Adiponectin gene variants and the risk of coronary heart disease: a 16-year longitudinal study

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Abstract

Objective: Circulating adiponectin levels have been shown to be associated with a risk of coronary heart disease (CHD). However, its primary role in protecting against the development of CHD remains controversial due to conflicting observations in prospective studies. To gain further insight into the primary role of adiponectin, our major objective was to investigate the relationship between single nucleotide polymorphisms (SNPs) of the adiponectin gene (*ADIPOQ*) and incident CHD in a population-based cohort with no CHD at baseline.

Design and methods: We conducted a 16-year longitudinal study in 2196 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS). During 33 862 person-years of follow-up, 184 subjects developed CHD (cumulative incidence rate = 5.4 per 1000 person-years). Nine *ADIPOQ* SNPs with potential functional relevance or shown to be associated with adiponectin levels and/or CHD were genotyped.

Results: Among the nine *ADIPOQ* SNPs, +276G>T (rs1501299) was independently associated with incident CHD in men but not in women, even after adjustments for traditional cardiovascular risk factors ($P_{\text{adjusted}} = 5.5 \times 10^{-3}$ to 0.023; hazard ratio = 1.39–1.54). Furthermore, there was a significant association of the T allele of +276G>T with a lower adiponectin level ($P = 0.027$; β (95% CI) = -0.05 (-0.10 , -0.01)).

Conclusions: This study demonstrated that +276G>T may be an independent predictor of CHD development. Our findings suggest that low adiponectin levels, as may be influenced by +276G>T, confer a higher risk of CHD, in keeping with a role of hypoadiponectinaemia in the development of CHD in the general population.

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Introduction

Adiponectin is one of the most abundant insulin-sensitising adipokines secreted by the adipocytes (1). Circulating levels of adiponectin are reduced in obesity, in particular visceral obesity (2, 3), and they predispose to endothelial dysfunction, atherosclerosis and

subsequent cardiovascular diseases (CVDs) in animal models. Hypoadiponectinaemia has been proposed as one of the mediators of increased cardiovascular risk in obesity (4). Adiponectin, encoded by *ADIPOQ*, is suggested to play a protective role in the development of coronary

heart disease (CHD) due to its anti-inflammatory, anti-oxidative, anti-apoptotic and anti-atherogenic properties (3, 5). The prospective relationship between circulating adiponectin level and the development of CHD has been extensively investigated. However, whether adiponectin levels are causally related to CHD development remains controversial. While high adiponectin levels are believed to be protective against CHD in healthy populations (6), more recent studies have suggested that high adiponectin levels are associated with a greater risk of CHD or cardiovascular mortality in older populations (7) or cohorts with prevalent CHD (8). As the up-regulation of adiponectin could act as a compensatory mechanism to limit further vascular injury (8), a high adiponectin level in the established disease state might reflect on the severity of the underlying vascular inflammation, and hence a positive association between the adiponectin level and cardiovascular mortality.

Studies on potentially functional *ADIPOQ* genetic variants may provide more insight into the primary role of adiponectin, if any, in protecting against the development of CHD. So far, only a few prospective studies conducted in Caucasians have investigated the genetic effects of *ADIPOQ* single nucleotide polymorphisms (SNPs) on CHD development (9, 10, 11). The primary objective of this study was to evaluate the impact of nine *ADIPOQ* SNPs on the risk of CHD in a 16-year longitudinal study cohort of healthy Southern Chinese. These SNPs were selected because of their potential functional relevance or reported influence on the risk of CHD or CVDs and/or adiponectin levels.

Subjects and methods

Subjects

The Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) is a population-based prospective study of cardiovascular risk factors in Hong Kong (12). In 1995–1996 (CRISPS1), 2895 Hong Kong Chinese were selected randomly by their telephone numbers to undergo a comprehensive assessment of cardiovascular risks. Subjects were contacted for reassessment in 2000–2004 (CRISPS2) and 2005–2008 (CRISPS3). The latest CRISPS4 follow-up assessment was commenced in July 2010. The current study involved a total of 2196 subjects who did not have a history of CHD at baseline (CRISPS1) and with their DNA samples available for genetic analysis. During 33 862 person-years of follow-up, 184 subjects (111 men and 73 women) who were without

CHD at baseline had developed CHD by the end of 2011, giving a cumulative incidence rate of 5.4 per 1000 person-years. A total of 2012 subjects (915 men and 1097 women) who were without CHD at baseline had remained without CHD at subsequent follow-up visit(s). CHD events were defined based on ICD-9 (402, 404, 410–414, 425–429), which included, among others, acute myocardial infarction (MI), heart failure and angina pectoris as described in our previous study (13). Information on the dates of the CHD events and discharge diagnosis were obtained from the patients and also verified from the hospital authority database or the patients' private practitioners. For those who had died, causes and dates of death were determined from the Hong Kong Death Registry database. Two physicians reviewed the medical diagnoses independently and disagreements between them were resolved by a third physician. The concordance between the two physicians was 0.98. Written informed consent was obtained from each participant and the study protocol was approved by the Ethics Committee of the University of Hong Kong.

Anthropometric and biochemical measurements

Anthropometric (including BMI, waist circumference (WC), systolic blood pressure (SBP) and diastolic blood pressure (DBP)) and biochemical parameters (including fasting plasma glucose (FPG), 2-h post-OGTT glucose (2hrG), triglyceride (TG), HDL-C, LDL-C and total cholesterol (TC)) were measured as described previously (14). Type 2 diabetes (DM) was defined as FPG ≥ 7.0 mmol/l or 2hrG ≥ 11.1 mmol/l or both, according to the World Health Organization 1998 diagnostic criteria (15) or on anti-diabetic medication. At baseline, 181 subjects (89 men and 90 women) had DM and 1923 subjects (904 men and 1017 women) were without DM. The presence of hypertension (HT) was indicated by BP $\geq 140/90$ mmHg or if the patient was receiving regular anti-hypertensive treatment. The presence of dyslipidaemia was indicated by fasting TG ≥ 1.69 mmol/l, HDL-C < 1.04 mmol/l in males and < 1.29 mmol/l in females, and LDL-C ≥ 3.4 mmol/l (16) or if the patient was taking lipid-lowering agents. As stored baseline plasma samples were no longer available from a large number of subjects, total adiponectin level was measured from available plasma samples collected during the CRISPS2 follow-up visit ($n=1676$), using an in-house sandwich ELISA kit established in our laboratory (intra- and inter-assay coefficient of variation values of 6.2–8.3 and 5.1–6.4% respectively) (17).

Genetic analysis

A total of nine *ADIPOQ* SNPs (rs16861194, rs266729 (−11377C>G), −10677C>T, rs1802052 (−10066G>A), rs822395 (−4034A>C), rs822396 (−3964A>G), rs12495941, rs2241766 (+45T>G) and rs1501299 (+276G>T)) were selected on the basis of previous publications suggesting them as functional (18, 19, 20), or shown to affect adiponectin levels (9, 21, 22, 23), or associated with CHD/CVD (9, 11, 24). Genotyping of these SNPs was carried out using the Sequenom iPLEX Gold genotyping assay at the Centre for Genomic Sciences, the University of Hong Kong. The average genotyping call rate and concordance rate were 99.8 and 97.5% respectively. The SNPs were tested for deviation from the Hardy–Weinberg Equilibrium (HWE) by the de Finetti program available online at <<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>>.

Statistical analyses

All statistical analyses were conducted using SPSS version 19.0. The associations of SNPs with incident CHD were evaluated using Cox proportional hazards regression (Cox regression) analyses under the additive model. Survival was calculated from the date of visit at baseline to the date of diagnosis of CHD or the date of last follow-up visit. In view of the known gender difference in adiponectin levels (25) and the observation of a higher incidence rate of CHD in men (7.1 per 1000 person-years vs 4.0 per 1000 person-years in women), we also performed gender-stratified analyses. A two-tailed $P<0.05$ was considered statistically significant. Baseline clinical parameters that were biologically likely to have an influence on the development of CHD or were statistically different ($P<0.05$) between the incident CHD and non-CHD groups were adjusted for in the multiple adjustment analyses. Three different sets of traditional risk factors were included in the multiple Cox regression analyses. Multiple adjustments for age, BMI, 2hrG, HOMA-IR, HDL-C, LDL-C, TG, SBP, DBP and smoking were made in Model 1; adjustments were made for age, BMI, FPG, 2hrG, HDL-C, LDL-C, TG, SBP, DBP and smoking in Model 2 and adjustments were made for age, BMI, DM, dyslipidaemia, HT and smoking in Model 3. Logistic regression analysis was employed to examine the association of +276G>T with DM at baseline. The association of +276G>T with plasma adiponectin level at CRISPS2 was evaluated by linear regression analysis. The associations of CRISPS2 adiponectin levels with the development of CHD were evaluated using Cox regression

analyses. Survival was calculated from the date of visit at CRISPS2 to the date of diagnosis of CHD or the date of last follow-up visit. Both combined and gender-stratified analyses were carried out. The study power was calculated using the Genetic Power Calculator (26).

Results

Baseline clinical characteristics

We examined the associations of nine *ADIPOQ* genetic variants with incident CHD in 2196 subjects with DNA samples available for genetic analysis. Among these subjects who were without CHD at baseline, 184 subjects (cumulative incidence rate=5.4 per 1000 person-years) developed CHD during 33 862 person-years of follow-up; 2012 subjects remained free of CHD at subsequent follow-up visit(s). Table 1 shows the baseline clinical characteristics of the subjects. In both genders, the incident CHD groups had worse traditional cardiovascular risk factors, such as the presence of DM, dyslipidaemia and HT, when compared with the non-CHD groups. However, there were no significant differences in regular exercise, alcohol drinking and family history of DM, HT and CHD.

Association with CHD development

The allele frequencies of the nine SNPs were comparable to those reported in HapMap or 1000 Genome Project (Table 2). The genotype distributions of these SNPs were in HWE ($P=0.071$ – 0.809). Among these SNPs, rs1501299 (+276G>T) showed a significant association with incident CHD ($P_{\text{unadjusted}}=0.042$; hazard ratio (HR) (95% CI): 1.26 (1.01–1.56)). When stratified by gender, the association of +276G>T variant with incident CHD remained significant in men ($P_{\text{unadjusted}}=0.020$; HR (95% CI)=1.39 (1.05–1.83)). However, no significant association was found in women ($P_{\text{unadjusted}}=0.816$; HR (95% CI)=1.04 (0.73–1.50)). No significant associations of the other SNPs with incident CHD were observed in either gender.

Independent association of +276G>T with incident CHD in men

The possible independent association of +276G>T with incident CHD in men was further analysed with adjustments for different sets of traditional risk factors, including DM and its related traits, as the +276G>T variant was independently associated with DM at baseline, even after adjustment for age, sex and BMI in this study

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Table 1 Baseline clinical characteristics of subjects. Data are expressed as mean $\pm$ s.d. or median with interquartile range.

Baseline parameters	All		Male		Female	
	Non-CHD	Incident CHD	Non-CHD	Incident CHD	Non-CHD	Incident CHD
<i>n</i>	2012	184	915	111	1097	73
Sex (male %)	45.5	60.3*	—	—	—	—
Age (years)	44.4 $\pm$ 11.7	56.3 $\pm$ 10.9*	44.9 $\pm$ 12.3	55.6 $\pm$ 10.8*	44.0 $\pm$ 11.2	57.2 $\pm$ 11.0*
BMI (kg/m ²)	24.0 $\pm$ 3.6	25.8 $\pm$ 3.3*	24.3 $\pm$ 3.3	25.8 $\pm$ 3.3*	23.9 $\pm$ 3.7	25.8 $\pm$ 3.3*
Waist circumference (cm)	M: 82.6 $\pm$ 9.1 F: 74.8 $\pm$ 9.0	M: 88.1 $\pm$ 9.5* F: 82.5 $\pm$ 8.4*	82.5 $\pm$ 9.1	88.1 $\pm$ 9.5*	74.8 $\pm$ 9.0	82.5 $\pm$ 8.4*
Fasting glucose (mmol/l)	5.3 $\pm$ 1.1	6.1 $\pm$ 2.3 [†]	5.4 $\pm$ 1.3	5.8 $\pm$ 1.8 [†]	5.2 $\pm$ 0.9	6.6 $\pm$ 2.9 [†]
2-h post-OGTT glucose (mmol/l)	6.6 $\pm$ 2.7	8.5 $\pm$ 5.0*	6.5 $\pm$ 3.1	7.9 $\pm$ 4.4*	6.7 $\pm$ 2.4	9.4 $\pm$ 5.9*
DM (%)	7.1	24.5*	7.8	18.9*	6.5	32.9*
DM family history (%) ^a	17.4	13.7	15.9	9.2	18.6	20.5
Fasting insulin (μ U/ml) ^b	4.8 (3.1–7.3)	5.7 (3.9–9.0)*	3.0 (2.0–4.6)	3.7 (2.6–5.3) [†]	3.3 (2.3–5.0)	5.1 (3.1–6.9)*
HOMA-IR ^a	1.1 (0.7–1.7)	1.5 (1.0–2.3)*	0.7 (0.4–1.1)	0.9 (0.6–1.3) [†]	0.7 (0.5–1.1)	1.2 (0.7–1.8)*
Dyslipidaemia (%)	62.4	82.1*	61.9	79.3*	62.9	86.3*
TC (mmol/l)	5.0 $\pm$ 1.0	5.4 $\pm$ 1.0*	5.1 $\pm$ 1.1	5.3 $\pm$ 0.9*	4.9 $\pm$ 1.0	5.6 $\pm$ 1.1*
HDL (mmol/l)	1.3 $\pm$ 0.3	1.2 $\pm$ 0.3*	1.2 $\pm$ 0.3	1.1 $\pm$ 0.3 [†]	1.4 $\pm$ 0.3	1.3 $\pm$ 0.3 [†]
LDL (mmol/l)	3.2 $\pm$ 0.9	3.6 $\pm$ 1.0*	3.3 $\pm$ 0.8	3.5 $\pm$ 0.8	3.1 $\pm$ 0.9	3.7 $\pm$ 1.1*
TG ^b (mmol/l)	1.0 (0.7–1.4)	1.3 (0.9–1.9) [†]	1.1 (0.8–1.6)	1.4 (1.0–2.0) [†]	0.6 (0.5–0.9)	0.9 (0.6–1.1) [†]
SBP ^c (mmHg)	117.6 $\pm$ 18.9	135.1 $\pm$ 23.3*	120.0 $\pm$ 17.0	133.3 $\pm$ 21.2*	115.5 $\pm$ 20.0	137.8 $\pm$ 25.9*
DBP ^d (mmHg)	74.1 $\pm$ 10.7	81.2 $\pm$ 11.9*	76.6 $\pm$ 10.0	81.6 $\pm$ 12.4*	72.0 $\pm$ 10.9	80.5 $\pm$ 11.0*
HT (%)	14.1	48.4*	14.8	45.9*	13.6	52.1*
HT family history (%) ^e	29.4	24.0	28.7	20.9	30.1	28.8
Taking anti-hypertensive drug	3.2	12.4*	3.2	8.8 [†]	3.3	17.9*
Regular exercise (%)	41.5	44.6	44.8	47.7	38.7	39.7
CHD family history (%)	10.4	13.8	10.9	5.5	16.2	17.8
Smoking (%)	22.6	37.2*	45.5	56.8 [†]	3.5	6.9
Alcohol drinking (%)	36.1	42.9	56.1	59.1	19.4	18.1

**P* value < 0.001; [†]*P* value < 0.05. CHD, coronary heart disease; DBP, diastolic blood pressure; DM, type 2 diabetes; F, female; HOMA-IR, homoeostasis model assessment index of insulin resistance; HT, hypertension; M, male; n, number; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

^aDiabetes in first-degree relatives.

^bNatural log transformed before analysis.

^cSBP + 10 mmHg if on anti-hypertensive drug.

^dDBP + 5 mmHg if on anti-hypertensive drug.

^eHypertension in first-degree relatives.

(*P*_{age, sex and BMI adjusted} = 2×10^{-3} ; OR (95% CI) = 1.45 (1.14–1.84)). Table 3 shows the results of the multiple Cox regression analyses in the male subjects. After adjustment for age, BMI, 2hrG, HOMA-IR, HDL-C, LDL-C, TG, SBP, DBP and smoking (Model 1), +276G>T showed a significant association with incident CHD in men (*P*_{adjusted} = 5.5×10^{-3} ; HR (95% CI) = 1.54 (1.13–2.08)). Figure 1 shows the cumulative survival curves for incident CHD in men, based on multiple adjustment Model 1 and stratified by the +276G>T genotypes. Male subjects with the TT genotype had a significantly higher risk of developing CHD than those with the GG or GT genotypes as shown in Fig. 1 (*P*_{adjusted} = 5.5×10^{-3}). The association was also significant in adjustment Model 2 (*P*_{age, BMI, FPG, 2hrG, HDL-C, LDL-C, TG, SBP, DBP and smoking adjusted} = 0.015; HR (95% CI) = 1.44 (1.08–1.93)). Furthermore, when the presence of DM, dyslipidaemia and HT were included in the adjustment model in addition to age, BMI and smoking (Model 3), the association persisted (*P*_{adjusted} = 0.023; HR (95% CI) = 1.39 (1.05–1.84)). By contrast,

as expected, no significant association of +276G>T with incident CHD was observed in women (Model 1: *P*_{adjusted} = 0.539, HR (95% CI) = 0.88 (0.58–1.33); Model 2: *P*_{adjusted} = 0.381, HR (95% CI) = 0.83 (0.56–1.13); Model 3: *P*_{adjusted} = 0.339, HR (95% CI) = 0.84 (0.58–1.21)).

Association of the +276G>T variant with plasma adiponectin level at CRISPS2

Adiponectin level was found to be significantly lower (*P* < 0.001) in men (median (interquartile range) = 5.62 (3.62–8.69) mg/l, *n* = 804) than in women (7.89 (5.37–11.78) mg/l, *n* = 872). We observed a significant association of the T allele of +276G>T with a lower adiponectin level (*P* = 0.027; β (95% CI) = −0.05 (−0.10, −0.01)) in 1676 subjects with available plasma samples. When the association was examined in men and women separately, we observed a significant association in men (*P* = 0.049; β (95% CI) = −0.07 (−0.14, 0.00)), but the association was not significant in women (*P* = 0.424;

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Table 2 Genotype distributions and results of Cox regression analyses.

SNP (1/2)	Genotype distribution		MAF		Unadjusted	
	Non-CHD (11/12/22)	Incident CHD (11/12/22)	Non-CHD	Incident CHD	HR (95% CI)	P value
rs1501299 (+276G>T) (G/T)						
All	1103/759/148	88/75/19	0.262	0.310	1.26 (1.01–1.56)	0.042
M	500/343/71	46/52/11	0.265	0.339	1.39 (1.05–1.83)	0.020^a
F	603/416/77	42/23/8	0.260	0.267	1.04 (0.73–1.50)	0.816
rs2241766 (T/G)						
All	1007/822/183	89/83/12	0.295	0.290	0.97 (0.78–1.22)	0.813
M	466/371/78	56/52/3	0.288	0.261	0.87 (0.65–1.18)	0.374
F	541/451/105	33/31/9	0.301	0.336	1.16 (0.82–1.62)	0.401
rs12495941 (G/T)						
All	680/966/362	56/95/32	0.421	0.434	1.05 (0.86–1.29)	0.633
M	327/417/168	31/58/21	0.412	0.455	1.15 (0.89–1.50)	0.278
F	353/549/194	25/37/11	0.427	0.404	0.91 (0.65–1.27)	0.592
rs822396 (A/C)						
All	1511/468/30	137/46/1	0.131	0.130	0.99 (0.73–1.35)	0.955
M	688/213/13	84/26/1	0.131	0.126	0.97 (0.65–1.45)	0.878
F	823/255/17	53/20/0	0.132	0.137	1.04 (0.64–1.67)	0.884
rs822395 (A/C)						
All	1441/527/41	130/53/1	0.151	0.149	0.98 (0.73–1.31)	0.900
M	654/243/16	81/29/1	0.151	0.139	0.92 (0.63–1.36)	0.674
F	787/284/25	49/24/0	0.152	0.164	1.08 (0.70–1.68)	0.720
rs182052 (G/A)						
All	698/966/342	64/93/25	0.411	0.393	0.93 (0.76–1.15)	0.510
M	319/436/157	41/53/15	0.411	0.381	0.89 (0.68–1.17)	0.400
F	379/530/185	23/40/10	0.411	0.411	1.00 (0.72–1.39)	0.992
–10677C>T^b (C/T)						
All	1755/248/8	156/25/3	0.066	0.084	1.26 (0.87–1.83)	0.225
M	797/114/4	90/20/1	0.067	0.099	1.48 (0.96–2.30)	0.078
F	958/134/4	66/5/2	0.065	0.054	0.86 (0.42–1.75)	0.671
rs266729 (C/G)						
All	1148/729/130	111/65/8	0.246	0.220	0.88 (0.69–1.12)	0.288
M	512/347/53	67/40/4	0.248	0.216	0.84 (0.61–1.16)	0.298
F	636/382/77	44/25/4	0.245	0.226	0.92 (0.63–1.34)	0.653
rs16861194 (A/G)						
All	1410/543/59	128/50/6	0.164	0.168	1.03 (0.78–1.34)	0.854
M	642/248/25	80/28/3	0.162	0.153	0.94 (0.65–1.35)	0.723
F	768/295/34	48/22/3	0.165	0.191	1.17 (0.78–1.76)	0.438

All significant associations are highlighted in bold.

1, Major allele; 2, minor allele; F, female; M, male; MAF, minor allele frequency.

^aRemained significant after adjustment for age and BMI.

^bNo rs number is assigned for this SNP.

β (95% CI) = -0.03 ($-0.09, 0.04$). [Supplementary Table 1](#), see section on [supplementary data](#) given at the end of this article, shows the comparison of adiponectin levels at CRISPS2 between different genotypes of +276G>T. Similar findings were obtained when subjects who had developed CHD by CRISPS2 were excluded.

Association of plasma adiponectin level at CRISPS2 with the development of CHD after a median interval of ~9.6 years

We further examined the association of plasma adiponectin levels at CRISPS2 with the development of CHD,

after a median interval of ~9.6 years. As 46 of the 1676 subjects with adiponectin levels available for analysis had developed CHD by CRISPS2, only 1630 subjects were included in the analysis. Over a median interval of ~9.6 years, 101 subjects (56 men and 45 women) had developed CHD, while 1529 subjects (720 men and 809 women) remained free of CHD. We did not observe any significant association between CRISPS2 adiponectin level and CHD development, in both the combined ($P=0.200$; HR (95% CI)=0.82 (0.60–1.11)) and gender-stratified analyses (men: $P=0.465$, HR (95% CI)=0.85 (0.60–1.30); women: $P=0.665$, HR (95% CI)=0.89 (0.54–1.48)).

Table 3 Multiple Cox regression analyses of *ADIPOQ* +276 G>T and incident CHD in the male subjects.

Risk factors	Model 1		Model 2		Model 3	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
+276G>T (T)	1.54 (1.13–2.08)	5.5×10^{-3}	1.44 (1.08–1.93)	0.015	1.39 (1.05–1.84)	0.023
Age (years)	1.06 (1.04–1.08)	<0.001	1.06 (1.04–1.08)	<0.001	1.06 (1.04–1.08)	<0.001
BMI (kg/m ²)	1.07 (1.00–1.15)	0.044	1.08 (1.02–1.15)	0.010	1.10 (1.03–1.16)	0.002
DM	–	–	–	–	1.01 (0.61–1.68)	0.976
FPG (mmol/l)	–	–	1.11 (0.90–1.37)	0.331	–	–
2hrG (mmol/l)	1.00 (0.95–1.05)	0.954	0.98 (0.90–1.06)	0.562	–	–
HOMA-IR ^a	1.06 (0.77–1.48)	0.711	–	–	–	–
Dyslipidaemia	–	–	–	–	1.43 (0.89–2.31)	0.143
HDL-C (mmol/l)	0.62 (0.26–1.50)	0.292	0.57 (0.24–1.32)	0.188	–	–
LDL-C (mmol/l)	0.91 (0.71–1.17)	0.467	0.92 (0.73–1.17)	0.504	–	–
TG (mmol/l) ^a	1.40 (0.85–2.31)	0.187	1.40 (0.86–2.28)	0.177	–	–
HT	–	–	–	–	2.03 (1.31–3.14)	0.002
SBP ^b (mmHg)	1.01 (0.99–1.03)	0.237	1.02 (1.00–1.03)	0.088	–	–
DBP ^b (mmHg)	1.01 (0.97–1.04)	0.744	1.00 (0.97–1.03)	0.791	–	–
Smoking	1.25 (0.96–1.88)	0.278	1.30 (0.88–1.92)	0.183	1.82 (0.88–1.91)	0.185

All significant associations are highlighted in bold.

Model 1: multiple adjustments made for age, BMI, 2hrG, HOMA-IR, HDL-C, LDL-C, TG, SBP, DBP and smoking. Model 2: multiple adjustments made for age, BMI, DM, dyslipidaemia, HT and smoking. 2hrG, 2-h post-OGTT glucose; DM, type 2 diabetes; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homoeostasis model assessment index of insulin resistance; HR, hazard ratio; HT, hypertension; SBP, systolic blood pressure; TG, triglyceride.

^aNatural log transformed before analysis.

^bSBP+10 mmHg and DBP+5 mmHg if on anti-hypertensive drug.

Discussion

In this study, we observed a significant association of +276G>T with CHD development in men in a general population, independent of conventional cardiovascular risk factors. Consistent with previous cross-sectional studies (27, 28, 29), the +276G>T variant showed a significant association with DM in our cohort. The current study demonstrated that the +276G>T variant was independently associated with incident CHD in men, even after adjustment for DM or its related traits, together with other potential confounding factors, in the different multiple adjustment models. We also observed that +276G>T was associated with a lower plasma adiponectin level in this Chinese population, as reported previously in studies among Italians (21) and Greeks (23). Our findings suggest that low adiponectin levels, as influenced by a genetic variant in the *ADIPOQ* gene, confer a higher risk of CHD, in keeping with a role of low circulating adiponectin levels in the development of CHD.

The T allele of +276G>T had previously been found to be associated with CHD in case-control cross-sectional studies among Chinese (24), Italians (21) and Greeks (23). The current study has further provided evidence for its association with an increased risk of developing CHD in a population-based cohort, likely through a reduction in adiponectin expression. Intriguingly, a significant association of the +276G>T variant with incident CHD

was only present in the male subjects of our cohort. The differences in cardiovascular risk profile between the two genders, such as lipid levels and blood pressure, may have contributed to the observed gender-specific association.

Figure 1

The cumulative survival curves for incident CHD in men, based on multiple Cox regression Model 1, with adjustment for age, BMI, 2hrG, HOMA-IR, HDL-C, LDL-C, TG, SBP, DBP and smoking. The cumulative survival curves were stratified by the +276G>T genotypes.

The higher CHD incidence rate in men (7.1 per 1000 person-years) compared with women (4.0 per 1000 person-years) may also be a contributing factor. The lack of a significant association in women might have been attributable to the smaller number of new CHD events. Adiponectin levels were known to be higher in women and later decline with abdominal adiposity, which also show gender-specific differences (25, 30). Indeed, adiponectin level was found to be higher in the female subjects of our cohort. Gender-specific differences in adiponectin levels have been shown to be strongly associated with serum androgen levels (25). Our group has previously demonstrated that testosterone selectively decreased the circulating concentrations of the high-molecular-weight form of adiponectin by impeding its secretion from the adipose tissue (17). Androgens have been shown to decrease plasma adiponectin and the androgen-induced hypoadiponectinaemia may lead to a higher risk of atherosclerosis in men (31). The unfavourable consequences of lower adiponectin levels could possibly lead to a higher risk of developing CHD in men than in women. Therefore, the genetic effect of *ADIPOQ* might be more readily detected in the high-risk male subjects.

In this long-term longitudinal study, we have demonstrated the independent association of *ADIPOQ* +276G>T with incident CHD in a general population. From previous prospective studies that examined the genetic effect of +276G>T on CHD (11) or CVD (9, 10) development, a US study based on diabetic men reported a significant association of this SNP with CVD development (9). In contrast to our findings, they reported that the T allele was associated with a lower risk of CVDs and increased adiponectin level (9). This discrepancy may be explained by the difference in inclusion criteria (subjects from the general population vs all DM patients and study endpoints (CHD vs CVD)). Two other longitudinal studies (10, 11) did not detect a significant association of +276G>T, but reported significant associations of rs266729 and rs17300539 (monomorphic in Han Chinese), with CVD development (10) and rs822395 with CHD risk (11). However, no significant associations of rs266729 and rs822395 were detected in this study. Ethnic differences in genetic composition and interaction, together with distinct environmental factors, may contribute to these variations in findings.

The +276G>T variant was located within intron 2 of *ADIPOQ*. The biological significance of this SNP and the mechanism through which this variant leads to altered adiponectin levels have not been fully elucidated. We postulated that it may potentially affect the

transcriptional activity or the splicing efficiency of the *ADIPOQ* gene. Previous studies have demonstrated that intronic polymorphisms can influence the transcription activity or splicing processes, even when the variants are located more than 30 bp away from the nearest splice junction (32). Intronic splicing control elements, which are involved in recognition of the appropriate splice site or in regulation of the splice site usage, have been found as far as 200 bp away from the splice site (33). Therefore, the +276G>T variant, which is located ~60 bp away from the nearest splice junction, may potentially affect the splicing efficiency. Future functional studies would be helpful to delineate the effect of this variant on adiponectin protein expression.

This study has several limitations. The relatively small number of incident CHD cases, despite the long follow-up period, has made this study slightly underpowered. Nonetheless, the current sample size could achieve over 80% power to detect a significant association of +276G>T with a large effect size of 1.54 in men, at a significance level of 0.05. On the other hand, the effects of the other *ADIPOQ* SNPs may be too modest for a significant association to be detected. This study was limited by the small number of hard endpoint cases, such as MI, and has therefore made it difficult for a definitive conclusion to be drawn. Furthermore, the current study would have been significantly strengthened with the detailed analyses of more CHD phenotypes. However, as data were retrieved from the hospital authority database, detailed information on coronary disease severity, such as the degree of coronary stenosis, were not available. Owing to the limited plasma samples available for the analysis of adiponectin levels at baseline, adiponectin levels obtained at CRISPS2 were used as an alternative to examine the association with the +276G>T variant and incident CHD in the current study. However, with the small sample size, in particular the limited number of incident CHD cases, as well as a shorter follow-up interval of ~9.6 years, we were unable to demonstrate a significant association between CRISPS2 adiponectin level and the development of CHD. Nevertheless, the HRs were in keeping with the protective effect of adiponectin against CHD development in a general population initially free of the disease. Furthermore, we have considered only the most, but not all, confounding factors for the development of CHD. Owing to the observational study design, detailed information on lifestyle modification or treatment interventions, including the effects of different drugs used and changing dosage during the study period, which may act as potential confounding factors, were not available for analyses.

Taken into consideration, these potential biases, effects of dysglycaemia (including DM, FPG and 2hrG), HT and dyslipidaemia were adjusted for in the multiple adjustment analyses. Other confounding factors in this observational study, such as the effect of attrition, may also lead to potential bias. The CRISPS cohort is a population-based study of the Hong Kong Southern Chinese. The findings from this study may not be generalised to other nations. Nonetheless, our data represent one of the largest longitudinal cohorts of Chinese subjects with a long follow-up period. With the growing epidemic of CHD risk factors, such as DM and hyperlipidaemia, in the Chinese population, the present findings may still be clinically significant. Further prospective studies in Chinese, as well as in other populations, would be useful to validate our results.

In conclusion, this study demonstrated that the +276G>T variant of *ADIPOQ*, associated with low circulating adiponectin levels, may be an independent predictor of CHD in community-based Southern Chinese men initially free of CHD. The current study has provided evidence that a low adiponectin level, as may be affected by a genetic variant in the *ADIPOQ* gene, confers a greater risk of CHD. Our data are suggestive of a protective role of adiponectin in CHD development in the healthy population. Our findings also support the notion that high adiponectin levels are associated with a lower risk of CHD in healthy populations, whereas high levels in established CVD may reflect a compensatory up-regulation of adiponectin. The +276G>T variant of *ADIPOQ* may affect the adiponectin gene expression and may act as a potential genetic marker for the prediction of CHD.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-14-0079>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

C Y Y Cheung and E Y L Hui contributed equally to this work and should be considered as co-first authors. P C Sham and K S L Lam contributed equally

to the supervision of this work and are co-corresponding authors. C Y Y Cheung, E Y L Hui, K S L Lam and P C Sham conceived and designed the experiments. K S L Lam initiated and supervised the study. C Y Y Cheung performed the experiments and analysed the data. C Y Y Cheung and E Y L Hui wrote the paper. A Xu, Y C Woo, C Y Yeung, K L Ong and C H Y Fong collected the data and provided advice on experiments and data analysis. K S L Lam, P C Sham, B M Y Cheung, E D Janus and H-F Tse critically revised the manuscript.

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