Serum autotaxin levels are associated with Graves’ disease

Takahiro Nojiri¹,²*, Makoto Kurano¹,³*, Osamu Araki⁴, Kazuki Nakawatari⁵, Masako Nishikawa³, Satoshi Shimamoto⁵, Koji Igarashi⁵, Kuniyuki Kano⁶, Junken Aoki⁶, Shinji Kihara², Masami Murakami⁶ and Yutaka Yatomi¹,³

¹Department of Clinical Laboratory, The University of Tokyo Hospital, Tokyo, Japan
²Department of Biomedical Informatics, Division of Health Sciences, Osaka University Graduate School of Medicine, Osaka, Japan
³Department of Clinical Laboratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
⁴Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Gunma, Japan
⁵Bioscience Division, TOSOH Corporation, Kanagawa, Japan
⁶Laboratory of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Miyagi, Japan

Abstract. Graves’ Disease is a representative disease of hyperthyroidism that presents with hyperthyroidism. Emerging evidence has shown the involvement of lysophosphatidic acid (LPA) and its producing enzyme, autotaxin (ATX), in the pathogenesis of various diseases; among them, the involvement of the ATX/LPA axis in some immunological disturbances has been proposed. In this study, we investigated the association between serum ATX levels and Graves’ disease. We measured the levels of serum total ATX and ATX isoforms (classical ATX and novel ATX) in patients with untreated Graves’ disease, Graves’ disease treated with anti-thyroid drugs, patients with subacute thyroiditis, silent thyroiditis, Plummer’s disease, or Hashimoto’s thyroiditis, and patients who had undergone a total thyroidectomy, as well as normal subjects. The serum total ATX and ATX isoform levels were higher in the patients with Graves’ disease, compared with the levels in the healthy subjects and the patients with subacute thyroiditis. Treatment with anti-thyroid drugs significantly decreased the serum ATX levels. The serum ATX levels and the changes in serum ATX levels during treatment were moderately or strongly correlated with the serum concentrations or the changes in thyroid hormones. However, the administration of T3 or T4 did not increase the expression or serum levels of ATX in 3T3L1 adipocytes or wild-type mice. In conclusion, the serum ATX levels were higher in subjects with Graves’ disease, possibly because of a mechanism that does not involve hyperthyroidism. These results suggest the possible involvement of the ATX/LPA axis in the pathogenesis of Graves’ disease.

Key words: Graves’ disease, Autotaxin, Anti-thyroid drugs, Lysophosphatidic acids, Thyroid hormones

GRAVES’ DISEASE is a representative disease of hyperthyroidism, and many subjects are afflicted with this disease worldwide. At present, an immunological disturbance is known to be involved in the pathogenesis of Graves’ disease: an autoimmune antibody against the thyroid stimulating hormone (TSH) receptor (TRAb) inappropriately stimulates the secretion of thyroid hormone, such as free T3 (fT3) and free T4 (fT4), from the thyroid [1]. However, the mechanisms underlying this immunological disturbance remain to be fully elucidated. For the treatment of Graves’ disease, the standard of care is the use of anti-thyroid drugs to inhibit the synthesis and secretion of thyroid hormone; in some subjects, however, the activity of Graves’ disease is difficult to control with medicine alone, and the use of radioiodine or ablative surgery may be required. Moreover, although many symptoms such as tachycardia and hyperhidrosis are derived from the activation of the sympathetic nervous system because of hyperthyroidism, some subjects with Graves’ disease suffer from an itching sensation, the mechanism of which remains to be completely eluci-
dated. The rather high reoccurrence rate of Graves’ disease is another difficulty in the management of subjects in remission. Therefore, investigating the pathogenesis of Graves’ disease is an important task in the development of novel treatments or biomarkers.

Autotaxin (ATX) is an enzyme that hydrolyzes lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) [2], and the involvement of ATX in several diseases has been reported, including liver fibrosis [3-5], pregnancy-induced hypertension [6], and malignancies [7-9]. Among them, the involvement of ATX and LPA in the pathogenesis of several immunological diseases has been reported, including rheumatoid arthritis [10, 11], inflammatory bowel syndrome, and multiple sclerosis [12]. Many series of elegant studies have demonstrated mechanisms for the possible association between ATX and the immune system [13]; for example, LPA facilitates the entry of T lymphocytes into lymph nodes [14] and the motility and migration of naïve T cells [15], while LPA also activates and enhances the production of immunoglobulin in B lymphocytes [16, 17]. As described above, the pathogenesis of Graves’ disease is thought to involve a disturbance in the complex immune system, and ATX has recently been reported to be associated with itching in subjects with primary biliary cirrhosis [18], which is also a symptom observed in Graves’ disease. Moreover, five alternative splicing isoforms of ATX have been identified as classical ATX (ATXα, ATXβ, ATXγ) and novel ATX (ATXδ and ATXε). Although classical ATX and novel ATX are widely expressed in human tissues, some reports have demonstrated difference in their expression tissues; relatively high expression levels of classical ATX were observed in the brain, placenta, ovary, and small intestine [19], while high levels of novel ATX were seen in the small intestine and spleen [20]. Therefore, there are possibility that the serum levels of ATX isoform might be differently modulated by Graves’ disease. These backgrounds prompted us to investigate the association between Graves’ disease and serum total ATX and ATX isoform levels.

Methods

Subjects

We collected serum samples from 58 healthy adult volunteers who had also provided written informed consent and 42 subjects with untreated Graves’ disease, 17 subjects with Graves’ disease who were receiving treatment with anti-thyroid drugs, 18 subjects with untreated subacute thyroiditis, 9 subjects with untreated silent thyroiditis, 6 subjects with untreated Plummer’s disease, 9 subjects with untreated Hashimoto’s thyroiditis, 12 subjects with treated Hashimoto’s thyroiditis, and 7 subjects who had undergone a total thyroidectomy but had not taken thyroid hormone. Among them, we collected samples from both before treatment and at one year after the treatment with anti-thyroid drugs from 38 subjects, of which 34 subjects were treated with thiamazole and 4 subjects were treated with propylthiouracil. The serum samples used in this study were residual samples of those obtained after the completion of routine laboratory analyses. The present study was conducted with the approval of the ethics review committee of The University of Tokyo (2602). The characteristics of the subjects were described in Table 1.

Measurement of serum ATX and its isoforms

The serum ATX antigen levels were determined using a two-site immunoenzymic assay with the TOSOH AIA system (TOSOH, Tokyo, Japan) [21]. Regarding ATX, since five alternative splicing isoforms of ATX have been identified as ATXα, ATXβ, ATXγ, ATXδ, and ATXε, we also measured the classical ATX (ATXα, ATXβ, and ATXγ) and novel ATX (ATXδ and ATXε) levels using enzyme immunoassays that we recently developed [22].

Cell experiments

3T3L1 fibroblasts (obtained from the JCRB cell bank) were cultured in DMEM (D5796; Sigma-Aldrich Co.) containing 10% fetal bovine serum (FBS, 10099-141; Gibco BRL, Eggstein, Germany) and 1% penicillin/streptomycin (15070-063; Gibco, Grand Island, NY). Two days after confluence, the differentiation of 3T3L1 fibroblasts into adipocytes was induced using 0.5 mM 3-isobutyl-1-methylxanthine (099-03411; WAKO Pure Chemical Industries), 1 μM dexamethasone (047-18863; WAKO Pure Chemical Industries), and 10 μg/mL of insulin (I1882; Sigma-Aldrich Co.). After three days, the medium was replaced with DMEM containing 10% FBS, 1% penicillin/streptomycin, and insulin (10 µg/mL). Thereafter, the medium was replaced with fresh DMEM containing 10% FBS and 1% penicillin/streptomycin every two days [23].

On the tenth day after the induction of differentiation into adipocytes, the cells were incubated in FBS-free DMEM containing various concentrations of T3 (T2877; Sigma-Aldrich, Co.) or T4 (T2376; Sigma-Aldrich, Co.). After 24 hours, total RNA was extracted using the GenElute Mammalian Total RNA Miniprep kit (Sigma-Aldrich Co.) and was subjected to reverse transcription using ReverTra Ace qPCR RT Master Mix (TOYOBO Co., LTD, Osaka, Japan). Real-time quantitative PCR was performed using the 7300 Real Time PCR System (Applied Biosystems, Foster City, CA) with hybridization probes and primers purchased from Applied
Biosystems: murine ATX (Mm00516572_m1), murine glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Mm99999915_g1), murine β-actin (Mm00607939_s1), and murine 18S (Mm03928990_g1). The expression level of genes of interest was adjusted using that of 18S as an endogenous control. To investigate the accumulation of fat in 3T3L1 adipocytes, we performed Oil-Red O staining as follows; we fixed cells, which were cultured on 6-well plate, with 10% formaldehyde solution and stained them with 60% isopropanol containing Oil-Red O at 1.8 mg/mL for 20 minutes. After we washed the cells with PBS twice, we extracted the stain from them, using 500 μL isopropanol, and measure the absorbance at 550 nm. The levels of human fT3 and/or fT4 in the medium were measured in SRL Inc. (Tokyo, Japan).

Animal experiments
C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan). Ten-week old C57BL6/J mice were injected intraperitoneally with either PBS or T3 at a dose of 0.1 mg/kg twice a day for ten days, based on the protocol used in a previous report [24]. The mice were then subjected to a 6-hour fast, and blood samples were subsequently collected. Serum murine ATX was measured using an ELISA as performed below: 96-well plates were coated with a rat anti-murine ATX monoclonal antibody 5E5 [25]. Then, 3 μL of sera were added to the plate. After 1 h, the samples were replaced with the rat anti-murine ATX monoclonal antibody S13A9 [25]. Murine ATX bound to the plate was detected with horseradish peroxidase-labeled streptavidin and 3,3′,5,5′-Tetramethylbenzidine (TMB). The standard curve for ELISA measurements of recombinant murine ATX is shown in Supplementary Fig. 1. Plasma LPA and LPC levels were determined using an LC-MS/MS method, as previously described [26]. The levels of human fT3 in the plasma were measured in SRL Inc. All the animal experiments were conducted in accordance with the

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<th>Table 1 Characteristics of subjects</th>
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<td>Age (year)</td>
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<td>Healthy</td>
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<td>Graves’ disease (untreated, pre-treatment)</td>
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<td>Subacute thyroiditis</td>
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Data are represented as the mean ± S.D. (95% confidence interval of the average) or number. N/A: not available.
guidelines for Animal Care and were approved by the animal committee of The University of Tokyo.

**Statistical analysis**

All the data were statistically analyzed using SPSS (Chicago, IL). The results were expressed as the mean ± SD. A comparison between two groups was performed using the Mann-Whitney U test, the values obtained from three groups in clinical studies were compared using an analysis of covariance (ANCOVA) to adjust for age followed by the Bonferroni test as a post-hoc test, and those in an in vitro experiments were compared using the Kruskal-Wallis test followed by the Games-Howell test as a post-hoc test. Correlations were determined using the Spearman correlation test, and comparisons between before and after treatment were analyzed using the paired t-test. A value of \( p < 0.05 \) was regarded as denoting statistical significance in all the analyses.

**Results**

**Serum ATX levels were higher in subjects with Graves’ disease**

First, we compared the serum total ATX and ATX isoform levels between healthy subjects and subjects with hyperthyroidism or hypothyroidism in male and female subjects separately, since the total ATX and ATX isoform levels are higher in women than in men [21]. As shown in Fig. 1A–C, the serum total and novel ATX levels were significantly higher in men with untreated Graves’ disease; in women, however, the serum total ATX, classical ATX, and novel ATX levels were significantly higher in both subjects with untreated and those with treated Graves’ disease (Fig. 2A–C). Regarding the ATX levels in the subjects with other diseases causing hyperthyroidism, we observed no significant difference between the healthy subjects and those with subacute thyroiditis or Plummer’s disease in both men and women, while the subjects with silent thyroiditis had significantly higher total ATX levels in both men and women, although the number of subjects with silent thyroiditis was relatively small (Figs. 1A–C and 2A–C). When we compared the ATX levels between subjects with Graves’ disease and those with subacute thyroiditis, we found that the serum novel ATX levels are significantly higher in men with untreated Graves’ disease and the serum total ATX, classical ATX, and novel ATX levels were significantly higher in women with untreated Graves’ disease than in the subjects with subacute thyroiditis (Fig. 2A–C).

We also measured the serum ATX levels in the subjects with hypothyroidism and observed that the serum total, classical, and novel ATX levels were significantly lower in female subjects who underwent a total thyroidectomy and had not taken thyroid hormones and female subjects with treated Hashimoto’s disease, while total ATX levels were significantly higher in female subjects with untreated Hashimoto’s disease, although the number of subjects with hypothyroidism was small (Fig. 2A–C).

When we compared the ratio of classical to novel ATX, we observed no differences in men, while the ratio was higher in women with untreated Graves’ disease than in healthy women (Figs. 1D and 2D).

**Treatment with anti-thyroid drugs decreased the serum ATX levels**

We collected serum samples at about one year after the start of treatment with anti-thyroid drugs from 38 of the enrolled subjects with untreated Graves’ disease and measured the serum ATX levels. As shown in Fig. 3A–C, treatment with anti-thyroid drugs successfully decreased the serum total ATX, classical ATX, and novel ATX levels, together with the fT3 and fT4 levels (9.32 pg/mL to 2.56 pg/mL, \( p < 0.001 \), and 3.33 ng/mL to 1.26 ng/mL, \( p < 0.001 \), respectively). Regarding the ratio of novel ATX levels to classical ATX levels, we observed that this ratio increased significantly after treatment with anti-thyroid drugs (Fig. 3D).

**Serum ATX levels were well correlated with thyroid hormone levels in both untreated and treated Graves’ disease**

Next, we investigated the correlation between the serum ATX levels and the levels of thyroid hormones to investigate the correlation between serum ATX levels and the state of Graves’ disease. As shown in Fig. 4, the serum ATX levels were strongly correlated with fT3 or fT4 in women with untreated Graves’ disease, while these correlations were not significant in men. In subjects with Graves’ disease who were receiving treatment, we observed that the serum ATX levels were moderately or strongly correlated with the fT4 levels in men and the fT3 and fT4 levels in women (Fig. 5). These results suggest the possibility that ATX is correlated with the severity of Graves’ disease. Actually, when the changes in the serum total, classical, and novel ATX levels were compared with the changes in the fT4 and fT3 levels before and after treatment with anti-thyroid drugs, close correlations were observed (Fig. 6). These correlations were observed when we analyzed male and female subjects separately (Supplementary Figs. 2 and 3). Regarding TRAb, serum TRAb levels were closely correlated with the serum ATX levels (\( r = 0.731, p < 0.001 \)) only in female subjects with untreated Graves’ disease (Fig. 7).

In other thyroid diseases, although we could not analyze each thyroid disease separately because of small number of subjects, when we investigated the correlation
Fig. 1  Serum ATX levels in male subjects with thyroid diseases
The serum total ATX, classical ATX, and novel ATX levels were measured in male subjects with untreated Graves’ disease (GD) ($n = 13$), treated GD ($n = 4$), subacute thyroiditis (SAT) ($n = 4$), silent thyroiditis (ST) ($n = 4$), Plummer’s disease (PD) ($n = 2$), untreated Hashimoto’s thyroiditis (HT) ($n = 5$), treated HT ($n = 7$), subjects who underwent a total thyroidectomy (Tx) ($n = 3$), and healthy subjects ($n = 25$). (A) Serum total ATX levels, (B) serum classical ATX levels, (C) serum novel ATX levels, and (D) the ratio of classical ATX to novel ATX levels. †$p < 0.01$ vs. GD-untreated and GD-treated, ‡$p < 0.01$ vs. GD-untreated, §$p < 0.01$ vs. GD-treated. The values were compared using ANCOVA to adjust for age followed by the Bonferroni test as a post-hoc test.
Serum ATX levels in female subjects with thyroid diseases

The serum total ATX, classical ATX, and novel ATX levels were measured in female subjects with untreated Graves’ disease (GD) \((n = 29)\), treated GD \((n = 13)\), subacute thyroiditis (SAT) \((n = 14)\), silent thyroiditis (ST) \((n = 5)\), Plummer’s disease (PD) \((n = 4)\), untreated Hashimoto’s thyroiditis (HT) \((n = 4)\), treated HT \((n = 5)\), subjects who underwent a total thyroidectomy (Tx) \((n = 4)\), and healthy subjects \((n = 33)\). (A) Serum total ATX levels, (B) serum classical ATX levels, (C) serum novel ATX levels, and (D) ratio of classical ATX to novel ATX levels. \(* p < 0.05\) vs. healthy subjects and SAT, \(\dagger p < 0.01\) vs. healthy subjects and GD-treated, \(\ddot{p} < 0.01\) vs. healthy subjects and GD-treated, \(\ddagger p < 0.01\) vs. healthy subjects and GD-treated, \(\dddot{p} < 0.01\) vs. healthy subjects, GD-treated, and SAT, \(\dddot{p} < 0.05\) vs. GD-treated. The values were compared using ANCOVA to adjust for age followed by the Bonferroni test as a post-hoc test.

Fig. 2  Serum ATX levels in female subjects with thyroid diseases
between the serum ATX levels and the fT4 or fT3 levels in the subjects with thyroid disease other than Graves’ disease, we observed significant positive correlation in female subjects (Supplementary Fig. 4).

Thyroid hormones might not regulate ATX levels

So far, we have demonstrated that the serum ATX levels were higher in subjects with Graves’ disease and that these levels decreased after treatment with anti-thyroid drugs. Moreover, we measured the serum ATX levels in subjects with other diseases causing hyperthyroidism and observed that the serum ATX levels in subjects with subacute thyroiditis were significantly lower than those in subjects with Graves’ disease (Figs. 1, 2). These results suggest that the serum ATX levels might not be elevated in patients with Graves’ disease as a result of hyperthyroidism, but rather due to a pathogenetic mechanism specific to Graves’ disease. However, we could not completely rule out the possibility that the serum ATX levels were regulated by thyroid hormones, since the serum ATX levels were lower in the subjects who had received a total thyroidectomy. Therefore, lastly, we investigated whether treatment with thyroid hormones increased the expression or serum levels of ATX using in vitro and in vivo experiments.

First, we treated 3T3L1 adipocytes with T3 or T4, since adipocytes are deemed to be a major origin of ATX [27]. The levels of fT3 and fT4 levels in the medium after 24 hour incubation were shown in Supplementary Table 1. As shown in Fig. 8A and B and Supplementary Fig. 5, T3 or T4 did not increase, but rather decreased the expression of ATX in 3T3L1 adipocytes, while they increased the expression of GAPDH and accelerated the accumulation of fat (Supplementary Fig. 6), which were concordant with the previous report [28].

We also treated wild-type mice with T3 and found that the treatment with T3 did not modulate either the serum ATX levels or the LPA levels, while the treatment with T3 increased fT3 levels in the mice to the levels higher than in Graves’ disease (Fig. 8C–G). These results suggest that the increased levels of thyroid hormone did not elevate the serum ATX levels, but several pathogenetic mechanisms of Graves’ disease might be involved in the elevation of the serum ATX levels.
Fig. 4  Correlations between serum ATX levels and serum concentrations of thyroid hormones in subjects with untreated Graves’ disease
The correlations between the serum total ATX levels and the serum fT4 (A, C) and fT3 levels (B, D) are shown for men (A, B) and women (C, D) with untreated Graves’ disease. A, n = 13; B, n = 12; C, n = 29; D, n = 25. Correlations were determined using the Spearman correlation test.

Fig. 5  Correlations between serum ATX levels and serum concentrations of thyroid hormones in subjects with Graves’ disease receiving treatment with anti-thyroid drugs
The correlations between the serum total ATX levels and the serum fT4 (A, C) and fT3 levels (B, D) are shown for men (A, B) and women (C, D) with Graves’ disease who were receiving treatment with anti-thyroid drugs. A, n = 18; B, n = 18; C, n = 41; D, n = 36. Correlations were determined using the Spearman correlation test.
Fig. 6 Correlations between the changes in the serum ATX levels and those of thyroid hormones before and after treatment with anti-thyroid drugs

The correlations between the changes in the serum total ATX (A, B), classical ATX (C, D), and novel ATX levels (E, F) and the changes in the serum fT4 (A, C, E) and fT3 levels (B, D, F) before and after treatment with anti-thyroid drugs are shown. A, $n = 38$; C, E, $n = 33$; B, $n = 31$; D, F, $n = 28$. Correlations were determined using the Spearman correlation test.

Fig. 7 Correlations between the serum ATX levels and TRAb

The correlations between the serum total ATX levels and the serum TRAb levels are shown for men (A) and women (B) with untreated Graves’ disease. A, $n = 13$; B, $n = 27$. Correlations were determined using the Spearman correlation test.
Discussion

In the present study, we demonstrated that the serum ATX levels were higher in subjects with Graves’ disease (Figs. 1, 2), that they were decreased by treatment with anti-thyroid drugs (Fig. 3), and that they were moderately or strongly correlated with thyroid hormone levels (Figs. 4–7). Regarding the association between ATX and thyroid disease, although the expression of ATX was reportedly higher in thyroid cancer [29], this is the first study to investigate the association between ATX and Graves’ disease or thyroid hormones.

Although a significant correlation between ATX and thyroid hormones was demonstrated to exist in subjects with Graves’ disease in the present study (Figs. 4–6), whether thyroid hormones might positively regulate the serum ATX levels or whether ATX might be influenced directly by the pathogenesis of Graves’ disease has been uncertain, since the thyroid hormone levels should be correlated with the activity of Graves’ disease. To investigate this issue, we measured the serum ATX levels in subjects with other diseases causing hyperthyroidism,
such as subacute thyroiditis, silent thyroiditis, and Plummer’s disease, and found that the serum ATX level was unchanged with the exception of the levels in subjects with silent thyroiditis. Considering that the number of subjects with silent thyroiditis was relatively small, it is reasonable to assume that thyroid hormone might not increase the ATX levels but that the pathogenesis of Graves’ disease itself or inflammation in thyroid, which is observed in Graves’ disease and silent thyroiditis, might be associated with the elevation in the serum ATX levels. Actually, we showed that thyroid hormones themselves might not increase ATX levels using in vitro and in vivo experiments (Fig. 8), although we should admit that the duration of treatment with thyroid hormones was rather short compared with the duration of hyperthyroidism observed in clinical practice. Other limitations in basic studies were that we only investigated the possibility of the involvement of TRα in the production of ATX because adipocytes express mainly TRα but not TRβ [28] and that, considering that T4 exerted less suppressive properties on the ATX expression than T3, the suppression of the ATX expression might not be directly caused by thyroid hormones in the in vitro experiments.

Regarding diseases that involve hypothyroidism, although the ATX levels observed in Hashimoto’s thyroiditis were not confirmative, the result that the subjects who received a total thyroidectomy had not taken thyroid hormones had lower ATX levels than healthy subjects does not agree this hypothesis. However, since the samples were collected from these subjects immediately after the thyroidectomy and the ATX level is known to be lower post-operatively, possibly because of malnutrition as previously reported [30], the lower levels of ATX in subjects after a total thyroidectomy do not necessarily suggest that thyroid hormones might regulate ATX. Anyway, considering these results together with the significant positive correlations between serum ATX levels and thyroid hormones in female subjects with thyroid diseases other than Graves’ disease (Supplementary Fig. 4) and the fact that the duration of hypothyroidism is in general longer in Graves’ disease than in other hyperthyroidism diseases, there remains possibility that hyperthyroidism itself regulate the ATX levels.

ATX is a producing enzyme for LPA [2], and LPA exerts its bioactivities, such as the migration, proliferation, or transformation of various cells, the activation of platelets [31, 32], the induction of inflammatory cytokines [33] or adhesion molecules [34, 35], the activation of immunological cells [13], and the development of pruritus [18], through six kinds of G protein-coupled receptors (LPA₁-₆) located on the cell membrane [36]. Among these biological properties of LPA, immunological activation and pruritus might be involved in the pathogenesis or symptoms of Graves’ disease.

Regarding the immunological disturbance that is observed in Graves’ disease, B cells secrete TRAb, which stimulates the release of thyroid hormones, while the involvement of many other immunological cells, such as dendritic cells, T cells, and thyroid epithelial cells, in the pathogenesis of Graves’ disease has been demonstrated [1]. Among these possible immunological disturbances and considering the immunological properties of LPA, the ATX/LPA axis might be involved in several proposed pathways for the pathogenesis of Graves’ disease; LPA might cause the proliferation of B cells, stimulating the secretion of immunoglobulin [16, 17]. LPA induces the chemotaxis of immature dendritic cells, while it reportedly inhibits lipopolysaccharide-stimulated dendritic cells [37]. LPA also stimulates the secretion of cytokines from T cells and promotes the adhesion of T cells [38] and the chemotaxis of T cells [14, 15]. These possible involvements of the ATX/LPA axis in the immunological disturbance observed in Graves’ disease suggest that an increase in serum ATX levels might provoke or cause a deterioration in Graves’ disease. Considering the previous report demonstrating that ATX secretion was induced in response to inflammation through the actions of inflammatory cytokines, chemokines, and platelet-derived growth factors in thyroid cancer [29], ATX/LPA axis and inflammation could form a vicious cycle in thyroid tissue to deteriorate the thyroiditis. However, the possibility that ATX might be regulated by thyroid hormone cannot be fully ruled out, as described above. Further basic studies are needed to investigate the association between Graves’ disease and the ATX/LPA axis.

Another interesting finding in this study was that the ratio of classical ATX to novel ATX was higher in women with Graves’ disease who were receiving treatment with anti-thyroid drugs than in healthy subjects. We also observed that treatment with anti-thyroid drugs significantly increased this ratio. Although the ratio of classical ATX to novel ATX has been measured in serum samples from patients with follicular lymphoma, liver fibrosis [22], acute coronary syndrome [39], and melanoma [40] and in ascites from subjects with gastric cancer [41], a significant modulation of this ratio has not been previously reported. As described in the Introduction section, although classical ATX and novel ATX are widely expressed in human tissues, some differences have been reported in their expression tissues [19, 20]; however, the predominant isoforms in the thyroid remain unclear, and anti-thyroid drugs might also possess unknown biased effects on the secretion of ATX isoforms from organs other than the thyroid.

Regarding the significance of the present findings,
both therapeutic and diagnostic applications can be expected. Further studies on the involvement of the ATX/LPA axis in the pathogenesis of Graves’ disease might clarify the possible usefulness of ATX inhibitor in the treatment of Graves’ disease. Although the diagnosis of Graves’ disease has been well established, further studies on the association between ATX and Graves’ disease, especially regarding patient prognosis or clinical symptoms, might help to elucidate the diagnostic usefulness of ATX in Graves’ disease. Regardless, since ATX has recently been demonstrated as a biomarker for various diseases, including liver fibrosis [5], the present findings suggest the importance of considering hyperthyroidism when using serum ATX levels as biomarkers for other diseases.

A limitation of this study is that the number of subjects with subacute thyroiditis, silent thyroiditis, Plummer’s disease, Hashimoto’s thyroiditis, and subjects who underwent a total thyroidectomy was rather small (Figs. 1 and 2) and further studies with larger number of subjects are necessary to conclude the association between ATX and thyroid diseases. Moreover, we did not measure the plasma levels of LPA, which actually exerts potent physiological activities, since a strict plasma sampling protocol is required to measure LPA, as shown in a previous report [42]. However, in general, the serum ATX levels are closely correlated with the plasma LPA levels, as we previously reported [3, 9, 43]. Therefore, we believe that the plasma LPA levels might also be higher in subjects with Graves’ disease. Another limitation is that the mechanism by which Graves’ disease increases the serum ATX level remains unclear. One possible candidate is TRAb, which is specifically increased in Graves’ disease, since either hyperthyroidism or inflammation might not be the cause of the elevation in the ATX levels as discussed above. Actually, the serum TRAb level was closely correlated with the serum ATX level in female subjects with untreated Graves’ disease (Fig. 7). Interestingly, the serum ATX levels were significantly correlated only with TRAb (r = 0.551, p = 0.002), and not with fT4 (r = 0.062, p = 0.744) or fT3 (r = 0.377, p = 0.053), after treatment with anti-thyroid drugs. Since TSH receptors exist in adipocytes [44], TSH receptor might be involved in the regulation of ATX level, however, we observed no significant correlation between the TSH levels and the serum ATX levels in Hashimoto’s thyroiditis (male: r = −0.292, p = 0.358, female: r = −0.250, p = 0.516). Further basic and clinical studies are needed to investigate these issues.

In summary, we have demonstrated that the serum ATX levels were higher in subjects with Graves’ disease, possibly through a mechanism that does not involve hyperthyroidism. These results might be useful for the development of novel therapeutic and/or diagnostic approaches to Graves’ disease.

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Disclosure

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