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A genetic variant in the gene encoding adrenomedullin predicts the development of dysglycemia over 6.4 years in Chinese

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ABSTRACT

Background: Adrenomedullin, a vasodilatory peptide, facilitates the differentiation of pre-adipocytes, and affects lipolysis and glucose uptake. We investigated the association of common single nucleotide polymorphisms (SNPs) in the gene encoding adrenomedullin (*ADM*) with dysglycemia in the Hong Kong Chinese population.

Methods: Four SNPs were genotyped in 1391 subjects without dysglycemia at baseline from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2, which had a median follow-up time of 6.4 years. Dysglycemia included impaired fasting glucose, impaired glucose tolerance, and diabetes according to the WHO 1998 criteria. At follow-up, 382 subjects had developed dysglycemia.

Results: In stepwise logistic regression, the SNP rs11042725 was a significant independent predictor of the development of dysglycemia (OR = 1.31, $P = 0.012$), together with baseline age ($P < 0.001$), plasma triglycerides ($P < 0.001$), body mass index ($P = 0.004$), 2-h glucose after oral glucose tolerance test ($P < 0.001$), homeostasis model assessment of insulin resistance index ($P = 0.045$), and follow-up duration ($P = 0.009$). The association was more significant in women ($P = 0.002$) and in subjects without regular exercise ($P = 0.001$).

Conclusions: Our study suggests a potential role of genetic variants in the *ADM* gene in the development of dysglycemia in our local Chinese population.

1. Introduction

Adrenomedullin is a vasodilatory peptide first isolated from human pheochromocytoma [1]. The gene encoding for adrenomedullin (*ADM*) is located on chromosome 11p15.4, and consists of four exons and three introns [2]. Adrenomedullin consists of 52 amino acids, and possesses vasorelaxant and hypotensive properties [1,3–5]. It is expressed ubiquitously in many different tissues, including adrenal medulla, lungs, heart, kidney, and pancreatic islets, and circulates in plasma in the low fmol/ml range [3–5]. We have previously demonstrated that adrenomedullin plays a regulatory role in both acute and chronic inflammatory responses and interacts with cytokines such as interleukin-6 and tumor necrosis factor-α [6–8].

Adrenomedullin was recently recognized as a novel adipokine since adipocyte is the major site of adrenomedullin production [5,9]. The plasma levels of adrenomedullin are also elevated in obesity [10–12], which may partly contribute to the increased levels observed in hypertension [13,14] and diabetes [15–17]. Adrenomedullin can inhibit adipocyte differentiation [18] and play a role in glucose metabolism [18–21]. Single nucleotide polymorphisms (SNPs) and a microsatellite marker located in the 3′end of the *ADM* gene have been shown to be associated with essential hypertension and renal diseases [22–26]. However, none of these studies have examined their associations with glycemia. Given the potential roles of adrenomedullin in glucose metabolism and the close relationship between raised blood pressure and dysglycemia in our local population [27], we investigated the association of common single nucleotide polymorphisms in the *ADM* gene with dysglycemia.

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2. Subjects and methods

2.1. Subjects

The Hong Kong Cardiovascular Risk Factor Prevalence Study-2 was a population-based prospective cohort study of cardiovascular risk factors in the Hong Kong Chinese population [27–30]. There were 1847 subjects from this cohort who had valid data on glycemic status at both baseline (1995–1996) and 6.4 year follow-up (2000–2004), and had DNA samples available for genotyping. In this study, we included 1391 subjects who did not have dysglycemia at baseline in the prospective analysis. Dysglycemia was defined as the presence of impaired glucose tolerance, impaired fasting glucose, or diabetes, according to the World Health Organization 1998 diagnostic criteria [31]. All the included study subjects underwent oral glucose tolerance test (OGTT) at both baseline and follow-up, except those taking anti-diabetic medication. All subjects gave written informed consent. The study protocol was approved by the Ethics Committee of the University of Hong Kong.

2.2. SNPs selection and genotyping

Tagging SNPs were selected in the *ADM* gene from the HapMap Han Chinese population (Phase II data, release 24). There were 2 tagging SNPs (rs3814700 and rs4910118) which captured all of the four SNPs from 5 kb region upstream to 2 kb downstream of the gene (position 10,278,218–10,287,499, GenBank accession number NC_000011, NCBI build 36) with $r^2 \geq 0.8$ and minor allele frequency (MAF) ≥ 5% (Fig. 1 and Supplementary Table 1). In addition, 2 SNPs (rs11042725 and rs34354539), not genotyped in HapMap, were selected from the NCBI database with reported MAFs ≥ 5% in Asian population. All the nucleotide sequences were based on the forward strand of sequence from the GenBank accession number NC_000011. Genotyping was performed using the MassARRAY system (Sequenom, San Diego, CA) with the iPLEX™ assay in the Genome Research Centre, University of Hong Kong. After genotyping, the SNPs rs3814700 and rs11042725 showed a high linkage disequilibrium (LD) ($r^2 > 0.8$) (Supplementary Fig. 1). Therefore, we only report the results for rs11042725 as it was reported to have functional effects [34].

2.3. Phenotypes and other variables of interest

Drinking was defined as alcoholic drinking at least once a week. Regular exercise was defined as having exercise for ≥30 min at least once a week in the past month. Smoking was defined as taking cigarettes currently. Other clinical parameters such as body mass index (BMI), blood pressure, plasma glucose, insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured or calculated as described previously [27–30].

2.4. Statistical analysis

Statistical analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Variables with skewed distributions were ln-transformed before analysis. Haploview version 4.1 was used to assess LD [32]. Haplotype analysis was performed using program PLINK (version 1.0.6) [33]. Multivariate logistic or linear regression models were used to estimate the odds ratios (OR) or unstandardized regression coefficients under the assumption of an additive effect of allele dosage. For single variant analysis, standardized regression coefficients (β) were also estimated. Baseline variables were used as covariates in regression analysis if they were biologically likely to affect glycemic status or were significantly different between subjects with and without dysglycemia. For variables that were highly correlated such as BMI and waist circumference, only one was entered into the regression analysis. Correction for multiple testing of four SNPs was performed using Bonferroni's method. Correction for testing of multiple phenotypes was not performed as the phenotypes tested were closely related to each other. The *P* values for interaction were estimated by including each multiplicative interaction term in the multivariate regression models in full sample after adjusting for the main effects of all covariates.

For haplotype analysis, only haplotypes with frequency >5% were tested. An omnibus test was first performed to assess the global *P* value of the overall variation at the locus. The effect of each of the specific haplotype was analyzed by comparing with all other haplotypes combined.

3. Results

3.1. Subject characteristics and genotyping

Among 1391 subjects without dysglycemia at baseline, 382 developed dysglycemia at follow-up. Subjects with dysglycemia at follow-up had significantly higher age, BMI, waist circumference, blood pressure, plasma triglycerides, plasma glucose, fasting insulin and HOMA-IR, and lower HDL cholesterol level at baseline (Table 1). In addition, a lower percentage of these subjects have regular exercise (i.e. more than once a week) at baseline.

The genotyping rates of the SNPs were all ≥99.9%. None of them showed significant deviation from Hardy-Weinberg equilibrium among all subjects or among case- and control-specific subgroups ($P > 0.20$). None of them showed significant difference in genotype

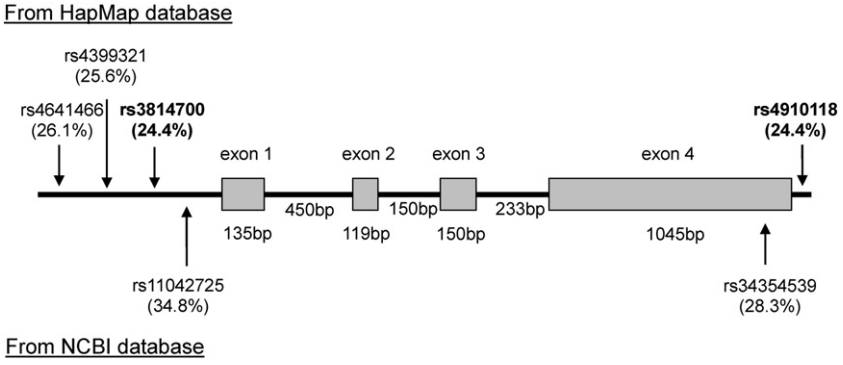

Fig. 1. A schematic diagram of the *ADM* gene. Exons and introns are represented by grey boxes and thin lines respectively with their sizes indicated below. The minor allele frequencies of the SNPs from the HapMap Han Chinese population or Asian population in the NCBI database are indicated in the brackets. The 2 tagging SNPs which captured all of the 4 SNPs (rs4641466, rs4399321, rs3814700, and rs4910118) in Han the HapMap database are shown in bold font. Two SNPs (rs11042725 and rs34354539) from the NCBI database, not genotyped in the HapMap project, were also selected for genotyping.