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Research Paper

Loss of Cardio-Protective Effects at the *CDH13* Locus Due to Gene-Sleep Interaction: The BCAMS Study

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ABSTRACT

Left ventricular mass index (LVMI) provides a metric for cardiovascular disease risk. We aimed to assess the association of adiponectin-related genetic variants resulting from GWAS in East Asians (loci in/near *CDH13*, *ADIPOQ*, *WDR11FGF*, *CMIP* and *PEPD*) with LVMI, and to examine whether sleep duration modified these genetic associations in youth. The 559 subjects aged 15–28 years were recruited from the Beijing Child and Adolescent Metabolic Syndrome study. Among the six loci, *CDH13* rs4783244 was significantly correlated with adiponectin levels ($p = 8.07 \times 10^{-7}$). The adiponectin-rising allele in rs4783244 locus was significantly associated with decreased LVMI ($p = 6.99 \times 10^{-4}$) after adjusting for classical cardiovascular risk factors, and further for adiponectin levels, while no significant association was found between the other loci and LVMI. Moreover, we observed a significant interaction effect between rs4783244 and sleep duration ($p = .005$) for LVMI; the genetic association was more evident in long sleep duration while lost in short sleep duration. Similar interaction was found in the subgroup analysis using longitudinal data ($p = .025$ for interaction). In this young Chinese population, *CDH13* rs4783244 represents a key locus for cardiac structure, and confers stronger cardio-protection in longer sleep duration when contrasted with short sleep duration.

1. Introduction

Left ventricular hypertrophy (LVH) is initially a compensatory response to chronic stress by cardiomyocytes, but individuals with LVH are at increased risk for cardiovascular diseases (CVD), even at young ages [2, 18]. LVM, reflecting left ventricular remodeling, is related to body size, sex, and age; as such, LVM index is calculated to minimize these effects and serves as an important marker for myocardial remodeling. LVH, reflected by increased left ventricular mass (LVM) and LVM indexes are influenced by both genetic determinants and environmental factors, including lifestyle [15]. In a recent genome wide association study (GWAS), investigators reported that the *cadherin 13* (*CDH13*) locus was associated with LVM in adults [3]. This gene has also been shown to be associated with adiponectin levels through its coding for T-cadherin, a receptor for high-molecular-weight species of adiponectin and widely expressed in vascular tissues and myocardium [7, 8]. It is known that adiponectin, a major adipokine, exhibits a board spectrum of biological effects, including anti-diabetic, anti-oxidant, and anti-atherosclerotic actions [4, 10], and low adiponectin levels have been reported to be a risk marker of cardiac remolding [4, 33, 36]. Studies that have documented the associations between *CDH13* genetic variations and other cardiometabolic profiles affected by adiponectin levels have provided evidence for crosstalk between this locus, T-cadherin and adiponectin in influencing cardiac remolding [5, 11, 14, 34, 38]; however, these metabolic links remain controversial and the underlying mechanisms warrant further studies. In addition to *CDH13*, a number of

Abbreviations: LVM, Left ventricular mass; LVM index, Left ventricular mass index; LVH, Left ventricular hypertrophy; GWAS, Genome wide association study; MetS, Metabolic syndrome; T2D, Type 2 diabetes; CVD, Cardiovascular diseases; BMI, Body mass index; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FBG, Fasting blood glucose; LDL-C, Low density lipoprotein cholesterol; HDL-C, High density lipoprotein cholesterol; TG, Triglyceride; HOMA-IR, Homeostasis model assessment of insulin resistance; IVSDT, Interventricular septal diastolic thickness; LVEDD, Left ventricular end-diastolic diameter; LVPWT, Left ventricular posterior wall thickness.

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adiponectin-associated loci, including in/near *ADIPOQ*, *CMIP*, *PEPD*, and *WDR11FGFR* etc. have been identified by GWAS [5, 7, 30, 42]. Although epidemiologic studies have reported that *ADIPOQ* variants are associated with metabolic syndrome (MetS), type 2 diabetes (T2D) and cardiovascular diseases (CVD) [9], the correlations between above-noted adiponectin-associated loci and cardiac remolding have not yet been fully evaluated.

In addition to genetic factors, sleep duration, a modifiable environmental factor, has been recently shown to be associated with LVM in a multiethnic elderly cohort [39]. Our previous findings from the cohort study of the 'Beijing Child and Adolescent Metabolic Syndrome' (BCAMS) have also demonstrated that short sleep duration is associated with increased LVM and LVM index in youth with risk for MetS [12], but this association is independent of traditional cardio-metabolic risk factors; although we also found short sleep duration was associated with cardio-metabolic risk factors in younger children at baseline from the same cohort [24]. We thus hypothesize that sleep modifications play a role in cardiac remodeling via genetic predisposition to LVH. Moreover, further study of the interactions between sleep and adiponectin-associated loci would improve the understanding of the underlying pathologic mechanisms and lead to prevention strategies to optimize cardiac remodeling.

Therefore, in our current study, we firstly aimed to determine the association of several GWAS–identified adiponectin-associated loci with parameters of cardiac structure as measured by echocardiography, a well-documented and reliable method [12, 23]. Secondly, we examined the interaction between genetic predisposition to cardiac remodeling and habitual sleep duration in this young population with risk for MetS.

2. Methods

2.1. Participants

The design of the BCAMS has been described in detail elsewhere [26, 40]. In brief, BCAMS study began in 2004, as a prospective cohort study of identifying cardiovascular risk factors from childhood to adulthood. The baseline population-based survey was conducted in a representative sample (n = 19,593, 50% boys) of school children in Beijing aged 6–18 years. In total, approximately 4500 participants were identified as being at a high risk of cardiovascular disease (CVD) due to having one of the following abnormalities: overweight/obesity as defined by body mass index (BMI between 85th percentiles in specific age and sex), high blood pressure (≥ 90th percentiles in specific age and sex), elevated lipids (total cholesterol ≥5.2 mmol/L, triglyceride ≥1.7 mmol/L), and/or fasting blood glucose (≥ 5.6 mmol/L) based on finger capillary blood tests. We conducted follow up studies in 2014. Participants were recruited consecutively through various modalities (phone, text, and/or email) and underwent medical examination at a center in the Beijing Chaoyang Hospital. Signed informed consent was obtained from all participants and/or their parents or guardians. The protocol for the follow-up examination was approved by the Ethics Committee at the Beijing Chaoyang Hospital, and conformed to standards indicated by the Declaration of Helsinki. A total of 559 individuals had complete follow-up data and thus were included into this analysis. The BCAMS study has been registered at www.clinicaltrials.gov (NCT03421444).

2.2. Clinical and Biochemical Measurements

All participants underwent a physical examination that involved measurements of height, weight, waist circumference (WC) and blood pressure in a sitting position after 15 min of rest. Standing height (to 0.1 cm) and weight (to 0.1 kg) were measured using a wall-mounted stadiometer. WC was measured by plastic tape as midway between the lowest rib and the top of the iliac crest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times during 10 min with a standard sphygmomanometer after 5 min of rest. BMI was calculated as weight divided by height squared. In addition, questionnaires were used to obtain information on lifestyle factors and health history [25]. Physical activity was assessed as weekly minutes of moderate-to-vigorous physical activity. Participants were asked to recall all their food intake in the previous week, including whole grains, meat, fruits, vegetables, dairy and snacks, and the average daily total caloric intake was calculated. Health history information included hypertension, diabetes, dyslipidemia, and kidney, heart and thyroid diseases plus medication. Cigarette smoking was defined as current, former, or never.

Blood samples were collected via an antecubital vein after a 10 h fasting. The fasting samples were also aliquoted and frozen for future analysis of adipokines. Blood glucose, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL—C), and triglycerides (TG) were measured on an autoanalyzer (Hitachi 7060C automatic biochemistry analysis system). Insulin and adiponectin were measured by monoclonal antibody-based sandwich enzyme-linked immunosorbent assay, which was developed in the Key Laboratory of Endocrinology, Peking Union Medical College Hospital [27–29]. The intra-assay coefficient of variation (CVs) for insulin and adiponectin were < 4.1% and < 5.4%, respectively. The inter-assay CVs were < 7.0% and < 8.5%, respectively. Insulin resistance was assessed by the homeostasis model assessment (HOMA-IR), calculated as fasting insulin (μIU/mL) × fasting blood glucose (mmol/L)/22.5 [32]. MetS was determined using the 2009 harmonized definition [1].

2.3. Genomic DNA Extraction and Genotyping

Genomic DNA was extracted from blood samples collected from each participant using the QIAamp DNA blood midikits (Qiagen). The six most strongly adiponectin-associated SNPs were selected from previously reported GWAS of adiponectin in East Asians [5, 7, 30, 42], *ADIPOQ* rs10937273, rs6773957, *CDH13* rs4783244, *WDR11FGF* rs3943077, *CMIP* rs2925979, and *PEPD* rs889140, and were genotype on the Sequenom Mass Array iPLEX genotyping platform in BioMiao Biological Technology Co, Ltd. [13, 25]. Repeated control samples were present in each genotyping plate, with the concordance rate being 100%. All these SNPs had genotyping efficiency >0.95 and were in Hardy-Weinberg equilibrium with p value >.008 (0.05/6).

2.4. Echocardiography

Ultrasound images were acquired by a non-invasive transthoracic echocardiogram using a LOGIQ P5 B-mode ultrasonogram equipped (LOGIQ P5, GE Ultrasound, Korea) with a 2.5–3.5 MHz probe. All images were obtained in the left decubitus position of participants to acquire parasternal long and short axis and apical four chamber views. The following measurements were measured by a sonographer who was blinded to group: interventricular septal diastolic thickness (IVSDT), left ventricular end-diastolic diameter (LVEDD), and left ventricular posterior wall thickness (LVPWT). The following variables were calculated: $\mathrm{LVM} = 0.8 \times \{1.04 \times [(\mathrm{LVEDD} + \mathrm{LVPWT} + \mathrm{IVSDT})^3 - (\mathrm{LVEDD})^3]\} + 0.6$. LVM index (a measure of hypertrophy) was calculated by dividing LVM by height in meters raised to 2.7 ($\mathrm{LVM/height}^{2.7}$) to minimize the effects of age, sex [6, 20].

2.5. Sleep Duration

Sleep duration was determined for each participant by a self-reported questionnaire (including bed time and wake up time). Sleep duration was asked by time bar reaching half-hourly from 5 to 13 h per day. In the study, sleep time was analyzed for continuous variable or classification variable as following: short sleepers (≤ 7 h/day), normal sleepers (> 7 to ≤9 h/day), and long sleepers (> 9 h/day) [12]. In addition, in subgroup retrospective analyses, subjects were reclassified into four groups according to the median of sleep durations at baseline

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