Thyroid Peroxidase Antibody is Associated with Plasma Homocysteine Levels in Patients with Graves' Disease

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ABSTRACT

Purpose Homocysteine is associated with cardiovascular, inflammation and autoimmune diseases. Previous studies have shown that thyroid peroxidase antibody is associated with homocysteine levels in hypothyroidism. The relationship between thyroid antibodies and homocysteine in hyperthyroidism remains unclear. In this study, we aimed to investigate the association of thyroid antibodies with homocysteine in patients with Graves' disease.

Methods This was a cross-sectional study including 478 Graves' disease patients who were consecutively admitted and underwent radioiodine therapy. Homocysteine, thyroid hormones, thyroid antibodies, glucose and lipids were measured.

Results Patients with homocysteine levels above the median were older and had unfavorable metabolic parameters compared to patients with homocysteine levels below the median. Thyroglobulin antibody or thyroid peroxidase antibody was associated with homocysteine levels (β = 0.56, 95 %CI 0.03-1.08, p = 0.04; β = 0.75, 95 %CI 0.23-1.27, p = 0.005). The relationship between thyroid peroxidase antibody and homocysteine remained significant when additionally adjusting for free triiodothyronine (β = 0.76, 95 %CI 0.24-1.28, p = 0.004). The presence of a homocysteine level above the median increased significantly with increasing thyroid peroxidase antibody quartiles in the logistic regression (OR = 1.74, 95 %CI 1.27-2.39, P for trend = 0.001). Homocysteine levels increased significantly with increasing thyroid peroxidase antibody quartiles (p = 0.005). Thyroid peroxidase antibody had no significant effect on other traditional cardiovascular risk factors.

Conclusions Thyroid peroxidase antibody is independently and positively associated with homocysteine levels in patients with Graves' disease. Thyroid peroxidase antibody may be associated with the cardiovascular risk of patients with Graves' disease through its effect on homocysteine.

Introduction

Thyroid hormones have a significant impact on heart function. Patients with thyroid disease are at high risk of cardiovascular diseases. In hyperthyroidism, the cardiovascular effects include an increase in resting heart rate, blood volume, myocardial contractility, and ejection fraction, systolic hypertension, systolic murmurs, increased left ventricular mass, exercise intolerance and angina pectoris [1]. Complications include atrial fibrillation with its risk of stroke, and high output heart failure. Subclinical hyperthyroidism patients also have an increased risk of developing atrial fibrillation [2, 3]. Even individuals with high normal thyroid function have an increased risk of atrial fibrillation as well as for atherosclerosis and myocardial infarction [4–6]. On the other hand, thyroid hormone deficiency results in lower heart rate and weakening of myocardial contraction and relaxation [7].

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Homocysteine (Hcy), which is a sulfhydryl-containing amino acid, is an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine [8]. A large amount of studies have shown that Hcy is an independent risk factor of cardiovascular diseases [9–12]. Immune activation and inflammation may be the cause of hyperhomocysteineamia. Recent study has indicated that there may be a bi-directional link between Hcy and immuno-inflammatory activation, in which immuno-inflammatory activation may contribute to Hcy increase, and Hcy, in its turn, may act as a pro-inflammatory and immuno-stimulating molecule putatively cooperating to the injury of the disease-specific target organs [13]. A positive relationship was found between the concentration of Hcy and some bio-humoral parameters of inflammation, such as the circulating levels of soluble receptors for different cytokines, C-reactive protein (CRP) and adhesion moleculars [14–16]. An association also exists between the level of Hcy and the accumulated disease activity [14].

Autoimmune thyroid disease is a cluster of autoimmune diseases which are commonly seen in clinic. A study has shown that Hcy level is higher in hypothyroid patients compared to those euthyroid patients [17]. In patients with iatrogenic hypothyroidism, those with Hashimoto's thyroiditis have significant higher Hcy levels than those without Hashimoto's thyroiditis [18]. There are significant abnormally high Hcy levels in thyroid peroxidase antibody (TPOAb) or thyroglobulin antibody (TGAb) positive subjects [8]. A recent study indicates that in subclinical hypothyroidism and chronic autoimmune thyroiditis patients with elevated TPOAb, when accompanied by increased Hcy, may be taken to indicate the presence of clinically unrecognized coronary heart disease [19]. All these studies have shown that autoimmune thyroid disease with or without hypothyroidism has an elevated Hcy level. TPOAb and TGAb as the autoimmune markers of autoimmune thyroid disease may be associated with the increase of Hcy.

Graves' disease is one of the common autoimmune thyroid diseases which cause hyperthyroidism. Patients with Graves' disease have an increased cardiovascular risk, whether their immune-inflammatory condition is associated with Hcy levels is not clear. The present study aims to investigate the association of Hcy with TPOAb, TGAb and thyrotrophin receptor antibody (TRAb) in Graves' disease patients.

Materials and Methods

Study population

This was a cross-sectional study including Graves' disease patients who were consecutively admitted and underwent radioiodine therapy in the department of Endocrinology and Metabolism in Zhongshan Hospital, Fudan University from May 2013 to August 2017. Of the 615 individuals who were screened, 137 subjects who did not measure plasma Hcy were excluded from the study. The final analysis comprised 478 subjects. All patients in our study underwent radioactive iodine uptake testing and thyroid ultrasound examination. Besides, of the 478 subjects in our study, TRAb was measured in 465 subjects. The diagnosis of Graves' disease in our study are based on clinical features, elevated levels of thyroxine and triiodothyronine, suppressed levels of thyroid stimulating hormone (TSH), a positive TRAb, an elevated radioactive iodine uptake and a diffused thyroid enlargement on ultrasound and isotope scanning.

Anthropometric and laboratory measurements

Medical history, medication information and smoking status were collected from the medical records. Height and weight were measured using a wall-mounted stadiometer with patients wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a mercury sphygmomanometer on the right arm of subjects in a seated position after a rest for at least 5 min. Waist circumference was measured at the midway between the lowest rib and the iliac crest with the subject standing. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. Waist-to-hip ratio (WHR) was calculated as waist circumference (m)/hip circumference (m).

Hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or current use of medication for hypertension. Diabetes mellitus (DM) was defined according to the American Diabetes Association Guideline [20], or current use of medication for diabetes. Hyperlipidemia was defined as total cholesterol (TC) ≥ 5.2 mmol/L, triglycerides (TG) ≥ 1.7 mmol/L or current use of medication for hyperlipidemia. Coronary artery disease and stroke were defined according to the medical history.

A morning blood sample was collected after at least 8 h of fasting. Assay for fasting plasma glucose (FPG), 2-h postprandial plasma glucose (2hPPG), TC, TG, high density lipoprotein cholesterol (HDL-C) and CRP were measured by a model 7600 automated bioanalyzer (Hitachi, Tokyo, Japan). The level of low density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation. Hcy was measured using enzymatic cycling assay (Diasys Diagnostic Systems, Shanghai CO., LTD). Glycosylated hemoglobin (HbA1c) was measured using high performance liquid ion exchange chromatography by the Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA). TSH, total triiodothyronine (TT3), total thyroxine (TT4), free triiodothyronine (FT3), free thyroxine (FT4), TPOAb, TGAb and TRAb were measured using the electrochemical luminescence method by Modular E170 automatic electrochemiluminescence analyzer (Roche Diagnostics Ltd., Germany).

Statistical analysis

All analysis was performed using SPSS (version 19.0, SPSS Inc, Chicago, USA). Two side tests were used. Significance level was defined as $P < 0.05$. Data are expressed as mean ± standard deviation (SD) or median and interquartile range for continuous variables and as percentage (%) for categorical variables. TGAb and TPOAb were SD-transformed for analysis. Values in the tables are expressed as untransformed data for ease of interpretation. Subjects were classified into two groups (group with Hcy level ≤ 9.55 mmol/L and group with Hcy level > 9.55 mmol/L) according to the median of Hcy. The characteristic comparisons between the two Hcy groups were assessed using t-test or Chi-square test. The associations of Hcy with clinical characteristics were assessed using Pearson linear correlation. Multivariate regression analysis was performed to evaluate the relationship between Hcy and thyroid antibodies. Subjects

were categorized into quartiles of TGAb concentration: ≤ 17.30 IU/ml, 17.31-153.50 IU/ml, 153.51-435.70 IU/ml, > 435.70 IU/ml; of TPOAb concentration: ≤ 26.47 IU/ml, 26.48-159.35 IU/ml, 159.36-413.08 IU/ml, > 413.08 IU/ml; and of TRAb concentration: ≤ 4.30 IU/ml, 4.31-9.40 IU/ml, 9.41-18.70 IU/ml, > 18.71 IU/ml. Logistic regression analysis was performed to evaluate the relationship between TPOAb, TGAb or TRAb quartiles and the presence of a Hcy level > 9.55 mmol/L. General linear analysis was performed to evaluate the relationship between TPOAb quartiles and cardiovascular risk factors.

▶ **Table 1** Clinical characteristics according to the median of Hcy levels in patients with Graves' disease.

Variables	All	Hcy level ≤ 9.55 mmol/L	Hcy level > 9.55 mmol/L	P value
Gender, n (M/F)	478,135/343	241,23/218	237,112/125	< 0.001
Age(years)	43.73 ± 14.04	41.38 ± 12.64	46.11 ± 14.98	< 0.001
Disease duration(years)	0.67(0.17,5.00)	0.75(0.17,5.00)	0.60(0.17,5.00)	0.60
Previous radioiodine therapy(%)	2.50	2.50	2.50	0.98
Comorbidities				
Hypertension(%)	9.20	5.40	13.10	0.004
Coronary artery disease(%)	1.00	0.80	1.30	0.64
Stroke(%)	0.40	0.40	0.40	0.99
Hyperlipidemia(%)	0.80	0.80	0.80	0.99
Diabetes mellitus(%)	7.00	3.90	9.60	0.09
Smoker(%)	9.60	4.10	15.20	< 0.001
Anthropometry parameters				
BMI(kg/m^2)	22.22 ± 3.29	22.18 ± 3.30	22.25 ± 3.30	0.86
Waist circumference(cm)	77.55 ± 9.75	75.59 ± 10.18	79.24 ± 9.12	0.05
Hip circumference(cm)	91.32 ± 7.47	90.86 ± 8.30	91.72 ± 6.71	0.55
WHR	0.85 ± 0.08	0.83 ± 0.08	0.86 ± 0.06	0.03
Cardio-metabolic parameters				
Heart rate(bpm)	90.92 ± 13.72	91.18 ± 13.16	90.65 ± 14.28	0.67
SBP(mmHg)	123.83 ± 14.67	121.65 ± 14.19	126.00 ± 14.86	0.001
DBP(mmHg)	73.76 ± 9.43	73.54 ± 8.64	73.97 ± 10.18	0.62
FPG (mmol/L)	5.21 ± 1.24	5.21 ± 1.01	5.22 ± 1.44	0.94
2hPPG(mmol/L)	7.56 ± 2.64	7.03 ± 1.89	8.11 ± 3.14	< 0.001
HbA1c(%)	5.50 ± 0.78	5.45 ± 0.73	5.56 ± 0.83	0.17
TC(mmol/L)	3.18 ± 0.71	3.20 ± 0.62	3.17 ± 0.78	0.62
TG(mmol/L)	0.99 ± 0.50	0.99 ± 0.45	1.00 ± 0.55	0.88
HDL-C(mmol/L)	1.18 ± 0.35	1.22 ± 0.33	1.15 ± 0.36	0.03
LDL-C(mmol/L)	1.55 ± 0.53	1.54 ± 0.50	1.57 ± 0.57	0.51
Thyroid function and antibodies				
TT3(nmol/L)	5.63 ± 2.29	5.57 ± 2.35	5.68 ± 2.24	0.64
TT4(nmol/L)	203.75 ± 59.56	201.66 ± 59.33	205.81 ± 59.85	0.47
FT3(pmol/L)	24.70 ± 12.32	24.48 ± 12.61	24.93 ± 12.05	0.69
FT4(pmol/L)	61.63 ± 26.09	60.74 ± 26.21	62.53 ± 26.00	0.46
TSH(uIU/mL)	0.01 ± 0.08	0.01 ± 0.03	0.01 ± 0.11	0.47
TGAb(IU/mL)	153.50(17.30,435.70)	142.55(19.95,400.65)	162.50(16.90,524.90)	0.65
TPOAb(IU/mL)	159.35(26.48,413.08)	134.80(21.75,337.20)	193.70(45.70,500.10)	0.03
TRAb(IU/L)	9.40(4.30,18.70)	9.25(4.08,17.50)	9.50(4.40,20.50)	0.49
Inflammation marker				
Hcy(mmol/L)	10.79 ± 5.80	7.64 ± 1.18	14.00 ± 6.80	< 0.001
CRP(mmol/L)	0.80(0.50,1.70)	0.70(0.40,1.5)	0.90(0.50,2.23)	0.04

Data were expressed as mean ± SD or median and interquartile range for continuous variables and as percentage (%) for categorical variables. Patients were classified into two groups according to the median of Hcy, group with Hcy ≤ 9.55 mmol/L and group with Hcy > 9.55 mmol/L. SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WHR: waist-to-hip ratio, FPG: fasting plasm glucose, 2hPPG: 2-h postprandial plasm glucose, HbA1c: glycosylated hemoglobin, TC: total cholesterol, TG: triglycerides, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, Hcy: homocysteine, TT3: total triiodothyronine, TT4: total thyroxine, FT3: free triiodothyronine, FT4: free thyroxine, TSH: thyroid stimulating hormone, TGAb: thyroglobulin antibody, TPOAb: thyroid peroxidase antibody, TRAb: thyrotrophin receptor antibody, CRP: C-reactive protein.

▶ **Table 2** Pearson correlation analysis between Hcy and clinical and laboratory parameters in patients with Graves' disease.

Variables	Coefficient	P Value
Age	0.11	0.02
Disease duration	-0.06	0.20
Heart rate	-0.06	0.22
SBP	0.14	0.002
DBP	0.01	0.81
BMI	0.09	0.16
Waist circumference	0.20	0.04
Hip circumference	0.08	0.41
WHR	0.20	0.04
TC	0.03	0.51
TG	0.003	0.94
HDL-C	-0.09	0.06
LDL-C	0.10	0.03
CRP	0.12	0.01
FPG	0.08	0.10
2hPPG	0.16	0.002
HbA1c	0.06	0.18
TT3	-0.02	0.72
TT4	-0.002	0.97
FT3	-0.04	0.39
FT4	-0.05	0.30
TSH	0.01	0.89
TGAb	0.20	<0.001
TPOAb	0.19	<0.001
TRAb	0.01	0.76

SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, WHR: waist-to-hip ratio, TC: total cholesterol, TG: triglycerides, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, CRP: C-reactive protein, FPG: fasting plasm glucose, 2hPPG: 2-h postprandial plasm glucose, HbA1c: glycosylated hemoglobin, TT3: total triiodothyronine, TT4: total thyroxine, FT3: free triiodothyronine, FT4: free thyroxine, TSH: thyroid stimulating hormone, TGAb: thyroglobulin antibody, TPOAb: thyroid peroxidase antibody, TRAb: thyrotrophin receptor antibody.

▶ **Table 3** Multivariate linear regression analysis for the association of thyroid antibodies with Hcy in patients with Graves' disease.

Variables	β	95%CI	P Value
TPOAb[a]	0.75	0.23-1.27	0.005
TPOAb[b]	0.76	0.24-1.28	0.004
TGAb[a]	0.56	0.03-1.08	0.04
TGAb[b]	0.52	−0.01-1.06	0.06
TRAb[a]	2.60	−37.32-42.51	0.90
TRAb[b]	−7.85	−0.03-0.01	0.26

TGAb and TPOAb were SD-transformed for analysis. [a] adjusting for gender, age, disease duration, SBP, DBP, BMI, FPG, 2hPPG, TC, TG, HDL-C, LDL-C, CRP and smoking status; [b] adjusting for gender, age, disease duration, SBP, DBP, BMI, FPG, 2hPPG, TC, TG, HDL-C, LDL-C, CRP, smoking status and FT3.

Results

We divided the patients into two groups according to the median of Hcy. The comparisons of characteristics between the groups were presented in ▶ **Table 1**. There were more men in group with Hcy level > 9.55 mmol/L. Subjects in group with Hcy level > 9.55 mmol/L were older and had higher WHR, SBP, 2hPPG, TPOAb and CRP levels, and lower HDL-C levels. Subjects in group with Hcy level > 9.55 mmol/L also had higher proportion of hypertension and more smokers. There was no significant difference of the disease duration, previous radioiodine therapy, and prevalence of coronary artery disease, stroke, hyperlipidemia and DM between the two groups. There was no significant difference of the concomitant medication use between the two groups. There were no significant difference of DBP, BMI, FPG, HbA1c, TT3, TT4, FT3, FT4, TSH, TGAb and TRAb levels between groups.

Pearson correlation analysis showed that Hcy levels were positively associated with age, SBP, waist circumference, WHR, LDL-C, CRP, 2hPPG, TGAb and TPOAb (▶ **Table 2**). The multivariate regression analysis showed that TPOAb was associated with Hcy level after adjusting for gender, age, disease duration, SBP, DBP, BMI, FBG, 2hPPG, TC, TG, HDL-C, LDL-C,CRP and smoking status (β = 0.75, 95 %CI 0.23-1.27, P = 0.005). The relationship between TPOAb and Hcy remained significant if additionally adjusting for FT3 (β = 0.76, 95 %CI 0.24-1.28, P = 0.004). TGAb was also associated with Hcy level after adjusting for gender, age, disease duration, SBP, DBP, BMI, FBG, 2hPPG, TC, TG, HDL-C, LDL-C,CRP and smoking status (β = 0.56, 95 %CI 0.03-1.08, P = 0.04). But the relationship between TGAb and Hcy became insignificant after additionally adjusting for FT3 (β = 0.52, 95 %CI -0.01-1.06, P = 0.06) (▶ **Table 3**).

The presence of a Hcy level > 9.55 mmol/L increased significantly with increasing TPOAb quartiles in the multivariate logistic regression after adjusting for gender, age, disease duration, SBP, DBP, BMI, FPG, 2hPPG, TC, TG, HDL-C, LDL-C, CRP and smoking status (OR = 1.74, 95 %CI 1.27-2.39, P for trend = 0.001). The association of TPOAb quartiles and the presence of a Hcy level > 9.55 mmol/L remained significant if additionally adjusting for FT3 (OR = 1.77, 95 %CI 1.28-2.44, P for trend = 0.001) (▶ **Table 4**). The association of TGAb or TRAb quartiles with the presence of a Hcy level > 9.55 mmol/L was not significant (▶ **Table 4**).

The general linear model analysis showed that Hcy levels increased significantly across the TPOAb quartiles after adjusting for gender, age, BMI and FT3 (p = 0.005). TPOAb had no significant effect on other traditional cardiovascular risk factors which included SBP, DBP, FPG, 2hPPG, HbA1c, TC, TG, HDL-C, LDL-C and CRP levels after adjusting for gender, age, BMI and FT3 (▶ **Fig. 1**).

Discussion and Conclusions

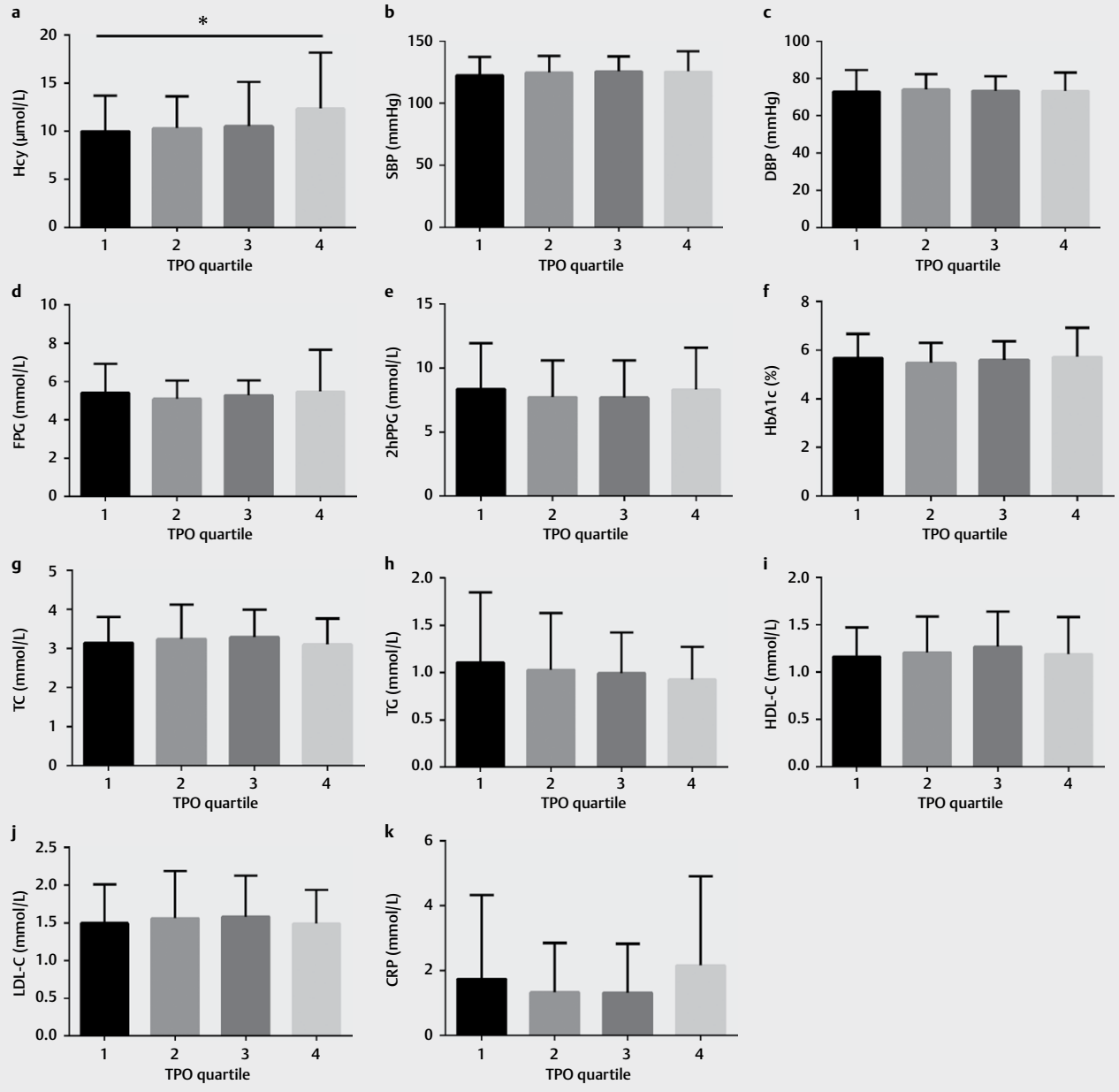
For the first time, this study showed an independent association of TPOAb with the level of Hcy in patients with Graves' disease.

Plasma Hcy levels are associated with major cardiovascular risks including gender, age, smoking, blood pressure, cholesterol and physical exercise, and the associations are dose-dependent [21]. Consistently, the Hcy levels are associated with age, SBP, waist circumference, WHR, LDL-C, CRP and 2hPPG in our study. Plasma concentration of Hcy in healthy humans is low and the level is between 5.0 and 15.0 μmol/L when assessed with the use of HPLC or 5.0-

▶ **Table 4** Logistic regression analysis for the association of thyroid antibodies with the presence of Hcy level > 9.55mmol/L in pateints with Graves' disease.

Variales	OR(95%CI)				P for trend
	Q1(ref.)	Q2	Q3	Q4	
TPOAb[a]	1	4.35(1.49-12.73)	3.18(1.05-9.64)	7.50 (2.63-21.38)	0.001
TPOAb[b]	1	4.26 (1.44-12.58)	3.33(1.10-10.08)	7.77(2.68-22.52)	0.001
TGAb[a]	1	1.33(0.49-3.62)	2.10(0.74-5.96)	2.08(0.79-5.49)	0.10
TGAb[b]	1	1.43(0.52-3.97)	2.20(0.77-6.29)	1.96(0.73-5.26)	0.14
TRAb[a]	1	1.29(0.48-3.50)	0.99(0.37-2.67)	1.17(0.45-3.02)	1.00
TRAb[b]	1	1.11(0.40-3.05)	0.90(0.33-2.46)	0.80(0.28-2.29)	1.03

[a] adjusting for gender, age, disease duration, SBP, DBP, BMI, FBG, 2hPPG, TC, TG, HDL-C, LDL-C, CRP and smoking status; [b] adjusting for gender, age, disease duration, SBP, DBP, BMI, FBG, 2hPPG, TC, TG, HDL-C, LDL-C, CRP, smoking status and FT3.



▶ **Fig. 1** Effects of TPOAb quartiles on cardiovascular risks. The general linear model showed that the effect of TPOAb quartiles on Hcy was significant after adjusting for gender, age, BMI and FT3 (p = 0.005), and there was no significant effect of TPOAb quartiles on SBP, DBP, FPG, 2hPPG, HbA1c, TC, TG, HDL-C, LDL-C and CRP levels after adjusting for gender, age, BMI and FT3.

12.0 µmol/L when immunoassay methods are used [12]. Hcy levels in our patients are 10.79 ± 5.80 mmol/L, which are within the normal range of the method used in our study. This result is different from previous studies that Hcy levels decreased in hyperthyroidism [22, 23]. The definition of hyperhomocysteineamia is based on a cut-off above the 90th or 95th percentile of the distribution of Hcy in the general population [9]. It's more appropriate to define hyperhomocysteineamia as a level of Hcy that correlates with a sudden increase in the risk of vascular events. Unfortunately, there is no definite threshold that correlates with a sudden increase in the risk of cardiovascular event and the relation between Hcy and risk appears to be linear [9].

Among the thyroid antibodies in Graves' disease, only TPOAb has an independent association with Hcy in our study. As we know that TRAb induces Graves' disease and stimulates thyroid hormone production, but there are no associations between Hcy and TRAb. TGAb is associated with Hcy in our study, but the relationship between TGAb and Hcy is dependent of thyroid function. Although the level of Hcy is not high in our study, there is a strong and positive association of Hcy with TPOAb levels, which is independent of other known cardiovascular risk factors and thyroid function. We also find that TPOAb is not associated with other cardiovascular risk factors. Therefore, it is possible that TPOAb induces an increase of Hcy in the normal range and leads to a higher cardiovascular risk in Graves' disease. Previous studies of subclinical hypothyroidism and chronic autoimmune thyroiditis patients also showed an association between Hcy and TPOAb, and the association is independent of thyroid function [17–19].

The underlying mechanism of the association between TPOAb and the levels of Hcy in Graves' disease remains unclear. The mechanisms, although not completely clarified, are probably multiple and intriguing [13]. Studies have shown that immune activation and inflammation may induce the increase of Hcy [13]. In contrast to TGAb, TPOAb is able to induce the complement system and cellular cytotoxicity. And there is an association between the concentration of TPOAb and the presence of lymphocytic infiltration of the thyroid gland [24, 25]. TPOAb might be related to the level of immunity of thyroid. So we suppose that immune and inflammation activities in Graves' disease may induce the increase of Hcy. A positive relationship was found between Hcy and some bio-humoral parameters of inflammation in rheumatoid arthritis, such as the circulating levels of soluble receptors for different cytokines, CRP, erythrocyte sedimentation rate and adhesion moleculars [14–16, 18, 26]. In our study, a positive relationship is also found between the levels of Hcy and CRP. But the relationship between TPOAb and the level of Hcy in Graves' disease is independent of CRP in our study. On the other hand, the accumulation of Hcy also can be attributed to the deficiency of folic acid, vitamins B6 or B12 [27]. In hyperthyroidism, Hcy is inversely related to folic acid, vitamin B12 and B2 levels [28]. Previous study has shown that thyroid antibody but not the thyroid function, is associated with folic acid or vitamin B12 levels [8, 29]. So the association between thyroidantibodies and Hcy may be mediated by folic acid or vitamin B12 levels. But it's a pity that folic acid and vitamin B12 levels are not measured in our study. In addition, patients with organ-specific autoimmune disease are prone to have other organ-specific autoantibodies [30]. The prevalence of parietal cell antibody positivity in general population is 7.8 % and is 19.5 % in the olds [31, 32]. In patients with autoimmune thyroid disease, the prevalence of parietal cell antibodies positivity is 20 % [33], and in patients with Graves' disease, the prevalence is 22 % [30]. Studies show that elevated levels of thyroid antibodies increase the risk of parietal cell antibodies positivity [33, 34]. On the other hand, about 30 % patients with positive parietal cell antibody have positive thyroid antibodies [35]. In addition, a study also shows patients with positive parietal cell antibody have higher levels of Hcy than patients with negative parietal cell antibody [36]. So there may be some underlying association between thyroid antibodies, Hcy and parietal cell antibody. Parietal cell antibody should be considered as a potential confounder in our study. However, parietal cell antibody is not measured in our study. Further studies are needed to investigate the underlying mechanism of the association of TPOAb with Hcy.

This study has some limitations that deserve to be acknowledged. First of all, it was a cross-sectional study. Further prospective researches with long-term follow-up and cardiovascular endpoints are needed to determine if there is a causal relationship between TPOAb and Hcy. Besides, not all the potential variables associated with Hcy were measured, such as erythrocyte sedimentation rate, folic acid, vitamin B12 and parietal cell antibody, which may introduce bias to our observations.

In this study, we measure the Hcy levels in patients with Graves' disease. We find an independent and positive relationship between TPOAb and Hcy levels. There is no significant association of TPOAb levels with other traditional cardiovascular risk factors. The association of TPOAb with Hcy may reflect the immune-inflammatory effect of Graves' disease on Hcy levels, which contributes to the cardiovascular risk of Graves' disease.

Conflict of Interest

The authors declare no conflict of interest.

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