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Original article

Gene and dietary calcium interaction effects on brachial–ankle pulse wave velocity

SeongIl Choi [a], Sukyoung Jung [b, c], Mi Kyung Kim [b, c, *], Jinho Shin [d], Min-Ho Shin [e, f], Dong Hoon Shin [g], Young-Hoon Lee [h], Byung-Yeol Chun [i], Kyung-Won Hong [j], Joo-Yeon Hwang [j]

[a] Department of Cardiology, Guri Hospital, College of Medicine, Hanyang University, Guri, South Korea
[b] Department of Preventive Medicine, College of Medicine, Hanyang University, Seoul, South Korea
[c] Institute for Health and Society, Hanyang University, Seoul, South Korea
[d] Division of Cardiology, Department of Internal Medicine, Hanyang University, College of Medicine, Seoul, South Korea
[e] Department of Preventive Medicine, Chonnam National University Medical School, Gwangju, South Korea
[f] Jeonnam Regional Cancer Center, Chonnam National University Hwasun Hospital, Hwasun, South Korea
[g] Department of Preventive Medicine, School of Medicine, Keimyung University, Daegu, South Korea
[h] Department of Preventive Medicine, Institute of Wonkwang Medical Science, Wonkwang University College of Medicine, Iksan, South Korea
[i] Department of Preventive Medicine, School of Medicine, Health Promotion Research Center, Kyungpook National University, Daegu, South Korea
[j] Center for Genome Science, National Institute of Health, Osong Health Technology Administration Complex, Chungcheongbuk-do, South Korea

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SUMMARY

Background & aim: Understanding the lifestyle and genetic factors that affect pulse wave velocity (PWV) may provide clues to preventing atherosclerotic cardiovascular events. The aim of this study is to investigate genome-wide genetic and dietary calcium (Ca) intake interaction effects on brachial–ankle pulse wave velocity (baPWV).

Methods: The baPWV was measured, and Ca intake was quantified by administering a food frequency questionnaire (FFQ) to 3198 participants, which included men and women (≥40 years) from the Korean **M**ulti-**R**ural communities **Cohort** study (MRCohort). The interaction effects of dietary Ca intake and 19 single-nucleotide polymorphisms (SNPs) on baPWV were assessed using the general linear models.

Results: Dietary Ca intake was not significantly associated with baPWV or any type of SNP among the subjects herein. In men, however, the *adducin1 (ADD1)* rs4961_C SNP had a significant dietary Ca intake –dependent effect on mean baPWV ($p_{interaction} = 0.002$). In women, the interaction of *zinc finger proteins 618 (ZNF618)* rs10817542_A with dietary Ca intake played a significant and key role in mean baPWV ($p_{interaction} = 0.001$). In the results of *ADD1* rs4961_C in men and *ZNF618* rs10817542_A in women, the minor allele-lowest Ca intake tertile (T1) group had significantly higher mean baPWV value than other subgroups of Ca intake tertile-genotype cross-classification whereas genotype was not a significant effector on mean baPWV values among highest Ca intake subgroups (T3).

Conclusions: The baPWV, a phenotype of arterial stiffness, can be modulated in subjects through regulation of dietary Ca intake, particularly in subjects with more vulnerable genotypes.

1. Introduction

Pulse wave velocity (PWV) is widely recognized as a noninvasive index of central arterial stiffness, which is associated with atherosclerosis. Pulse wave velocity is increased in various atherosclerotic cardiovascular diseases (CVDs) [1]. Additionally, because PWV also acts on the independently potent predictor of cardiovascular mortality and morbidity in healthy subjects [2] and the general population [3], PWV is now considered the gold standard noninvasive method for assessing the arterial stiffness in the research field of atherosclerotic CVD [4,5]. Thus, an understanding of the various types of factors that affect PWV may provide important clues to preventing the occurrence of atherosclerotic

* Corresponding author. Department of Preventive Medicine, Medical School Building A-Room 517-2, College of Medicine, Hanyang University, 17 Haengdang Dong, Sungdong Gu, Seoul 133-791, South Korea. Tel.: +82 2 2220 0667; fax: +82 2 2293 0660.
E-mail address: kmkkim@hanyang.ac.kr (M.K. Kim).

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cardiovascular events, slowing their progression, and improving prognosis.

Vascular alterations are influenced by "extrinsic factors" such as hormones, salt, and glucose regulation [6], as well as by "genetic factors" [1,5]. Among extrinsic factors, the ingestion of high levels of calcium (Ca) in drinking water has been reported to decrease atherosclerosis [7–9], and a reduction in coronary artery disease mortality was also observed with high Ca intake in both the Iowa Women's Health Study [10] and a cohort of Swedish men [11]. Moreover, based on the negative correlation between PWV and radial bone mineral density (BMD), adequate dietary Ca intake is recommended to prevent aortic calcification [12].

While it is true that the mechanisms of dietary Ca intake effects on atherosclerosis and atherosclerotic cardiovascular events are difficult to elucidate and that the relationship of arterial stiffness to BMD has not been clearly established, significant aspects of these relationships remain unexplained due to the interaction of genetic factors with dietary Ca intake in subjects [1,13].

Studies have been conducted to determine which genetic factors modulate the variability in PWV by using various tools such as gene expression studies, heritability, linkage analyses, and genome-wide association studies [1]. Recent genome-wide association studies have identified genetic polymorphisms that are associated with increased arterial stiffening [1,5]. Furthermore, in terms of significant differences in genetic susceptibilities to chronic complex diseases such as atherosclerosis and CVD [14], it is increasingly recognized that genetic susceptibility to arterial stiffening is influenced by lifestyle (e.g., dietary salt consumption, Ca, micronutrients, and exercise) [1].

To the best of our knowledge, no existing study had investigated the interaction effects of dietary Ca intake and genetic polymorphisms on PWV, a noninvasive index of central arterial stiffness. Therefore, the aim of this study is to identify the single-nucleotide polymorphisms (SNPs) associated with brachial–ankle pulse wave velocity (baPWV) through interaction with dietary Ca intake.

2. Subjects and methods

2.1. Study population

This study population was participants of the Korean **M**ulti-**R**ural communities Cohort Study (MRCohort), as a part of the **Ko**rean **G**enome **E**pidemiology **S**tudy (KoGES). The Korean Multi-Rural communities Cohort was initiated to investigate risk factors for CVDs and 8702 subjects who aged ≥40 years at enrollment were recruited from three centers located in Yangpyeong (in the middle part of Korea), Namwon (in the southwestern part), and Goryeong (in the southeastern part) between 2005 and 2008. Villages within each site were selected by multistage cluster sampling and then the district leaders such as heads of villages encouraged residents, who were on the list of households, to participate. Most participants were housewives and farmers.

At first, the dataset of **CA**rdio**V**ascular disease **A**ssociation **S**tudy (CAVAS-G1) was adopted to undertake this large-scale genome-wide association study analysis. Among 8702 participants, we excluded subjects with history of heart disease, cerebrovascular disease, type 2 diabetes, hyperlipidemia, or cancer and those receiving antihypertensive medication on the self-reported questionnaire. Also those with systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg on the measurement of blood pressure and those with fasting plasma glucose ≥ 126 mg/dL were excluded from study population. As a result, only 4052 subjects were eligible for genotyping. Among them, 3626 subjects passed the genotype calling and quality control process. Additionally, subjects who reported implausible dietary intakes (<500 or >4000 kcal/d, n = 15), missing above 10 food items or values of cooked rice (n = 8), missing data of alcohol intake (n = 4), smoking status (n = 3), anthropometric measurements (n = 16), education (n = 10), exercise (n = 26), or baPWV (n = 346) were additionally excluded. Ultimately, 3198 subjects (1175 men and 2023 women) were included in the analysis.

This study protocol was approved by the Institutional Review Boards of Korea National Institute of Health. All participants were informed of the study contents and the written consent for use of their data. Written informed consent was obtained from all subjects.

2.2. Assessment of general characteristics

In order to overcome the limitations of a multi-center study and to collect uniform data at the 3 study centers, standard protocols for each questionnaire survey and examination procedures were developed and the same personnel from the coordinating center trained all interviewers and examiners. Standard protocols for questionnaire survey were described in more detail elsewhere [15]. Subjects were interviewed by trained interviewers with a standardized questionnaire to determine general characteristics including information on demographics, education, smoking status, alcohol consumption, exercise, medical and reproductive histories of women. The criterion of higher education was defined as ≥12 years of schooling and regular exercise as ≥3 sessions per week and ≥30 min per session. When it comes to smoking, non-smokers were defined as subjects who had never smoked or smoked below 400 cigarettes during their lifetime. Subjects who had smoked at least 400 cigarettes during their lifetime were classified as 2 groups, ex-smokers and current smokers, on the basis of current smoking status. With regards to alcohol consumption, non-drinkers were defined as subjects who had never had 1 drink during their lifetime. Subjects who had had ≥1 drink during their lifetime were categorized into 2 groups, ex-drinkers and current drinkers, on the basis of current alcohol intake. Drinkers were requested about their average frequency and amount of alcohol consumption to estimate daily alcohol consumption. Total daily alcohol consumption was calculated from the sum of all alcoholic beverages consumed, expressed as grams of alcohol per day (g/d).

2.3. Assessment of anthropometric and biochemical blood profile

Various anthropometric indices such as weight, height, waist circumference and blood pressure were measured by using standardized protocols. The weight of each subject was assessed with a metric weight scale to the nearest 0.01 kg and the height was measured with a standard height scale to the nearest 0.1 cm while the subject was wearing light clothing with no shoes. Waist circumference was measured, to the nearest 0.1 cm, halfway between the lowest rib margin and the iliac crest. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Two consecutive blood pressure measurements were performed in a seated position after a minimum 5-min rest according to a standard protocol. SBP and DBP were measured with a standard mercury sphygmomanometer (Baumanometer, WA Baum Co., Inc., Copiague, NY, USA) using the first and fifth Korotkoff sounds, to the nearest 2 mmHg. If the two SBP or SBP measurement were different above 5 mmHg, an additional measurement was performed. Based on the recommended technique for measuring blood pressure in Canadian Hypertension Education Program [16], the first reading was discarded and the mean value of the last two measurements was used for the analyses.

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