β_1 -adrenergic and Muscarinic Acetylcholine Type 2 Receptor Antibodies are Increased in Graves' Hyperthyroidism and Decrease During Antithyroid Therapy

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Key words

autoantibodies, G-protein-coupled receptor, β_1 receptor, angiotensin II type 1 receptor, muscarinic type 2 receptor, cardiac biomarkers

received 28.09.2020

revised 02.12.2020

accepted 22.12.2020

published online 12.01.2021

Bibliography

Exp Clin Endocrinol Diabetes 2021; 129: 783–790

DOI 10.1055/a-1342-2853

ISSN 0947-7349

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Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

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Supplementary Material is available under <http://doi.org/10.1055/a-1342-2853>

ABSTRACT

Objective To determine the association between autoantibodies to G-protein-coupled receptors with effect on the cardiovascular system and the cardiac biomarker N-terminal pro-brain natriuretic peptide reflecting heart function in Graves' disease.

Design and Methods Sixty premenopausal women with Graves' disease were analyzed for IgG autoantibodies against β_1 -adrenergic, muscarinic acetylcholine type 2 and angiotensin II type 1 receptors using enzyme-linked immunosorbent assays based on cell membranes overexpressing receptors in their native conformations. N-terminal pro-brain natriuretic peptide and heart symptoms were analyzed in hyperthyroidism and after 7.5 months of antithyroid treatment. Matched thyroid healthy controls were also assessed.

Results Serum levels of antibodies against the β_1 -adrenergic and the muscarinic acetylcholine type 2 receptors were higher in hyperthyroid patients than in controls (median β_1 -adrenergic receptor antibodies 1.9 [IQR 1.3–2.7] vs. 1.1 [0.8–1.7] $\mu\text{g/mL}$, $P < 0.0001$; muscarinic acetylcholine type 2 receptor 20.5 [14.0–38.3] vs. 6.0 [3.2–9.9] U/mL, $P < 0.0001$). These antibodies decreased in euthyroidism ($P < 0.01$), but were still higher than in controls ($P < 0.01$). Angiotensin II type 1 receptor levels did not differ. N-terminal pro-brain natriuretic peptide was higher in hyperthyroidism (240 [134–372] vs. <35 [<35–67] ng/L, $P < 0.0001$), normalized after treatment and did not correlate with autoantibodies.

Conclusion Autoantibodies against the β_1 -adrenergic and the muscarinic acetylcholine type 2 receptors were increased in Graves' patients, decreased with treatment, but did not correlate with cardiac function. However, an autoimmune effect on the heart cannot be excluded in subpopulations, as the functional properties of the analyzed antibodies remain to be determined.

Introduction

Graves' disease (GD) is caused by autoantibodies produced by B cells that have escaped central and/or peripheral immunological tolerance mechanisms. These autoantibodies activate the thyroid-stimulating hormone (TSH) receptor (TRAb), leading to thyroid hormone overproduction [1]. Besides classical symptoms like weight loss, anxiety, heat production and tremor, hyperthyroidism is associated with cardiac symptoms like tachycardia, dyspnoea and exercise intolerance. Changes in the circulatory system can be detected by echocardiography [2, 3] or by analysis of N-terminal pro-brain natriuretic peptide (NT-proBNP) [4], a sensitive marker of cardiac wall stress [5] with a high negative predictive value (0.94–0.98) [4, 5]. Another functional cardiac biomarker is troponin I (TNI), which indicates heart muscle damage [6]. GD may adversely affect the heart via several mechanisms [4]: (i) chronic tachycardia/atrial fibrillation [7]; (ii) direct stimulation of the heart by triiodothyronine (T3) [8, 9]; and (iii) imbalance between the sympathetic and vagal systems [10]. Furthermore, beside TRAb, antibodies targeting other receptors may also affect the cardiovascular system.

The TSH receptor belongs to the family of G-protein-coupled receptors (GPCRs). Autoantibodies binding to GPCRs (GPCRAB) are described in many diseases [11, 12], but the reason why these receptors are important targets for autoimmunity is not clear. Besides the TSH receptor, the GPCR family includes many additional receptors, including the adrenergic, muscarinic, and angiotensin receptors that are important regulators of the circulatory system. Autoantibodies to the angiotensin II type 1 receptor (ATR1Ab), β_1 -adrenergic receptor (B1RAB), and muscarinic acetylcholine type 2 receptor (M2RAB) have been described in several different diseases and have been shown to activate their receptors and affect cellular functions [11, 13, 14]. In GD patients with heart disease, increased levels of (ATR1Ab) [2], (B1RAB) and (M2RAB) [15, 16] are reported with proposed pathogenic effects. B1RAB and M2RAB facilitate atrial fibrillation, which is in line with animal studies indicating a causal effect on rhythm [17]. However, knowledge of the presence and impact of these GPCRAB in a general GD population without obvious clinical cardiac dysfunction is still lacking, as is knowledge of their response to antithyroid therapy (ATD). It is also principally important if other antibodies than those targeting the thyroid gland can be detected at increased levels in GD patients, as there are complications in GD where mechanisms are not fully understood.

In many previous studies, GPCRAB have been analyzed using non-standardized enzyme-linked immunosorbent assays (ELISAs) using small peptides of the receptor as target antigens or denatured antigen rather than entire GPCR in its native structural conformation [2, 14, 16]. Since antibodies often target conformational epitopes in the receptors, this may limit the ability to detect relevant antibody specificities [18–20]. Standardized ELISAs using cell membranes overexpressing the receptors in their native conformation as target antigens are now available for evaluation of GPCRAB, which enables the detection more physiologically relevant antibody specificities [21–24].

We hypothesized that GPCRAB, existing in parallel to classic thyroid autoimmunity, impair cardiac function in hyperthyroid Graves' patients without evident cardiac disease. Our aim was to analyze these autoantibodies longitudinally in hyperthyroidism and euthyroidism in a general population of premenopausal GD patients

using standardized cell-based ELISA analyses, to evaluate cardiac function biomarkers, and reveal any potential autoimmune impact on the heart.

Subjects and Methods

Participants

From September 2011 to March 2018, 65 premenopausal women with newly diagnosed GD were included in the CogThy study, a study focusing on the mental consequences of GD, at Sahlgrenska University Hospital, Gothenburg, Sweden [25]. In this sub-study, data were used for the first 60 patients enrolled until September 2017.

Patients were eligible for the CogThy study if they were premenopausal with newly diagnosed first-time GD with free thyroxine (FT4) levels ≥ 50 pmol/L (reference range: 12–22 pmol/L) and/or total T3 ≥ 6.0 nmol/L (reference range: 1.3–3.1 nmol/L) in combination with positive TRAb and/or a technetium scintigraphy with a diffuse uptake. Exclusion criteria were pregnancy, other serious disease including heart failure, thyroid-associated ophthalmopathy with expected steroid treatment, previous/present steroid treatment, contraindications for magnetic resonance imaging of the brain, amiodarone-induced GD, if > 2 weeks after starting ATDs, or inability to follow the study protocol.

Patients were included within 2 weeks from the start of ATD treatment (► **Table 1**) when still hyperthyroid (► **Table 2**). All patients received standard GD treatment for this age group, i.e. 18 months of ATD treatment or thyroid surgery. One patient was treated with radioactive iodine (RAI).

We approached 103 patients who met the criteria for participation and 58 % were enrolled. Reasons for non-enrolment are presented as **supplementary data** (Supportive ► **Figure 1**).

Patients were divided into four age groups (18–27, 28–36, 37–45, and 46–55 years) and the same numbers of controls were recruited to each age group. Eligible controls were premenopausal, non-pregnant women with no cardiac, thyroid, or autoimmune diseases. The controls comprised 38 women who gave blood samples for the purpose of this study and 22 participating in a vaccine trial who had consented the use of serum samples in other studies after sample anonymization [26] (► **Table 1**).

Ethics

The study was approved by the regional Ethical Review Board in Gothenburg, Sweden, and was performed according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Methods

Questions of relevance for the cardiovascular system (palpitations, shortness of breath, and swollen hands/feet) from the thyroid-specific Patient-Reported Outcome (ThyPRO) quality of life questionnaire [27] were used at inclusion and after 15 months.

Serum and plasma samples were stored at -80°C until analysis for GPCRAB, NT-proBNP, TNI, and thyroid-stimulating immuno-

globulin (TSI). FT4, FT3, TSH, and TRAb were analyzed in fresh samples. Controls were analyzed once for GPCrAb, NT-proBNP, and TNI.

► **Table 1** Baseline characteristics and follow-up data of women with Graves' disease.

Group and parameter	Inclusion	Follow-up at 7.5 months
Patients, <i>n</i>	60	43
Age (yr)	32 [26–39] ^a	32 [27–40] ^a
BMI (kg/m ²)	22.2 [19.9–24.9]	24.4 [21.9–27.8]
Time from blood test at diagnosis to inclusion (days)	13.5 [11.0–19.8]	–
Time from starting antithyroid drug to blood test at inclusion (days)	8 [6–12]	–
Duration of symptoms before diagnosis (months) ^b	4 [3–7]	–
Previous smokers, <i>n</i> (%)	23 [38]	–
Smokers at inclusion, <i>n</i> (%)	8 [13]	–
Patients treated with antithyroid drugs, <i>n</i> (%)	57 [95]	31 [72]
Thiamazole	55 [92]	28 [65]
Propylthiouracil	2 [3]	3 [7]
Patients who had thyroidectomy ^c , <i>n</i> (%)	0	10 [23]
Patients who had radioactive iodine treatment ^d , <i>n</i> (%)	0	1 [2]
Patients treated with beta-blockers ^e , <i>n</i> (%)	45 [75]	4 [9]
Controls, <i>n</i>	60	
Age (yr)	32 [26–41] ^a	–
Abbreviations: BMI, body mass index; IQR, interquartile range; yr, years. ^a Range 20–54 years; ^b <i>n</i> = 58; ^c Median (IQR) from inclusion to surgery was 5 (3.8–5.5) months and to follow up 3.5 (1.5–4.3) months; ^d Radioactive iodine given for 2.5 months after inclusion; ^e Metoprolol or propranolol.		

Thyroid hormones were analyzed with electrochemiluminescence methods: FT3 (reference range: 3.1–6.8 pmol/L), FT4 (reference range: 12–22 pmol/L), and TSH (reference range: 0.30–4.2 mIU/L), all with Roche Cobas 8000® (Roche, Mannheim, Germany).

TRAb and TSI were analyzed with chemiluminescence methods: TRAb (reference: < 1.8 IU/L) using BRAHMS KRYPTOR® (Thermo Fisher Scientific, Hennigsdorf, Germany) and TSI (reference: < 0.55 IU/L) using Immulite® (Siemens, Erlangen, Germany).

B1Rab, ATR1Ab, and M2Rab were analyzed using 96-well microtiter plates coated with fixed membranes of Chinese hamster ovary cells overexpressing the respective receptor according to the manufacturer's instructions (CellTrend, Luckenwalde, Germany) and as described previously [23, 28]. Briefly, sera were diluted 1:100 and incubated for 2 h at 2–8 °C. IgG antibodies were detected by adding anti-IgG antibodies labelled with horseradish peroxidase. After 1 h incubation at room temperature, plates were developed using tetramethyl benzidine substrate and the absorbance was analyzed after 20 minutes at 450 nm after addition of sulphuric acid. We used the cut-offs suggested by the manufacturer to signify positive antibody levels (ATR1Ab > 17.0 U/mL and B1Rab > 2.2 µg/mL). As the M2R ELISA had no suggested cut-off, we used an arbitrary cut-off (> 9.0 U/mL) for positivity, giving comparable frequencies of positive samples (30 %) as analysis of ATR1Ab in the first 50 recruited controls.

NT-proBNP and TNI were analyzed with immunochemical methods using Immulite® (Siemens). The cut-off for NT-proBNP was 125 ng/L according to the European Society of Cardiology (ESC) heart failure guidelines [4] and for TNI 0.04 µg/L, representing the 99th percentile in healthy individuals, as recommended by the ESC guidelines for acute coronary syndromes [6].

Statistical analyses

The Mann-Whitney test was used for between-group comparisons, the Wilcoxon matched pairs test for longitudinal comparisons within the GD group, and Spearman's rank test for correlation analyses. Frequencies were analyzed with Fisher's exact test. Data is presented as median and interquartile range (IQR). All significance tests

► **Table 2** Cardiac symptom scores and laboratory test results at diagnosis, inclusion, and follow-up. Values presented as median (IQR).

Parameter	Reference range	Diagnosis (<i>n</i> = 60)	Inclusion (<i>n</i> = 60)	Follow-up at 7.5 months (<i>n</i> = 43)
Cardiac symptom scores ^a				
Palpitations	–	–	3 (2–4)	0 (0–1)
Shortness of breath	–	–	3 (1–3)	0 (0–1)
Swollen hands/feet	–	–	0 (0–2)	0 (0–1)
Thyroid hormones				
Serum FT3 (pmol/L)	3.1–6.8	26.0 [22.0–34.0] ^b	13.0 [9.5–16.0]	4.1 [3.7–4.6]
Serum FT4 (pmol/L)	12–22	63.0 [54.0–81.3]	38.0 [28.3–48.8]	17.0 [15.0–19.0]
Serum TSH (mIU/L) ^c	0.3–4.2	0.01 [0.01–0.01]	0.01 [0.01–0.01]	1.1 [0.1–2.6]
Thyroid antibodies				
Serum TRAb (IU/L)	< 1.8	–	10.2 [5.4–25.2]	2.1 [0.9–4.7]
Serum TSI (IU/L)	< 0.55	–	7.6 [4.0–16.1]	1.4 [0.9–2.6] ^d
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; TRAb, TSH receptor antibodies; TSH, thyroid-stimulating hormone; TSI, thyroid-stimulating immunoglobulin; IQR, interquartile range. ^a <i>n</i> = 59 at inclusion and <i>n</i> = 43 at 15 months follow-up; ^b <i>n</i> = 49; ^c TSH concentration is reported as 0.01 mIU/L when below detection limit; ^d <i>n</i> = 38.				

were two-tailed with a significance level at 0.05. We used Graph-Pad Prism 7 software (San Diego, California).

Results

Symptoms of cardiac involvement

None of the patients had atrial fibrillation or clinical heart failure. At inclusion, 69 % of patients experienced grade 3/4 palpitations, 54 % grade 3/4 shortness of breath, and 24 % grade 3/4 swollen hands/feet. After treatment of hyperthyroidism < 10 % of patients experienced grade 3/4 symptoms at follow-up (► Table 2).

► **Fig. 1** Changes in levels of **a** TSH receptor antibodies (TRAb) and **b** thyroid-stimulating immunoglobulins (TSI) from inclusion to follow-up after treatment at 7.5 months in Graves' disease patients ($n = 43$). Horizontal solid lines indicate median levels and dotted lines the recommended positive cut-offs.

Thyroid hormone and antibody levels

At diagnosis, FT4 was > 50 pmol/L in 92 % and FT3 > 15 pmol/L in 100 % of patients. At inclusion, after a median 8 days of ATD treatment (► Table 1), all patients were still hyperthyroid (► Table 2). TRAb was positive in all but two patients who had diffuse uptake on technetium scintigraphy as a sign of GD. Levels of TRAb and TSI are presented in ► Table 2 and ► Fig. 1.

After 7.5 months on ATD treatment, median values for thyroid hormones decreased markedly (► Table 2); only 9 % and 5 % of patients still had elevated FT4 or FT3, respectively. TSH remained below cut-off in 30 % of patients after treatment and was above cut-off in 12 % of patients. TRAb remained positive in 65 % of patients and TSI in 82 % (► Fig. 1).

GPCRab and cardiac biomarkers

B1Rab and M2Rab levels were higher in patients than in controls at inclusion, whereas there was no difference in ATR1Ab levels (► Fig. 2a–c). B1Rab was positive in 38 % of patients compared to 15 % for controls ($P < 0.01$). Corresponding results for M2Rab were 87 and 32 %, respectively ($P < 0.0001$), whereas no difference was observed for positive ATR1Ab samples (18 and 25 %, respectively; $P > 0.05$). Patients treated with beta-blockers had comparable levels of GPCRab as patients without beta-blocker treatment (data not shown).

Among patients at inclusion, 78 % had NT-proBNP levels above the cut-off for positivity compared to 5 % for controls ($P < 0.0001$) (► Fig. 2d). Only 15 % of patients and none of the controls had TNI concentrations above the detection limit ($0.006 \mu\text{g/L}$). All patients and controls had TNI levels below the cut-off for positivity ($0.04 \mu\text{g/L}$) (data not shown).

After 7.5 months of treatment, B1Rab and M2Rab levels decreased; median fold change 1.2 (range, 0.9–1.6) for B1Rab and 1.7 (range, 1.3–2.3) for M2Rab (► Fig. 3a,b), but remained higher than in controls ($P < 0.01$ for both antibodies). This corresponded to positive B1Rab in 26 % of patients versus 15 % for controls ($P > 0.05$) and positive M2Rab in 72 % of patients versus 32 % for

► **Fig. 2** Individual levels of antibodies targeting **a** β_1 -adrenergic receptors (B1Rab), **b** muscarinic acetylcholine type 2 receptors (M2Rab), and **c** angiotensin II type 1 receptors (ATR1Ab), or concentration of **d** N-terminal pro-brain natriuretic peptide (NT-proBNP) in Graves' disease patients in hyperthyroidism and thyroid healthy controls (each $n = 60$). Horizontal solid lines indicate median levels and dotted lines the positive cut-off values used in the study. NS, not statistically significant.

► **Fig. 3** Changes in levels of antibodies targeting **a** β_1 -adrenergic receptors (B1Rab), **b** muscarinic acetylcholine type 2 receptor antibodies (M2Rab), and **c** angiotensin II type 1 receptor antibodies (ATR1Ab), and **d** N-terminal pro-brain natriuretic peptide (NT-proBNP) from inclusion to follow-up after treatment at 7.5 months in Graves' disease patients ($n=43$). Horizontal solid lines indicate median levels and dotted lines the positive cut-off values used in the study.

controls ($P < 0.0001$). ATR1Ab was different to B1Rab and M2Rab (► **Fig. 3c**); this antibody specificity increased slightly from baseline after 7.5 months (median 1.1-fold change, $P < 0.01$) but remained below the cut-off in most patients. However, ATR1Ab levels increased markedly in five patients (median 3-fold increase, range 2.3–4.7).

When patients regained euthyroidism, we observed substantial decreases in NT-proBNP, which returned to levels similar to those in controls ($P > 0.05$) (► **Fig. 3d**) and all patients had TNI concentrations under the detection limit (data not shown).

Correlations

Levels of different GPCrAb were correlated in patients at inclusion and at 7.5-month follow-up and in controls (► **Fig. 4a–i**). The strongest and most consistent correlations ($r \geq 0.6$, $P < 0.001$) were between B1Rab and M2Rab (► **Fig. 4a,d,g**). Most other correlations were weaker, but still statistically significant. Out of the 60 patients analyzed at inclusion, 40% were positive for one, 42% for two, and 7% for all three antibodies, with the most frequent combination being M2Rab- and B1Rab-positivity (38%). All patients positive for B1Rab were also positive for M2Rab and 10/11 patients positive for ATR1Ab were positive for M2Rab. Only 12% of patients were negative for all three antibodies.

There were no statistically significant correlations between the GPCrAb and NT-proBNP levels (► **Fig. 2Sa–c**), TNI levels or cardiac symptoms (data not shown) in hyperthyroid patients at inclusion. GPCrAb or cardiac biomarkers were not correlated with age (data not shown). At inclusion, ATR1Ab levels correlated with FT3 ($r = 0.36$, $p < 0.01$) and FT4 ($r = 0.03$, $p < 0.05$), and B1Rab with FT3 ($r = 0.31$, $P < 0.05$). GPCrAb levels did not correlate with TRAb or TSI (data not shown).

Discussion and Conclusion

We have demonstrated remarkably higher levels of B1Rab, M2Rab, and the cardiac biomarker NT-proBNP in otherwise young and healthy women with Graves' thyrotoxicosis than in matched controls. After regaining euthyroidism, B1Rab and M2Rab levels decreased, but were still higher than in controls even though thyroid hormones and cardiac biomarkers had normalized.

An argument for specific autoimmune activity targeting the heart is that apparently cardiac healthy GD individuals may develop unexplained heart failure or atrial fibrillation [29]. GD is considered to arise from a breakdown of self-tolerance to the thyroid antigens, with the TSH-receptor being the main antigen [30]. The presence of antibodies targeting other antigens in tissues apart from the thyroid suggests a more generalized breakdown of tolerance, potentially as a result of defective regulatory B or T cells [30, 31]. Thyroid antibodies are also common in other autoimmune diseases [32, 33], and a recently published genome-wide association study found that variants of *FLT3*, that encodes a key regulator of development of monocytes and dendritic cells, is strongly associated with autoimmune thyroid disease as well as other autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis [34]. This supports a common problem with T cell tolerance induction or maintenance. However, we found no correlation between TRAb or TSI versus GPCrAb, suggesting that production of these antibodies may be induced and/or regulated separately. Further studies are needed to clarify when and where antibodies to these different autoantigens are induced and subsequently produced.

In contrast, levels of the different GPCrAb correlated with each other and the co-presence of antibodies with different specificities was common. A common mechanism may initiate antibody pro-

► **Fig. 4** Correlations between antibody levels targeting (A,D,G) β_1 -adrenergic receptor (B1Rab), and muscarinic acetylcholine type 2 receptor (M2Rab), (B,E,H) B1Rab and angiotensin II type 1 receptor (ATR1Ab), and (C,F,I) M2Rab and ATR1Ab in Graves' disease patients at **a–c** inclusion in hyperthyroidism ($n=60$) and **d–f** at follow-up after 7.5 months ($n=43$), or in **g–i** healthy controls ($n=60$). The dotted lines are the cut-off levels for positivity used in the study.

duction, but there may also be cross-reactivity between antibodies to different GPCRs. Immune absorption studies suggest that B1Rab and M2Rab are immunologically distinct from TRAb [15], and results from competitive ELISA tests indicate that B1Rab and M2Rab do not cross-react [35]. However, recent results obtained using the same type of ELISAs that we used suggest the existence of physiological networks of anti-GPCR antibodies that may be disrupted in different diseases [28]. It is, therefore, of great interest that ATR1Ab behaved differently compared to the other antibody specificities in our study. It will be important to follow the patients with elevated ATR1Ab levels over time to see if these individuals run a higher risk of long-term complications of GD, as higher levels are reported more often among GD patients with cardiovascular manifestations [2].

To our knowledge, this is the first study in GD patients that has used cell membrane-based ELISAs for analysis of GPCRAb. Using peptide-based ELISAs, levels of B1Rab, M2Rab, and ATR1Ab were previously reported to be significantly elevated in patients with cardiac dysfunction, but not in GD patients with normal heart function, compared to healthy controls [2, 16]. This is in contrast to our results, where elevated B1Rab and M2Rab levels were detected in a general population of GD patients. Since cell membrane-based ELISAs allow detection of antibodies targeting conformational epitopes in the complex GPCR structure, such assays have been described as measuring more physiologically relevant antibodies than peptide-based ELISAs [14, 19, 20]. These results suggest that important antibody specificities may not have been detected in previous studies of GD patients. However, we could not confirm any

correlation between antibody levels and cardiac biomarkers or cardiac symptoms, indicating that the antibodies may not have a direct effect on the heart, or that only a subpopulation of patients had functionally active antibodies, as indicated in sera from GD patients with atrial fibrillation [15, 16]. Further studies are required to determine if the antibodies detected in our patients have functional activity.

Although our study group included relatively young women without evident cardiac dysfunction, NT-proBNP was elevated in almost all patients during severe hyperthyroidism. Increased levels of NT-proBNP may be derived from an increased systemic arterial stiffness, volume overload, and the effect of increased heart rates [36, 37]. However, in a few studies, thyroid hormone has been reported to induce BNP gene expression in rat cardiomyocytes and BNP/NT-proBNP secretion in humans [38, 39]. If NT-proBNP is elevated in some patients from reasons other than cardiac dysfunction, this may explain the absence of an association between GPCrAb and NT-proBNP.

The main strengths of this study are the case-controlled study design, the longitudinal approach, and the use of ELISA tests using membrane-bound receptors. This study is, however, limited by the fact that the functional properties of the antibodies remain to be elucidated, that we did not analyze antibodies during a longer follow-up, and that there was a 28% dropout rate. The cut-off values used for positivity in the GPCrAb ELISA tests also need further validation.

Through this study, new longitudinal data on GPCrAb have been gained using more clinically relevant ELISA tests suggesting broader and more complex autoimmune reactions in GD patients than previously reported. This will lay ground for more research on its possible connections to unexplained complications in autoimmune thyroid diseases. Important observations regarding NT-proBNP as a potentially non-reliable biomarker of heart dysfunction in GD patients has also been made. Although our data does not directly support the hypothesis that GPCrAb cause cardiovascular effects in a general GD population, an autoimmune effect of antibodies on the heart in hyperthyroidism is not excluded. Further studies are needed with a longer follow-up to determine whether the GPCrAb contribute to the circulatory changes seen in GD and to analyze if GD patients develop autoantibodies against additional GPCRs or other autoantigens that may contribute to pathology in this patient group.

Acknowledgements

We are obliged to Jenny Tiberg, nurse for the CogThy study at the Centre of Endocrinology and Metabolism, Sahlgrenska University Hospital and to colleagues at the Department of Endocrinology, Sahlgrenska University Hospital for recruiting patients for this study.

Conflict of Interest

HFN has received lecturing fees from Siemens Inc., Sanofi, Astra Zeneca, and Bristol Mayer Squibb. All the other authors declare no conflict of interests.

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