. When conducting a weight-loss intervention, special attention should be paid to the possibility that a self-control problem might be interfering with weight loss for participants who express the characteristic of external LOC and who have spouses, close family members or friends who are obese or have untreated obesity-related disease. Moreover, it is possible that participants' attitudes towards future obesity risks (i.e. obesity-related behaviour, sedentary lifestyle, impatience, indifferent attitude towards risk aversion, obesity-related family eating traditions and food choices) can be measured with the SRPO in future weight-control studies (Yakusheva *et al.*, 2010; Pachucki *et al.*, 2011; Takada *et al.*, 2011). Effective weight-loss interventions that incorporate the acquisition of social support through a reliable social network should be used as an aid for self-control and a strong commitment to weight loss. Additionally, participants should be encouraged to develop a reliable social network that helps them maintain healthier habits.

Limitations

This study had some limitations. First, the items on the number of convenient athletic facilities and employer support were deleted because of their extremely poor response rates. Many participants could not respond to questions about employer direction and support because they were retired or owned their own businesses. However, the literature shows that these two factors play an important role in preventing obesity (Sallis *et al.*, 1992). Second, the participants in the Takada *et al.* (2011) study were recruited through a public advertisement, so there may have been a self-selection bias, although participants were subsequently randomized into the tele-care and self-help groups. If the SRPO is to be employed in a particular population, demographic characteristics, in particular, potential confounding factors such as age and job status should be taken into consideration. Third, as this scale was constructed by selecting items from other scales, the direct correlation between weight loss and scores on this scale may not strongly reflect the relationship between weight loss and self-control in

social relationships, because we did not examine correlations between weight loss and other variables that have been previously shown to be related to weight loss. Fourth, it is necessary to determine the intrinsic differences between individuals who can and cannot develop and maintain strong self-control for healthy behaviours and devise a means to measure these differences.

Applications

The SRPO has moderate validity, reliability and clinical utility in examining how social relationships support self-control with regard to weight loss or obesity prevention. Thus, it can be used as a screening tool in weight-loss interventions. The SRPO can also be used to examine the social environment and self-control problems in obese people, factors that should be considered when conducting a weight-loss intervention since obese people may have self-control problems that interfere with weight-loss plans (Kan, 2007).

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CLINICAL INVESTIGATIONS

Tooth Loss and Atherosclerosis: The Nagahama Study

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Abstract: *Several epidemiologic studies have suggested that oral disease is a risk factor for cardiovascular disease (CVD). However, whether a clinically significant association exists between the 2 disorders remains controversial. Here, we investigated the association between tooth loss, as an indicator of oral disease, and arterial stiffness, as a marker of atherosclerosis, in Japanese adults. Cross-sectional data were collected for 8,124 persons aged 30 to 75 y with no history of tooth loss for non-inflammatory reasons, such as orthodontic treatment, malposition, and trauma. Participants received a comprehensive dental examination and extensive in-person measurements of CVD risk factors, and arterial stiffness was evaluated using the cardio-ankle vascular index (CAVI). We examined the association between CAVI and tooth loss using general linear models with adjustment for age, sex, body mass index, smoking status, hemoglobin A1c, and a history of insulin or hypoglycemic medication depending on the model. In addition, we performed an analysis that included interaction terms of the centered variables tooth loss, sex, and age. The results of the multiple regression analysis that included the interaction terms detected that the relationship between CAVI and tooth loss was dependent on sex, with only men showing a positive correlation (β for interaction = 0.04; 95% confidence interval, 0.02–0.06). The findings from this study suggest that a linear relationship exists between tooth loss and degree of arterial stiffness and that the association differed depending on sex.*

Key Words: arterial stiffness, epidemiology, inflammation, periodontal disease, cross-sectional analysis, cardiovascular diseases.

Introduction

Cardiovascular disease (CVD) is the most common cause of death and disability in industrialized nations and has a high cost to society. The coincidence of cardiovascular and oral disease is relatively high, and numerous studies have reported that a positive association exists between these 2 diseases (Beck et al. 1998; Beck et al. 2001; Hujoel et al. 2001; Desvarieux et al. 2003; Pussinen et al. 2003; Desvarieux et al. 2004; Ylostalo et al. 2006; Tonetti et al. 2007; Tu et al. 2007; Dietrich et al. 2008; Senba et al. 2008; Choe et al. 2009; de Oliveira et al. 2010; Kim et al. 2010). However, as a significant relationship was not detected in several studies (Hujoel et al. 2000; Lavelle 2002; Colhoun et al. 2008), it remains controversial whether a clinically significant association exists between the 2 diseases (Hujoel et al. 2000; Lavelle 2002; Lockhart et al. 2012).

Inflammation plays an important role in the pathogenesis of CVD. Systemic inflammation may represent the underlying mechanism that links oral and cardiovascular diseases. Oral disease, such as periodontal disease, is characterized by chronic systemic inflammation and often results in tooth loss due to the breakdown of periodontal tissue. Therefore, tooth loss is a useful proxy for the accumulated burden of inflammatory disease (Desvarieux et al. 2003; Houshmand et al. 2012).

Elderly people and men carry a disproportionate burden of CVD and oral disease. Therefore, age and sex adjustment must be performed when evaluating the potential link between oral disease and CVD. Moreover, as oral disease and CVD share common risk factors, including smoking, diabetes, hypertension, and obesity, the potential for confounding is substantial. Thus, the power for detecting effect modification is limited in small population studies.

Arterial stiffness, as assessed by the cardio-ankle vascular index (CAVI), is

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a measure of CVD (Kadota et al. 2008). CAVI was developed to overcome the dependency of pulse-wave velocity (PWV) measurements on blood pressure. The underlying principle of CAVI measurement is based on the stiffness parameter β and is calculated using the Bramwell-Hill formula, which is basically independent of blood pressure (Yambe et al. 2004; Shirai et al. 2006; Takaki et al. 2008; Shirai et al. 2011). Thus, CAVI is an easily administered arterial stiffness screening test that ensures good reproducibility as a diagnostic tool for CVD. Here, we investigated the relationship between tooth loss and arterial stiffness using baseline survey data in a population-based cohort.

Methods

Ethics Statement

This study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee, the Ad Hoc Review Board of the Nagahama Study, and the Nagahama Municipal Review Board of Personal Information Protection. Appointments for health examinations were made by telephone by the municipal government staff, and participant registration was performed at the site of the health examinations. Written informed consent was also obtained from all participants prior to their health examination.

Study Design and Population

The Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience (the Nagahama Study) is a population-based prospective cohort study of a broad range of chronic illnesses and was conducted in Nagahama City, Shiga Prefecture, Japan (Tabara et al. 2013). The present study is a prospective study that collected data by means of questionnaires, anthropometric and physiologic measures, biochemical measurements of blood samples, genomic information, and oral examinations. The Nagahama Study participants were recruited from apparently healthy community residents living in Nagahama City, a largely rural city of approximately 125,000 inhabitants in Shiga Prefecture, which is located in central Japan. A baseline survey was conducted between fiscal years 2008 and 2010. Information on the project was provided to potential participants by newsletters, newspaper flyers, and brochures and on the homepages of the local government and citizen organizations. Information sessions for the residents were also held by researchers and city employees, who explained the project to interested residents. Residents of Nagahama City who fulfilled the following criteria were recruited for the cohort study: 1) age between 30 and 74 years old at the time of recruitment, 2) able to participate in the health examinations independently, 3) no difficulties in communication in Japanese, 4) no serious diseases/symptoms or health issues, and 5) voluntarily decided to participate in the project. We performed a complete case analysis, so only participants with complete information for subjective measures of tooth loss, CAVI, and all other examined covariates were included in the analytical sample. As no variables were missing from more than 5% of the total number of cases, and the missing data were random, the missing data are not considered to have markedly influenced the outcomes of the analysis. A total of 1,670 participants were excluded from the adjusted analyses because of missing data, and participants who reported that tooth loss was due to orthodontic treatment, malpositioned teeth, or trauma were also excluded. Therefore, the analyses reported in the present study included a total of 8,124 participants.

Risk Factor Assessment

Trained physicians and research assistants administered standardized questionnaires, performed anthropomorphic measurements, and collected fasting blood specimens using standardized protocols. Subjects were interviewed and completed a questionnaire regarding sex, age, cardiovascular risk factors, and other medical conditions.

Height and weight measurements were determined with calibrated scales. Body mass index (BMI) was calculated using the obtained height and weight data. Trained nurses measured blood pressure using a calibrated automated sphygmomanometer (HEM-9000; Omron Healthcare Co., Ltd., Kyoto, Japan). All measurements were taken at least twice in a sitting position, and the last measurement among the data measured without error was used in the analysis. Fasting blood glucose level, high-density lipoprotein (HDL) cholesterol (HDL-C), low-density lipoprotein (LDL) cholesterol (LDL-C), and hemoglobin A1c (HbA1c) were measured using the collected blood samples from all subjects. Participants were categorized as current smokers, former smokers, or never smokers based on self-report.

Hypertension was defined as a systolic blood pressure (SBP) of ≥140 mm Hg or a diastolic blood pressure (DBP) of ≥90 mm Hg, or the self-report of history of antihypertensive drug use. HbA1c values (%) are reported according to the National Glycohemoglobin Standardization Program. Diabetes mellitus was defined by a history of insulin or hypoglycemic medication, or a fasting glucose level ≥126 mg/dL or random plasma glucose level ≥200 mg/dL, or HbA1c ≥6.5 (HbA1c ≥6.1%), according to the Japan Diabetes Society criteria (Seino et al. 2010).

Measurement of CAVI

CAVI was recorded using a Vasera VS-1500 vascular screening system (Fukuda Denshi Ltd., Tokyo, Japan) with the participant resting in the supine position, as described in a previous report (Shirai et al. 2006). Briefly, electrocardiograph electrodes were placed on both wrists, a microphone for detecting heart sounds was placed on the sternum, and cuffs were wrapped around both arms and ankles. After automatic measurements, obtained data were analyzed using VSS-10 software (Fukuda Denshi Ltd.), and values of the right and left CAVI were calculated. Averages of the right and left CAVI were used for analysis.

Dental History and Oral Examination

At baseline, subjects were interviewed and underwent a complete examination of the oral cavity administered by 1 of 2

Table 1.
Characteristics of Study Participants

Variable	Men (n = 2680)	Women (n = 5444)	All Participants (N = 8124)
Age, y	56.0 (28.9–83.1)	53.3 (27.2–79.5)	54.2 (53.9–54.5)
Tooth loss	4.3 (0–18.3)	3.2 (0–14.4)	3.6 (0–15.8)
CAVI	7.9 (5.4–10.3)	7.2 (5.2–9.3)	7.4 (5.2–9.7)
Hypertension	1,066 (39.8)	1,549 (23.5)	2,420 (29.8)
Diabetes	226 (8.4)	170 (2.6)	377 (4.6)
Former smoker	1,184 (44.2)	454 (8.3)	1,638 (20.2)
Current smoker	827 (30.9)	345 (6.4)	1,172 (14.4)
BMI, kg/m^2	23.4 (17.3–29.5)	21.8 (15.3–28.3)	22.3 (15.8–28.9)
SBP, mm Hg	128.6 (91.9–160.3)	119.4 (85.6–153.3)	122.4 (88.2–156.7)
DBP, mm Hg	79.9 (58.4–101.3)	73.5 (52.1–94.9)	75.6 (53.3–97.8)
HDL-C, mg/dL	57.7 (26.2–89.2)	68.7 (36.3–101.2)	65.1 (31.3–98.8)
LDL-C, mg/dL	123.0 (60.4–185.7)	123.8 (61.36–186.2)	123.5 (61.1–181.0)
HbA1c	5.2 (3.9–6.5)	5.1 (4.2–6.0)	5.1 (4.0–6.2)
Glucose, mg/dL	94.3 (51.9–136.7)	88.8 (63.2–114.4)	90.6 (58.1–123.2)
Antihypertensive medication	598 (22.3)	843 (15.5)	1,441 (17.7)
Hypoglycemic medication	141 (5.3)	88 (1.6)	229 (2.8)
Insulin	14 (0.5)	14 (0.3)	28 (0.3)

Values are presented as the mean (reference range: mean −2 SD to mean +2 SD) or n (%).
BMI, body mass index; CAVI, cardio-ankle vascular index; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

trained, calibrated dentists, who were randomly assigned to subjects. The same 2 dentists performed the dental examinations during the study period. During the oral examination, the number of missing teeth was counted. Congenitally missing and impacted teeth were excluded from the count of tooth loss. Third molars were excluded from counts of tooth loss, because third molars tend to be completely impacted or congenitally missing.

Statistical Analysis

Continuous variables are reported as means (reference range: mean −2 SD to mean +2 SD), and categorical variables are given as counts (percentages). The number of teeth showed right-skewed distributions and was therefore logarithmically transformed before analyses and back transformed to the original scale when presented. We examined the association between CAVI and tooth loss using general linear models with adjustment for age, sex, BMI, smoking status, HbA1c, and a history of insulin or hypoglycemic medication depending on the model, having excluded the presence of multicollinearity. In addition, the models were compared with and without interaction terms. To investigate possible effect modification with sex, age, and tooth loss, we added interaction terms using the centered variables tooth loss, sex, and age (<60 and ≥60 years). We checked the linear relationship identified in this multiple regression model by visual examination of plots of standardized residuals.

Probability values of less than 0.05 were considered indicative of statistical significance. Statistical analyses were performed using the STATA version 11 software package (Stata Corp., College Station, TX).

Results

General Characteristics

Table 1 lists demographic information of the study participants, including data for the known risk factors of arteriosclerosis and tooth loss. The mean (reference range) age of the 8124 participants was 54.2 y (27.7–80.8 y), and 67.0% were women. Men were significantly older than women (56.0 [28.9–83.1] vs. 53.3 [27.2–79.4] y) and had a high prevalence of arteriosclerosis risk factors, including hypertension, diabetes, smoking status, and obesity. CAVI values were higher for men than for women (7.9 [5.4–10.3] vs. 7.2 [5.2–9.3], respectively). The mean

Table 2.
Multivariable Linear Regression Models for CAVI and Tooth Loss Adjusted for Age, Sex, BMI, Smoking Status, Hemoglobin A1c, a History of Insulin or Hypoglycemic Medication, and Interactions

	Unadjusted Model		Adjusted Model[a]			
			Model 1		Model 2	
	β	95% CI	β	95% CI	β	95% CI
Tooth loss	0.47	(0.45 to 0.50)	0.04	(0.02 to 0.06)	0.03	(0.01 to 0.06)
Sex	−0.65	(−0.70 to −0.60)	−0.48	(−0.53 to −0.43)	−0.48	(−0.53 to −0.43)
Age	0.06	(0.06 to 0.06)	0.06	(0.05 to 0.06)	0.06	(0.05 to 0.06)
Tooth loss × sex[b]					0.05	(0.02 to 0.09)
Tooth loss × age category[c]					−0.04	(−0.08 to 0.01)

Tooth loss was logarithmically transformed.
CAVI, cardio-ankle vascular index; CI, confidence interval; BMI, body mass index.
[a]Multivariable linear regression analysis, adjusting for body mass index, smoking status, hemoglobin A1c, and a history of insulin or hypoglycemic medication.
[b]Sex (0: male, 1: female).
[c]Age category (0: <60 years, 1: ≥60 years).

(reference range) tooth loss was higher in men 4.3 (0–18.3) than in women 3.2 (0–14.3).

Association between Tooth Loss and CAVI

Table 2 shows the results of the regression modeling for the association between tooth loss and CAVI, as reported by coefficient β (β) and 95% confidence intervals (CIs). For the unadjusted analysis, a significant relationship was detected between tooth loss and CAVI (β = 0.47; 95% CI, 0.45–0.50).

Multiple regression modeling after adjustment for age, sex, BMI, smoking status, HbA1c, and a history of insulin or hypoglycemic medication was also performed (model 1, Table 2). The adjusted multiple regression analysis detected a significant positive association between CAVI and tooth loss (β = 0.04; 95% CI, 0.02–0.6). As the residuals were randomly scattered around 0 (horizontal line) and exhibited a relatively even distribution, the association between CAVI and tooth loss appeared to be linear (data not shown).

Interaction Effects of Sex, Age, and Tooth Loss

We introduced the interaction between sex, age, and tooth loss in the multiple regression analysis by examining the association between CAVI and tooth loss (model 2, Table 2). The analysis revealed that the relationship between CAVI and tooth loss differed depending on sex, with only men showing a positive correlation (β for interaction = 0.04; 95% CI, 0.02–0.6).

Discussion

The present large-scale epidemiologic study has identified that a significant positive correlation exists between tooth loss and arterial stiffness, even after adjustment for age, sex, and other confounding factors. Notably, we found an association between tooth loss and CAVI, although the association differed depending on sex. Our data provide evidence that oral disease and CVD may be positively related in men, who had higher rates of tooth loss and arterial stiffness than did women.

Previous reports examining the association between tooth loss and CVD have treated tooth loss as a nominal variable and have primarily focused on the presence or absence of CVD (Choe et al. 2009; Desvarieux et al. 2004). In contrast, here we treated both tooth loss and the primary outcome of atherosclerosis, arterial stiffness, as numeric variables. Our analysis revealed that the severity of atherosclerosis is linearly related to tooth loss, which often results from the breakdown of periodontal tissue caused by periodontal disease. As tooth loss is a marker of current and long-term cumulative effects of periodontal disease (Desvarieux et al. 2003; Houshmand et al. 2012), our findings suggest that periodontal disease may play a role in the pathogenesis of atherosclerosis progression. In this study, we excluded noninflammatory reasons for tooth loss such as traumatic or orthodontic procedures, because we considered that noninflammatory tooth loss may bias the association between tooth loss and CAVI. However, we considered that the influence of excluding noninflammatory tooth loss on the findings from this study was not significant, because the number of individuals who were excluded for noninflammatory tooth loss was small.

Several potential mechanisms have been proposed in the literature for the association between periodontal disease, including tooth loss, and CVD. The findings from animal and epidemiologic studies suggest that infectious agents, including those associated with periodontal disease, increase

inflammatory cytokine production and platelet aggregation (Herzberg and Meyer 1996), which contribute to arteriosclerosis and thrombosis. In the present study cohort, age was the most important covariate for the relationship between tooth loss and arterial stiffness. Elderly people are more likely to develop periodontal disease and ensuing tooth loss, and they have increased arterial stiffness. We detected a positive association between tooth loss and CAVI and also found that the association differed depending on sex.

We investigated the factors of sex, age, and tooth loss as potential effect modifiers and found that sex that appeared to modify the association of interest. The identified sex difference in the association between clinical periodontal disease, tooth loss, and systemic disease has several potential explanations (Desvarieux et al. 2004; Demmer et al. 2008). Findings from clinical studies and laboratory research have suggested that estrogen is associated with beneficial cardiovascular effects in women (Kannel et al. 1976; Barrett-Connor and Bush 1991) as it reduces the development of atherosclerotic plaque. However, it is also possible that oral inflammation has little or no causal relationship with arteriosclerosis in women.

Progression of atherosclerosis is closely related with increased pulse pressure (Nichols et al. 1985; Sako et al. 2009), which therefore represents an important surrogate marker of arterial stiffness. Pulse pressure is a function of SBP and DBP (pulse pressure [P] = SBP – DBP) and was incorporated into the equation used to calculate CAVI as follows: CAVI = $a\{(2\rho/\Delta P) \times \ln (SBP/DBP)PWV^2\} + b$, where ΔP = SBP – DBP, ρ is blood density, a and b are constants to match aortic PWV. Therefore, pulse pressure is an independent determinant of CAVI (Okura et al. 2007). In CAVI, the rate of increase is reportedly approximately 0.05 per year (Shirai et al. 2011). In the present study, the coefficient β of the multiple regression analysis was 0.04; the loss of 5 teeth corresponds to the amount that CAVI increases in a 4-y period. This finding suggests that the relationship between CAVI and tooth loss is clinically significant.

This study has several limitations that are inherent to cross-sectional analyses. First, the relationships reported here, while robust, should not be interpreted as causal. Our cross-sectional study design lacked information on the time sequence of events and therefore did not permit identification of causal relationships. To confirm the relationship between tooth loss and subclinical atherosclerosis, it is necessary to follow a cohort of middle-age adults until death. Second, we did not measure dietary habits. Increased tooth loss often leads to decreased masticatory performance and a change of dietary habit, which is related to risk factors of atherosclerosis, such as diabetes and hypertension. For this reason, we conducted multivariate analyses with adjustment for potential confounding factors, including hypertension and diabetes. Finally, information related to socioeconomic factors, such as education and income, were not collected in this cohort. Although previous studies have attempted to delineate the influence of socioeconomic differences on mortality, morbidity, and risk factors of disease, the Japanese population may not necessarily reflect the same pattern of relationships observed in other developed countries (Kagamimori et al. 2009). For example, an association between higher education and health is not strongly expressed among the Japanese population (Kagamimori et al. 2009; Lahelma et al. 2010). This may be partly due to the fact that a compulsory insurance system covers all people living in Japan, thereby minimizing differences in access to health care based on socioeconomic status.

In conclusion, our results suggest that the progression of atherosclerosis is linearly related to increased tooth loss and further strengthen the suggested association between these 2 factors. Notably, the age and sex differences in atherosclerosis prevalence seemed to be related to not only the distribution but also the differing contributions of oral inflammatory disease to atherosclerosis across sexes. These findings have profound clinical and public health implications, as they provide further evidence that implementing strategies for preventing periodontal disease, which is both preventable and treatable, might help prevent atherosclerosis. Preventable and treatable contributors of CVD would add to the existing options available to clinicians and public health practitioners for the control of CVD. Educating patients in methods for preventing periodontal disease and improving personal oral hygiene is expected to benefit not only their oral but also their systemic health.

Author Contributions

K. Asai, contributed to conception and design, performed the experiments, contributed to data analysis, drafted manuscript; M. Yamori, contributed to conception and design, performed the experiments, drafted manuscript, initially revised manuscript; T. Yamazaki, contributed to conception and design, performed the experiments, contributed to data analysis, initially revised manuscript; A. Yamaguchi, S. Kosugi, contributed to conception and design, critically revised manuscript; K. Takahashi, contributed to conception and design, performed the experiments, critically revised manuscript; A. Sekine, contributed to conception and design, contributed reagents/materials/tools for analysis, critically revised manuscript; F. Matsuda, T. Nakayama, contributed to conception and design, performed the experiments, initially revised manuscript; K. Bessho, contributed to conception and design, critically revised manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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Comprehensive Replication of the Relationship Between Myopia-Related Genes and Refractive Errors in a Large Japanese Cohort

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Purpose. We investigated the association between refractive error in a Japanese population and myopia-related genes identified in two recent large-scale genome-wide association studies.

Methods. Single-nucleotide polymorphisms (SNPs) in 51 genes that were reported by the Consortium for Refractive Error and Myopia and/or the 23andMe database were genotyped in 3712 healthy Japanese volunteers from the Nagahama Study using HumanHap610K Quad, HumanOmni2.5M, and/or HumanExome Arrays. To evaluate the association between refractive error and recently identified myopia-related genes, we used three approaches to perform quantitative trait locus analyses of mean refractive error in both eyes of the participants: per-SNP, gene-based top-SNP, and gene-based all-SNP analyses. Association plots of successfully replicated genes also were investigated.

Results. In our per-SNP analysis, eight myopia gene associations were replicated successfully: *GJD2*, *RASGRF1*, *BICC1*, *KCNQ5*, *CD55*, *CYP26A1*, *LRRC4C*, and *B4GALNT2*.Seven additional gene associations were replicated in our gene-based analyses: *GRIA4*, *BMP2*, *QKI*, *BMP4*, *SFRP1*, *SH3GL2*, and *EHBP1L1*. The signal strength of the reported SNPs and their tagging SNPs increased after considering different linkage disequilibrium patterns across ethnicities. Although two previous studies suggested strong associations between *PRSS56*, *LAMA2*, *TOX*, and *RDH5* and myopia, we could not replicate these results.

Conclusions. Our results confirmed the significance of the myopia-related genes reported previously and suggested that gene-based replication analyses are more effective than per-SNP analyses. Our comparison with two previous studies suggested that BMP3 SNPs cause myopia primarily in Caucasian populations, while they may exhibit protective effects in Asian populations.

Keywords: refractive error, myopia, genome-wide association study, Japanese, gene-based replication

Myopia is one of the most common ocular disorders worldwide. Recent studies reported that the prevalence of myopia is much higher in East Asian populations (40%–70%) than in Caucasian populations (20%–42%).[1–3] Additionally, the prevalence of high myopia, which could give rise to various ocular complications and lead to blindness, also is much higher in East Asian populations.[4–8] However, the regional and/or ethnic differences in the genetic background of myopia between Asians and Caucasians have not been fully investigated.

Previously, several candidate loci have been identified using family-based linkage analyses or twin studies; however, the mechanisms underlying myopia development have not been fully elucidated through these findings.[9] Research on myopia-related genetic regions has progressed greatly after genome-wide association studies (GWASs) have been performed for myopia.[10] To date, more than 10 GWASs have identified several genes associated with myopia or related phenotypes; two of these were *15q14* and *15q25*, which showed potent and consistent associations beyond regional and racial variations.[11–20]. Among them, the two largest GWASs, published in 2013 by the Consortium for Refractive Error and Myopia (CREAM) and the 23andMe database, identified as many as 51 genes that account for most of the myopia-related genes that have ever been reported by GWASs.[19,20]

In the CREAM GWAS discovery stage, 21 single-nucleotide polymorphisms (SNPs) from the Caucasian dataset and eight SNPs from the combined datasets of Caucasians and Asians showed significant associations with refractive error. Although 23andMe performed GWASs for the age of myopia onset using only Caucasians, these two studies showed remarkable overlaps in the associated SNPs and even in their effect sizes.[21] Further replication studies in different populations would narrow down

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Method	Target	Comment	Range of interest	Significant P-value
Per-SNP replication	Purple circle in B (SNP)	Reported SNP	Single SNP	0.05
Gene-based top-SNP analysis	Purple circle in C (SNP)	Top SNP within the gene	Gene ±10kb	0.05/Number of tag-SNPs
Gene-based all-SNP analysis	All SNPs within the gene	Using VEGAS software	Gene ±50kb	0.05

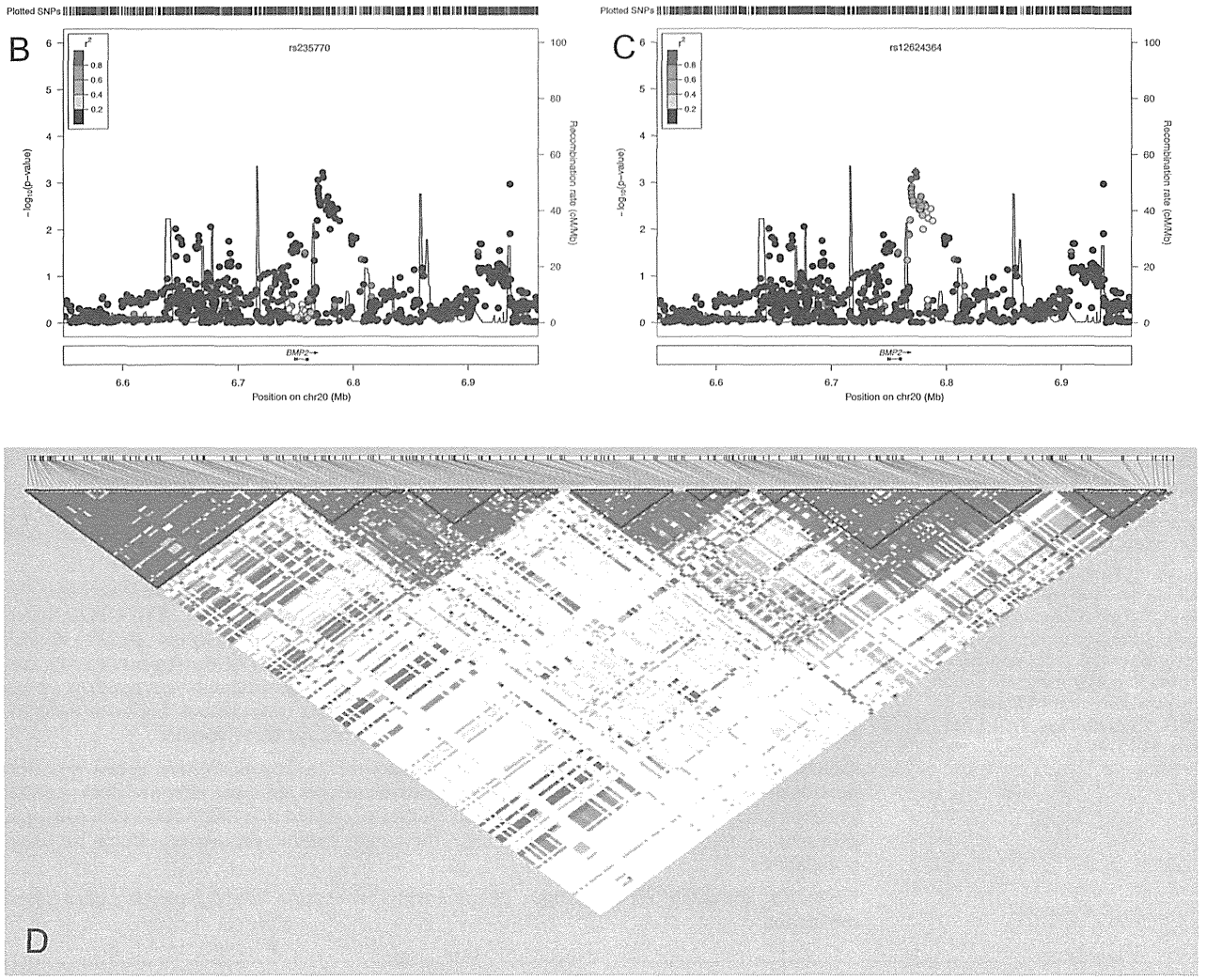

FIGURE 1. Description and illustration of three replication methods used in our analysis, using *BMP2* as an example. (**A**) Definitions of the three methods are summarized. (**B**, **C**) Association plots of the SNPs near *BMP2* in our dataset, showing the target SNPs of per-SNP analysis (**B**) and gene-based top-SNP analysis (**C**). Genetic regions ± 200 kb are shown in each plot. (**D**) An LD plot within the genetic region ± 50 kb of BMP2, comprised of 240 SNPs in our dataset. Totals of 17 haplotype blocks and 19 SNPs were not included in any of the blocks. Thus, the number of tag-SNPs was counted to be 36 (= 17 + 19). The top SNP of BMP2 that is shown in (**C**) should be corrected by the number of tag-SNPs and a P value of < 0.0014 (= 0.05/36) would be significant for gene-based top-SNP analysis.

the target genes and help elucidate variable genetic backgrounds in myopia across ethnicities.

In this study, we analyzed myopia-related genes that were reported by these two GWASs as disease-susceptible polymorphisms related to refractive error in a relatively large Japanese cohort. We analyzed 51 genes, even ones with marginal significance or without successful replication in their dataset, so that the genes that contribute dominantly to Asian myopia would not be eliminated. In addition, three replication methods including gene-based approaches were performed to avoid excluding the genes by the heterogeneous distribution of SNP associations or different linkage disequilibrium (LD) patterns across ethnicities.

METHODS

All procedures used in this study adhered to the tenets of the Declaration of Helsinki. The institutional review board and ethics committee of Kyoto University Graduate School and the Faculty of Medicine Ethics Committee, the Ad Hoc Review Board of the Nagahama Cohort Project, and the Nagahama Municipal Review Board of Personal Information Protection approved the protocols

TABLE 1. Characteristics of the Study Population According to Age

	30–39 y	40–49 y	50–59 y	60–69 y	70–75 y	Total
Patients, *n*	1047	367	518	874	276	3082
Age,* y	34.60 ± 2.76	44.24 ± 2.87	55.17 ± 2.88	64.4 ± 2.93	72.2 ± 1.57	51.02 ± 14.09 (30–5)
Sex, *n* (%)						
Male	294 (28.1)	124 (33.8)	152 (29.3)	338 (38.7)	121 (43.8)	1029 (33.4)
Female	753 (71.9)	243 (66.2)	518 (70.7)	536 (61.3)	155 (56.2)	2053 (66.6)
MSE,* D (range)	−2.63 ± 2.53	−2.75 ± 2.82	−1.84 ± 2.81	−0.75 ± 2.52	0.20 ± 2.28	−1.72 ± 2.78
	(−13.25–7.44)	(−15.38–2.19)	(−15.69–6.69)	(−14.81–4.38)	(−13.31–4.63)	(−15.69–7.44)
Right eyes	−2.66 ± 2.58	−2.82 ± 2.90	−1.90 ± 2.89	−0.78 ± 2.60	0.18 ± 2.44	−1.76 ± 2.85
Left eyes	−2.59 ± 2.52	−2.68 ± 2.79	−1.78 ± 2.84	−0.71 ± 2.56	0.22 ± 2.40	−1.68 ± 2.80
AL,* mm (range)	24.51 ± 1.30	24.54 ± 1.40	23.98 ± 1.37	23.70 ± 1.19	23.41 ± 1.11	24.10 ± 1.34
	(20.47–28.92)	(21.92–28.99)	(21.03–29.80)	(21.07–28.37)	(21.29–28.83)	(20.47–29.80)
Right eyes	24.53 ± 1.32	24.57 ± 1.44	24.00 ± 1.38	23.72 ± 1.21	23.42 ± 1.14	24.12 ± 1.36
Left eyes	24.48 ± 1.30	24.51 ± 1.37	23.96 ± 1.40	23.69 ± 1.20	23.39 ± 1.11	24.08 ± 1.35

* Age, MSE, and AL are shown in mean ± SD.

of this study. All participants were fully informed of the purpose of and procedures involved in this study, and written informed consent was obtained from each participant.

Study Populations

The individuals studied were healthy Japanese volunteers enrolled in the Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience dataset (The Nagahama Study, $n = 9809$). This community-based prospective multi-omics cohort study has been described in detail previously.[22,23] This cohort was recruited from the general population living in Nagahama City, a large rural city of 125,000 inhabitants in the Shiga Prefecture, located in the center of Japan. All participants voluntarily joined the study, which resulted in the difference in the number of participants of each sex. All eligible participants were included in this study and underwent ophthalmic evaluations: automatic objective refractometry and corneal curvature calculation (Autorefractor ARK-530; Nidek, Tokyo, Japan), axial length (AL) measurement (IOL Master; Carl Zeiss, Jena, Germany), and fundus photography using a digital retinal camera (CR-DG10; Canon, Tokyo, Japan) in a darkened room.[24] History of cataract surgery, ocular surgery other than cataract surgery, and ocular laser treatment including photocoagulation were obtained through a questionnaire. Anthropometric parameters and genomic information also were available. We excluded participants with history of any intraocular procedures that could distort the mean spherical equivalent (MSE). Only participants with analyzable spherical equivalent refraction in both eyes were included in this study.

Genotyping and Imputation

The DNA samples were prepared and genotyped as described previously.[25] Briefly, 3712 samples were genotyped using at least one of the three genotyping platforms, HumanHap610K Quad Arrays, HumanOmni2.5M Arrays, or HumanExome Arrays (Illumina, Inc., San Diego, CA, USA). To ensure high-quality genotype data, a series of quality control (QC) filters were applied to the data in each platform: sample success rate (>90% for HumanHap610K Arrays, >95% for HumanOmni2.5M Arrays, and >99% for HumanExome Arrays), individual call rate (>99%), minor allele frequency (MAF) cutoffs (>0.01), *P* value for the Hardy-Weinberg test of equilibrium ($>1 \times 10^{-6}$), and estimated relatedness (PI-HAT < 0.35). After these preliminary QC procedures were performed using PLINK (ver. 1.07; available in the public domain at http://pngu.mgh.harvard.edu/~purcell/plink/), SNP genotype imputation was conducted for these samples using the MaCH program (version 1.0.10; available in the public domain at http://www.sph.umich.edu/csg/abecasis/MACH/) with 500 Markov sampler rounds and 200 haplotype states.[26] Genotypes of East Asian samples in the 1000 Genomes Project (release3) were set as reference sequences and standard QC was applied again to the postimputed dataset (sample success rate [>90%], individual call rate [>90%], MAF cutoffs [>0.01], and HWE *P* value [$>1 \times 10^{-7}$]). The SNPs with low imputation quality ($r^2 < 0.5$) were excluded from the following association analysis.

Myopia-Related Genes and SNPs and the Methods Used for Replication

From the previously reported results for myopia in the two largest GWASs, we included 51 genes that showed associations in at least one GWAS, even without successful replication in their dataset. These genes included 61 SNPs (henceforth referred to as "myopia-related genes and SNPs"). To replicate these myopia-related genes and SNPs, we conducted GWAS for

TABLE 2. Characteristics of the Study Population According to Sex

	Male	Female	P†
Patients, *n*	1029	2053	
Age,* y	53.02 ± 14.24	50.02 ± 13.91	<0.001
MSE,* D (range)	−1.56 ± 2.68 (−14.75–7.44)	−1.80 ± 2.82 (−15.69–6.69)	0.023
Right eyes	−1.59 ± 2.75	−1.85 ± 2.90	0.020
Left eyes	−1.53 ± 2.71	−1.75 ± 2.83	0.039
AL,* mm (range)	24.37 ± 1.32 (21.06–9.80)	23.96 ± 1.33 (20.47–8.99)	<0.001
Right eyes	24.39 ± 1.35	23.98 ± 1.35	<0.001
Left eyes	24.34 ± 1.31	23.94 ± 1.34	<0.001

* Age, MSE, and AL are shown in mean ± SD.
† Student's *t*-test.

MSE refraction of both eyes using our dataset. These association results were adapted to the replication analysis in three different approaches: one was SNP-based replication and the others involved gene-based replications. Each method is illustrated in Figure 1. For the per-SNP replication method, we directly examined myopia-related SNPs or SNPs with complete LD ($r^2 = 1$) in our dataset. The LD between associated SNPs and SNPs from 1000 Genomes Pilot 1 of CHB/JPT was calculated using the SNAP software (available in the public domain at http://www.broadinstitute.org/mpg/snap/ldsearch.php). A *P* value of <0.05 was considered statistically significant. The SNPs were excluded from this analysis if neither the original SNP nor the SNP with complete LD was included in our dataset. For gene-based replications, we conducted two methods: one was gene-based top-SNP replication and the other was gene-based all-SNP replication reflecting association signals of all SNPs. For gene-based top-SNP replication, we selected SNPs that showed the strongest association for MSE within each genetic region ±50 kb of myopia-related genes. The *P* value was multiplied by the number of tagging SNPs and a corrected *P* value of <0.05 was considered statistically significant. All of the imputed SNP genotypes in our dataset were imported into Haploview 4.2 to obtain the r^2- and D'-based LD plots for each genetic region. Haplotype blocks were defined by the confidential blocks and the number of tagging SNPs was manually counted from these LD plots.[27] For gene-based all-SNP replication, we used the VEGAS software (available in the public domain at http://gump.qimr.edu.au/VEGAS/) that incorporated information from all SNPs within each genetic region ±50 kb.[28] Gene associations with MSE were calculated from the list of SNPs and their *P* values in our dataset. This software provides powerful information on whether multiple risk variants exist within a gene.[29,30]

Statistical Analysis

The associations between MSE and SNP genotypes were analyzed as a quantitative trait using linear regression analysis in PLINK, assuming additive regression models with adjustment for age, sex, and principal components. Statistical significance of each replication method was assessed as stated above. Deviations from the Hardy-Weinberg equilibrium (HWE) in genotype distributions were assessed using the HWE exact test. We further highlighted regional association signals near the replicated genes to visualize the effect of different LD across ethnicities using LocusZoom.[31]

RESULTS

A total of 3655 individuals passed a series of QC filters after genotyping, and 3082 individuals were analyzed, excluding those with conditions as stated above. The evaluated genomic variances were 6,746,251 SNPs after imputation and QC. The demographics of the study population are shown in Table 1. The age of the subjects ranged from 30 to 75 years, with spherical equivalent refraction ranging from −15.38 to +7.44 diopters (D) with an MSE of −1.69 ± 2.78 D. Subgroup analysis of MSE by age suggested that the refractive status could be shifted to a hyperopic state in older populations. In addition, female subgroups had significantly ($P = 0.023$) higher myopic refraction compared to male subgroups (Table 2), suggesting that the analysis should be performed with adjustment for age and sex in the linear regression analysis. The genomic inflation factor (λ) in our cohort was 1.055 after including the first two principal components as covariates, suggesting proper adjustment for population stratification.

In the per-SNP replication of the 61 myopia-related SNPs, 16 SNPs were not available in our dataset. Of those, 13 (81%) showed extremely low MAF (≤0.0056) in JPT samples of the 1000 Genomes database build 37 (Supplementary Table S1). We analyzed 45 originally reported myopia-related SNPs and one SNP (rs4458448 in the BMP3 region) that showed complete LD ($r^2 = 1$) to the original SNP (rs1960445 in the BMP3 region), and found that 11 SNPs in nine genetic regions showed $P < 0.05$ for the association with MSE (Table 3 and Supplementary Table S2). In the BMP3 region, rs4458448 did not show significant association with MSE, while rs5022942 had a significant *P* value of 0.0496. However, the association direction of rs5022942 was opposite to the original SNP results and we did not regard *BMP3* as significantly replicated (Supplementary Table S3). In the gene-based top-SNP replication, 12 genetic regions showed $P < 0.05$ after Bonferroni correction by the number of tagging SNPs (Table 4). In the gene-based all-SNP replication study performed using VEGAS software, eight genes showed $P < 0.05$ (Table 5). A total of 15 genetic regions showed $P < 0.05$ in at least one of the three analyses and were considered to be myopia-associated genes in the Japanese (Table 6). Among these, genetic regions near *KCNQ5, GJD2, RASGRF1, BICC,* and *CD55* showed $P<0.05$ in all analyses, and regions near *BMP4, SH3GL2,* and *B4GALNT2* showed $P < 0.05$ in two of the three analyses. Our findings were compared to the results of two previous GWASs in Supplementary Table S4. Association plots of the eight genes that were replicated by per-SNP replication are shown in Figure 2. Three of them showed peak association signals with high LD in the originally reported SNPs (Fig. 2A), while the other five genes did not (Fig. 2B). Figure 3 shows association plots of seven genetic regions that were only replicated by gene-based analyses and failed to be replicated by per-SNP analysis. Peak association signals and the originally reported SNPs had separated chromosomal positions in our dataset. We further evaluated the effect of different LD structures on the