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Single nucleotide polymorphism determines walking speed in later life among community-dwelling Japanese women.

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Aims: Slow walking speed in older subjects was reported to be associated with an increased risk of cardiovascular mortality. On the other hand, minor allele frequency of the single nucleotide polymorphism (SNP) rs3782886 is reported to be positively associated with coronary artery disease. Therefore, the rs3782886 genotype might be associated with walking speed later in life. However, no studies have reported on the influence of rs3782886 on walking speed in elderly subjects.

Methods: To evaluate the influence of SNP rs3782886 on walking speed in later life, we conducted a cross-sectional study of 562 elderly women aged 60 years and over who had undertaken a general health check-up between 2014 and 2015. Faster walking speed was defined by a questionnaire which asked, "Do you walk faster than your contemporaries?" (yes, no).

Results: Of the total study population, with regard to the rs3782886 genotype, 356 subjects showed major homo (A/A), 177 hetero (A/G) and 29 minor homo (G/G). Independent of known cardiovascular risk factors, with major homo as the reference group, the adjusted odds ratio and 95% confidence interval for faster walking speed were 0.92 (0.54, 1.58) for hetero and 0.39 (0.16, 0.97) for minor homo.

Conclusion: Independent of classical cardiovascular risk factors, the SNP rs3782886 was found to be associated with faster walking speed, as defined by a questionnaire, in elderly Japanese women. This result represents an efficient tool to clarify the mechanism of rs3782886 as a risk factor for cardiovascular disease.

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Key words: elderly women, rs3782886, SNP, walking speed

Introduction

A previous prospective study of 3,208 community-recruited elderly men and women aged ≥65 reported a strong association between slow walking speed and increased risk of cardiovascular mortality [1]. Additionally, a Nurse's Health Study of 13,535 women reported an association between increased energy expenditure from walking and increased survival [2].

On the other hand, minor allele frequency of SNP rs3782886 is reported to be positively associated with the risk of coronary artery disease [3]. Since coronary artery disease is a major life threatening condition, rs3782886 may be associated with walking speed later in life. SNP rs3782886 is likely to present only in Asian populations according to HapMap data [4], which has been described in detail elsewhere [5]. SNP rs3782886 does not cause amino acid substitutions but instead reduces gene transcriptional activity [3], and the minor alley frequency of rs3782886 in the Japanese population is reported to be

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29.3% [6]. Therefore, SNP rs3782886 could have an influence on waking speed particularly in Asian populations such as the Japanese.

However, no studies have clarified the association between rs3782886 and walking speed in the general elderly population. We therefore conducted a cross-sectional study of 562 elderly women aged 60 years and over who had undertaken a general health check-up between 2014 and 2015.

Materials and Methods

Subjects and methods

This study was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences (project registration number 14051404). Written consent forms were available in Japanese to ensure comprehensive understanding of the study objectives, and informed consent was provided by the participants. The study population comprised 623 women residents aged ≥60 years from the western rural communities of the Goto Islands, who undertook an annual medical check-up from 2014 and 2015 as recommended by the Japanese government.

To avoid the influence of chronic disease and malnutrition, subjects with a low body mass index (BMI) (<19.0kg/m^2) (n=61) were excluded, leaving 562 subjects with a mean age of 73.1 years (standard deviation (SD): 7.4; range: 60-92) enrolled in the study.

Data collection and laboratory measurements

Trained interviewers obtained information on clinical characteristics. Body weight and height were measured with an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan), and BMI (kg/m^2) was calculated. Systolic and diastolic blood pressure were recorded at rest. Triglycerides (TG) and creatinine were measured enzymatically. HDL-cholesterol (HDL) was measured using a direct method, while hemoglobin A1c (HbA1c) was measured using the latex coagulation method at SRL, Inc. (Tokyo, Japan). Glomerular filtration rate (GFR) was estimated by using an established method, but with three variations recently proposed by a working group of the Japanese Chronic Kidney Disease initiative [7]. According to this adaptation, GFR (ml/min/1.73 m^2) = 194× (serum creatinine (enzyme method))$^{-1.094}$×(age)$^{-0.287}$×(0.739 for women). Genomic DNA was extracted from 2ml of whole peripheral blood with GENE PREP STAR NA-480 (KURABO). Subject DNA was typed for SNP rs3782886 (BRAP on chromosome 12q24.12) using the HybProbe method with LightCycler 480 (Roshe). Faster walking speed was defined by a questionnaire which asked, “Do you walk faster than your contemporaries?” (yes, no).

Statistical analysis

Clinical characteristics of the SNP rs3782886 genotype were compared. Differences in mean values or prevalence of potential confounding factors based on SNP rs3782886 genotypes were calculated. P values were calculated with a regression model for mean values, and a logistic regression model was used for proportion.

Logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to determine the influence of SNP rs3782886 on faster walking speed.

Adjustments for confounding factors were made into three models. In the first model (Model 1), we adjusted only for age. In the second model (Model 2), we included other possible confounding factors, that is, BMI (kg/m^2), smoking status (never-smoker, former smoker, current smoker), alcohol consumption [never-drinker, former drinker, current drinker (<23g/week, 23g/week≤ <46g/week, 46g/week≤ <69g/week, 69g/week≤)], systolic blood pressure (mmHg), serum triglycerides (mg/dL), serum HDL-cholesterol (mg/dL), HbA1C (%) and GFR (ml/min/1.73 m^2). And in Model 3, we made further adjustment for history of ischemic heart disease and stroke, since both rs3782886 and walking speed are likely associated with cardiovascular disease, which may influence the association between those two factors. All statistical analyses were performed with the SAS system for Windows (version 9.4; SAS Inc., Cary, NC). All p-values for statistical tests were two-tailed, with values of <0.05 regarded as being statistically significant.

Results

Characteristics of the present study population are shown in Table 1. Current drinker status was found to be significantly associated with genotype.

Table 2 shows the OR and 95% CI for faster walking speed in relation to rs3782886 type. Independent of known cardiovascular risk factors, rs3782886 minor was inversely associated with faster walking speed. Compared with non-minor homo (A/A and A/G), the fully-adjusted OR and 95% CI of faster walking speed for minor homo (G/G) was 0.39 (0.16, 0.95).

Table 1. Characteristics of the study population by rs3782886 genotype

	rs3782886			p value
	major homo (A/A)	hetero (A/G)	minor homo (G/G)	
No. of participants	356	177	29	
Age, years	72.2 ± 7.4	72.8 ± 7.4	72.5 ± 7.3	0.657
Body mass index (BMI), kg/m^2	23.5 ± 2.9	24.0 ± 3.3	22.8 ± 2.7	0.093
Systolic blood pressure, mmHg	140 ± 18	141 ± 18	138 ± 15	0.779
Diastolic blood pressure, mmHg	81 ± 11	80 ± 11	80 ± 10	0.599
Current drinker, %	14.6	6.2	0.0	0.002
Current smoker, %	1.4	1.1	0.0	0.796
History of ischemic heart disease, %	7.0	7.3	0.0	0.328
History of stroke, %	2.0	4.0	6.9	0.174
Serum triglycerides (TG), mg/dL	108 ± 63	109 ± 56	80 ± 34	0.239
Serum HDL-cholesterol (HDL), mg/dL	62 ± 16	60 ± 16	65 ± 19	0.284
Hemoglobin A1c (HbA1c), %	5.7 ± 0.5	5.6 ± 0.4	5.7 ± 0.4	0.274
Serum creatinine, mg/dL	0.67 ± 0.14	0.70 ± 0.15	0.66 ± 0.11	0.130
Glomerular filtration rate (GFR), mL/min/1.73m^2	67.7 ± 14.0	65.1 ± 13.8	68.0 ± 13.1	0.127

values: mean ± standard deviation.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for faster walk speed in relation to rs3782886 genotype.

<table>
<tr><th rowspan="3"></th><th colspan="3">rs3782886</th><th rowspan="3"><i>P</i></th></tr>
<tr><th colspan="2">non-minor homo</th><th rowspan="2">minor homo (G/G)</th></tr>
<tr><th>major homo (A/A)</th><th>hetero (A/G)</th></tr>
<tr><td>No. at risk</td><td>356</td><td>177</td><td>29</td><td></td></tr>
<tr><td>No. of cases (percentage)</td><td>312 (87.6)</td><td>151 (85.3)</td><td>21 (72.4)</td><td></td></tr>
<tr><td>Model.1</td><td>1.00</td><td>0.83 (0.49, 1.40)</td><td>0.37 (0.16, 0.89)</td><td>0.057</td></tr>
<tr><td></td><td colspan="2">1.00</td><td>0.40 (0.17, 0.93)</td><td>0.034</td></tr>
<tr><td>Model.2</td><td>1.00</td><td>0.92 (0.54, 1.58)</td><td>0.39 (0.16, 0.97)</td><td>0.128</td></tr>
<tr><td></td><td colspan="2">1.00</td><td>0.41 (0.17, 0.98)</td><td>0.044</td></tr>
<tr><td>Model.3</td><td>1.00</td><td>0.92 (0.54, 1.59)</td><td>0.38 (0.15, 0.94)</td><td>0.122</td></tr>
<tr><td></td><td colspan="2">1.00</td><td>0.39 (0.16, 0.95)</td><td>0.038</td></tr>
</table>

Model.1: adjusted only for age. Model.2: further adjusted for body mass index, systolic blood pressure, alcohol consumption, smoking status, triglycerides, HDL-cholesterol, HbA1c and GFR. Model. 3: Model.2 + further adjusted for history of ischemic heart disease and stroke.

Discussion

The major finding of the present study showed that independent of classical cardiovascular risk factors, the SNP rs3782886 minor homo genotype is significantly inversely associated with faster walking speed in elderly Japanese women.

The underlying mechanism in which SNP rs3782886 bestows a disadvantage in walking speed in elderly women has not yet been clarified. SNP rs3782886 is known to be located in the Breast Cancer Suppressor Protein Associated Protein (BRAP) gene on chromosome 12q24. And higher expression of BRAP with a minor allele (G allele) is associated with increased risk of atherosclerosis [8] by enhancing the degree of inflammation through activation of the NF-κB protein [9]. Therefore, the minor allele of rs3782886 is associated with coronary artery disease [3]. Since coronary artery disease attributable to atherosclerosis is a leading cause of mortality in many countries [10] and slow walking speed in the elderly is strongly associated with an increased risk of cardiovascular mortality [1], rs3782886 genotype is likely associated with walking speed later in life. Studies showing that inflammation induces slow gait speed in elderly subjects [11], and the activation of inflammatory cascades by BRAP [8] also support the above-mentioned mechanism.

Since walking is a well-known aerobic exercise, reduced hematopoietic activity should also result in a lower walking speed. In fact, we found that anemia (defined as hemoglobin <11 g/dL) is significantly inversely associated with a faster walking speed (fully adjusted OR and 95%CI of faster walking speed for anemic subjects is 0.35(0.14, 0.89). Additionally, although statistical power did not reach significance, SNP rs3782886 minor homo is positively associated with anemia; with non-minor homo (A/A and A/G) as the reference group, the fully adjusted OR and 95%CI of anemia was 1.92 (0.38, 9.74) for minor homo (G/G). Anemia is common in patients with acute coronary syndrome [12], while the minor allele frequency of SNP rs3782886 is associated with coronary artery disease [3]. These studies also might partly support the aforementioned mechanism. Further investigation with a larger study population is necessary.

Previously, we reported that SNP rs3782886 minor homo is significantly inversely associated with reduced tongue pressure in the elderly [13]. Additionally, reduced tongue pressure is associated with sarcopenia [14]. The present study showed that tongue pressure is significantly positively associated with faster waking speed; the 1 standard deviation increment in tongue pressure (9.9 kPa) for faster waking speed was 1.79 (1.23, 2.61) for Model 1, 1.70 (1.14, 2.55) for Model 2 and 1.71 (1.14, 2.57) for Model 3. Therefore, sarcopenia also might influence the association between the present SNPs and faster walking speed.

Although the minor allele of SNP rs3782886 is reported to be positively associated with coronary artery disease [3], the background mechanism of this association is not yet clear. In the present study, we established that SNP rs3782886 minor homo is inversely associated with a faster walking speed. Since slow walking speed in the elderly is strongly associated with increased risk of cardiovascular mortality [1], our present finding should serve as an efficient tool to clarify the mechanism of SNP rs3782886 as a risk factor for cardiovascular disease.

One potential limitation of this study is that walking speed was defined solely by a questionnaire. Further studies using actual walking speed will be necessary. Additionally, no information on dementia was available in the present study; therefore, we could not evaluate the influence of dementia despite its possible association with gait abnormality [15]. Finally, since this study included only 15 male minor homo subjects, and all of these subjects are defined as having a faster waking speed, we could not evaluate the association between SNP rs3782886 and walking speed in men. Further investigation using a larger study population will be necessary.

Conclusion

In conclusion, independent of classical cardiovascular risk factors, SNP rs3782886 minor homo is significantly inversely associated with a faster walking speed in the elderly.

Competing interests

The authors declare no conflicts of interest.

Human and animal rights and informed consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics Committee for Human Use of Nagasaki University obtained ethical approval.

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References

1. Dumurgier J, Elbaz A, Ducimetière P, Tavernier B, Alpérovitch A, Tzourio C. Slow walking speed and cardiovascular death in well functioning older adults: prospective cohort study. BMJ. 2009;339:4460.
2. Sun Q, Townsend MK, Okereke OI, Franco OH, Hu FB, Grodstein F. Physical activity at midlife in relation to successful survival in women at age 70 years or order. Arch Intern Med 2010;170:194-201.
3. Ozaki K, Sato H, Inoue K, et al. SNPs in BRAP associated with risk of myocardial infarction in Asian populations. Nat Genet. 2009;41:329-333.
4. NCBI retiring HapMap Resource [Home page on the Internet]. [Cited January 5, 2018] Available from: http://www.hapmap.org
5. Thorisson GA, Smith AV, Krishnan L, Stein LD. The International HapMap Project Web site. Genome Res. 2005; 15:1592-1593.
6. Yamada Y, Sakuma J, Takeuchi I, et al. Identification of polymorphisms in 12q24.1, ACAD10, and BRAP as novel genetic determinants of blood pressure in Japanese by exome-wide association studies. Oncotarget 2017;8:43068-43079.
7. Imai E. Equation for estimating GFR from creatinine in Japan. Nihon Rinsho. 2008; 66: 1725-1729. [Article in Japanese]
8. Liao YC, Wang YS, Guo YC, et al. BRAP activates inflammatory cascades and increases the risk for carotid atherosclerosis. Mol Med 2011;17:1065-1074.

9. Karin M, Delhase M. The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling. Semin Immunol. 2000;12:85-98.
10. Breslow JL. Cardiovascular disease burden increases, NIH funding decreases. Nat Med. 1997;3:600-601.
11. Brown PJ, Roose SP, Zhang J, et al. Inflammation, depression, and slow gait: A high mortality phenotype in later life. J Gerontol A Biol Sci Med Sci. 2016;71:221-227.
12. Bhavanadhar P, Srinivasan VR, Oruganti SS, Adiraju KP. A prospective study on prevalence and causes of anaemia in patients with acute coronary syndrome. J Clin Diagn Res 2016;10:OC01-5.
13. Shimizu Y, Sato S, Noguchi Y, et al. Impact of single nucleotide polymorphism on short stature and reduced tongue pressure among community-dwelling elderly Japanese participants: a cross-sectional study. Environ Health Prev Med. 2017;22:62.
14. Maeda K, Akagi J. Decreased tongue pressure is associated with sarcopenia and sarcopenic dysphagia in the elderly. Dysphagia 2015;30:80-87.
15. Verghese J, Lipton RB, Hall CB, et al. Abnormality of gait as a predictor of non-Alzheimer's dementia. N Engl J Med. 2002;347:1761-1768.