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Plasma adiponectin levels, *ADIPOQ* variants, and incidence of type 2 diabetes: A nested case-control study

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ABSTRACT

Aims: To clarify the associations between plasma adiponectin levels and *ADIPOQ* variants with type 2 diabetes incidence in a general Japanese population.

Methods: We conducted a case-control study nested within the Japan Public Health Center-based Prospective Study. We measured plasma adiponectin levels and genotyped +45T > G (rs2241766) and +276 G > T (rs1501299) in the *ADIPOQ* gene among 417 incident diabetes cases and 1197 control subjects matched by age, sex, and area.

Results: After potential confounding factor adjustment, the multivariable-adjusted diabetes odds ratios (ORs) were 0.59 (95% confidence interval [CI]: 0.51–0.68) and 0.68 (95% CI: 0.60–0.78) per 1 standard deviation increment in the log-transformed levels of total- and high-molecular-weight (HMW) adiponectin levels, respectively. However, the *ADIPOQ* variants were not significantly associated with plasma adiponectin levels (for total adiponectin, +45 $P = 0.15$ and +276 $P = 0.08$) and diabetes risk (+45 $P = 0.70$ and +276 $P = 0.72$) under the additive genetic model.

Conclusions: Our prospective findings suggest that both total and HMW adiponectin levels are strongly and inversely associated with diabetes risk after adjustment for potential confounding factors; however, the *ADIPOQ* variants +45 and +276 are not associated with adiponectin levels and diabetes risk in the general Japanese population.

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1. Introduction

Adiponectin has been shown to improve insulin sensitivity [1] and its circulating levels have been consistently inversely associated with the risk of type 2 diabetes [2–6]. Human adiponectin, which is expressed exclusively in adipose tissue [1], exists in three multimeric forms, high-molecular-weight (HMW), middle-molecular-weight, and low-molecular-weight [7,8]. Of these, HMW is thought to represent the major active form of adiponectin in peripheral tissues and some studies have suggested that HMW adiponectin levels may be more strongly associated with type 2 diabetes than are total adiponectin levels [9,10].

A potential protective effect of adiponectin against type 2 diabetes suggests that variants in the adiponectin gene (*ADIPOQ*), which is located at the chromosomal locus 3q27 [1], might be associated with the risk of type 2 diabetes; accordingly, over the past decade, much interest has been drawn to the role of genetic variations in *ADIPOQ* in the development of type 2 diabetes [11–15]. A case-control study of 384 cases and 480 controls in Japan first reported that among 14 genotyped polymorphic variants in the *ADIPOQ* gene, two variants, +45T > G in exon 2 (rs2241766; silent mutation) and +276G > T in intron 2 (rs1501299), were significantly associated with the prevalence odds of type 2 diabetes [11]. In addition, the +276 variant was significantly associated with adiponectin levels among participants with obesity but without diabetes, although the association was insignificant in the sample including participants without obesity [11]. Since this first report, more than 30 studies, many from Asian populations, have examined the association between +45 and/or +276 variants and type 2 diabetes [14–16]. These have yielded mixed results for the +45 single nucleotide polymorphism (SNP); some case-control studies, many from Chinese populations [16,17], reported significant positive association between the SNP +45 G allele and type 2 diabetes [16–20]. However, case-control studies in Japanese populations failed to replicate this finding for the +45 SNP [21,22]. Furthermore, most [12,13,23,24], but not all [25], cohort studies, of which all were from Western populations, found no significant association between the +45 variant and the incidence of type 2 diabetes. These inconsistencies in results suggest the need for cohort studies that investigate the association in Japanese populations from which the significant association for the +45 variant were first reported. For the +276 variant, all studies, with the exception of case-control studies in a Polish [26] and a Chinese population [27], failed to replicate the positive association between the SNP +276 G allele and type 2 diabetes [14–16,21,22,28]. However, to the best of our knowledge, no prospective cohort study in Japan has investigated the association of the +45 and +276 variants with type 2 diabetes risk.

Therefore, we conducted this nested case-control study in the Japan Public Health Center-based Prospective (JPHC) Study in order to (1) examine whether and to what extent the circulating levels of total and HMW adiponectin are associated with the risk of type 2 diabetes in a general Japanese population; and (2) investigate whether these *ADIPOQ* variants are associated with the incidence of type 2 diabetes in a large cohort of the general Japanese population. This prospective investigation provides an opportunity to clarify the role of +45 and +276 variants in the development of type 2 diabetes in a large, well-characterized general Japanese population.

2. Material and methods

2.1. Study population

Fig. 1 shows the flowchart of the selection of the participants. The JPHC Study was initiated in 1990 for cohort I and spanned from 1993 to 1994 for cohort II. All subjects were from 11 public health center (PHC) areas, aged 40–59 years in 1990 (cohort I: Iwate, Akita, Nagano, Okinawa, and Tokyo) and 40–69 years in 1993 (cohort II: Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka) at the time of their first survey. Specific details of the study design of the JPHC Study are described elsewhere [29]. Thus, the JPHC Study consisted of 11 PHC areas throughout Japan with a total of 140,420 subjects (68,722 men and 71,698 women). A questionnaire survey was conducted at baseline and at the 5- and 10-y follow-ups. We excluded subjects with a non-Japanese nationality ($n = 51$), precommencement emigration ($n = 206$), incorrect birth date ($n = 6$), or duplicate registration ($n = 4$). We also excluded the PHC areas from Tokyo and Osaka because data on diabetes incidence were not available ($n = 23,488$). Furthermore, we excluded those who did not respond to the baseline or 5-y follow-up questionnaire ($n = 36,544$), and those who reported histories of cancer, stroke, ischemic heart disease, or chronic liver disease at the 5-y follow-up ($n = 7582$), which left 72,546 eligible subjects (32,934 men and 39,612 women). At the 5-y follow-up, 24,929 subjects (8819 men and 16,110 women) provided a 10 mL blood sample during the health check-up conducted by each PHC area. Of these, 17,587 subjects (6062 men and 11,525 women) did not have diabetes defined by the criteria described below at the 5-y follow-up and were thus followed until the 10-y follow-up. Among the subjects without diabetes at the 5-y follow-up, 5797 (1982 men and 3815 women) participated in the JPHC Diabetes Study, a prospective sub-cohort of the JPHC Study, which involved hemoglobin A1c (HbA1c) measurements and an additional questionnaire concerning diabetes and lifestyle [30]. For those who participated in the JPHC Diabetes Study, diabetes was defined by HbA1c levels $\geq$6.5% ($\geq$48 mmol/mol), taking glucose-lowering medications, or diabetes diagnosed by a physician (Group 1). For those who did not participate in the JPHC Diabetes Study (4080 men and 7710 women), participants were considered as having diabetes if they reported a diagnosis of diabetes or taking glucose-lowering medications via a self-administered questionnaire (Group 2). Because the age of onset in our study population ranged from 45 years old or older, all incident cases of diabetes were considered as type 2 diabetes.

Over five years, 471 subjects (220 men and 251 women; 317 in Group 1 and 154 in Group 2) developed incident type 2 diabetes. Controls were matched in a 1:3 ratio to cases by age (within 3 years), sex, cohort, PHC area, city or town and village