

Original Article

Reverse 'See-Saw' Relationship between Graves' Disease and Myasthenia Gravis; Clinical and Immunological Studies

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Although the association of two distinct autoimmune diseases, Graves' disease (GD) and myasthenia gravis (MG), is rare, the relationships of clinical and immunological activities between the two diseases remain unknown. In the present study, we investigated whether there exist any relationships between clinical and immunological activities of GD and MG as well as any common characteristics of their HLA antigens in five patients with concomitant association with GD and MG. The present study clearly showed positive relationships between the clinical activities of GD and MG in all five cases. Except for two cases, one with undetectable acetylcholine receptor antibody and another with few sample number, there were positive relationships between two circulating auto-antibodies against TSH receptor and acetylcholine receptor as well as their immunological and clinical activities in the remaining three cases. Furthermore, the present serological HLA typing study revealed that all five cases had common HLA-DQ3. Therefore, our study clearly demonstrates a reverse 'see-saw' relationship between GD and MG based on their clinical and immunological features, and suggests that HLA-DQ3 may play a potential pathogenic role in the concomitant

development of the two diseases.

Key words: HLA typing, anti-TSH receptor antibody, anti-acetylcholine receptor antibody

Introduction

Since the first case report of a patient with Graves' disease (GD) associated with myasthenia gravis (MG) by Rennie¹, a close association between two distinct autoimmune diseases has been suggested². McEachern and Parnell proposed that there exists a negative relationship, so-called 'see-saw' relationship, of the clinical activities between the two diseases³, i.e. when MG gets worse, GD gets better, and vice versa. On the other hand, a positive relationship, so-called reverse 'see-saw' relationship, of their clinical activities has often been reported^{4,5}. Recent development of immunological methods to measure circulating anti-acetylcholine receptor antibody (AChRAb) and thyrotropin receptor antibody (TRAb) have been widely used for the diagnosis of MG and GD, respectively^{6,7}. Thus, measurement of these disease-specific auto-antibodies could be a useful tool for accurate assessment of the relationships of the immunological activities between two diseases.

During the past five years, we have experienced five cases of GD associated with MG, in which GD had occurred before or at the same time as MG developed. In the present study, we studied whether or not there is a 'see-saw' relationship between their clinical and

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Received October 21; Accepted December 3, 2004

immunological activities, and whether there is a common genetic background between two diseases by HLA typing analysis.

Patients

Clinical features of all 5 cases with GD and MG studied are summarized in Table 1. They were three females and two males, aged 15~57-year-old (mean 36.4-year-old). While the duration of GD was variable ranging from 0 to 17 years, MG was diagnosed in four after the onset, and in one at the same time of GD. The diagnosis of GD was established by physical findings (goiter, tachycardia, finger tremor, weight loss, etc.), laboratory findings including elevation of thyroid hormones levels (total triiodothyronine [T3] and/or free thyroxine [FT4]), suppression of TSH levels ($<0.1 \mu\text{U/ml}$), and the presence of TRAb. The diagnosis of MG was established by muscle weakness, positive Tensilon test, provocative electromyography, and the presence of AchRAb except for Case 1.

Methods

Measurements of auto-antibodies and HLA typing

TRAb was measured by COSMIC III assay kit (RSR Limited, Cardiff, U.K.) using porcine thyroid membranes as a source of TSH receptor. The data was expressed as the percentage of TSH binding inhibitory activity of immunoglobulins; the normal range was $\pm 10\%$. AchRAb was measured by COSMIC II assay kit (RSR Limited, Cardiff, U.K.) using human muscle cell line TE671 as a source of acetylcholine receptor. This assay has high sensitivity (0.07 nmol/L) because it contains both embryonic type (γ) and adult type (ϵ) receptor; the normal range is below 0.3 nmol/L. For analysis of HLA typing, class I antigen (A, B, C) and class II antigen (DQ, DR) were determined by the lymphocyte cytotoxicity test.

Assessment of clinical activities

The clinical activities of both diseases were assessed by their typical signs, and scored as the absence (-) and the presence of severe (+++), moderate (++) , and mild (+) manifestations. Clinical activity of GD was scored as follows: (+++) more than two out of the following five signs (weight loss, tachycardia,

Table 1. Clinical features of 5 cases with Graves' Disease (GD) and Myasthenia Gravis (MG)

Case	1	2	3	4	5
Age / Sex	57 / female	55 / male	20 / female	15 / female	35 / male
Preceding disease	GD	GD	GD	GD	Simultaneous
GD : Symptoms and signs	Weight loss Tachycardia Tremor	Weight loss Tachycardia Restlessness	Palpitation (Tachycardia) Restlessness Tremor Hidrosis	Exophthalmos, Weight loss Hidrosis	Palpitation (Tachycardia) Weight loss
Duration of GD (year)	7	17	6	3	0
Treatment for GD*	ATD, Io	ATD, RAI	ATD, Io, PP	ATD, PSL	ATD
Discontinuation of ATD	+	+	+	-	-
MG : Symptoms and signs	Diplopia, Ptosis	Bi-ptosis, Weakness	Bi-ptosis, Dyspnea Dysarthria, Weakness	Ptosis	Weakness
Osserman type	Ocular	Generalized	Acute fulminating	Ocular	Generalized
Treatment for MG**	PSL, Am	Py, Tm	Py, PSL, Tm	Py, Am	Tm
Thymectomy / pathology	(-)	(+) / (thymic atrophy)	(+) / thymoma(-)	(-)	(+) / thymoma(-)

* ATD: antithyroid drugs, PP: plasmapheresis, PSL: prednisolone, Io: iodine, RAI: radioactive iodine

** Py: pyridostigmine, Am: ambenonium, Tm: thymectomy

tremor, restlessness, hidrosis); (++) more than one; (+) only one; (-) no sign. Clinical activity of MG with generalized type (Cases 2, 3, 5) was scored as follows: (+++) manual muscle testing (MMT) level less than 3; (++) level 3; (+) level 4; (-) normal MMT level. However, the clinical activity in ocular type MG (Cases 1 and 4) was assessed by the degree of myasthenic ocular signs.

Statistical analysis

Changes of clinical, hormonal, and immunological parameters were represented by their scores and values simultaneously determined during the clinical course in each case, which were compared with each other by statistical analysis using Spearman's rank correlation test; p values less than 0.05 were considered statistically significant.

Clinical courses

Case 1: She was diagnosed as GD in 1996, but had had two histories of discontinuation of anti-thyroid drugs (ATD). Methimazole (MMI) was administered from June 2001, but was discontinued due to drug-induced liver dysfunction and inorganic iodine was started. Hyperthyroidism was ameliorated. However, two months later, she became hyperthyroid again, and suffered from diplopia and blepharoptosis at the same time. As Tensilon test was positive, she was diagnosed as ocular type MG (type I), although AchRAB was negative and thymoma was not detected. Ambenonium (Am) and prednisolone (PSL) were started for treatment of MG. Her myasthenic symptoms gradually improved and also her thyrotoxic symptoms improved along with the decrease in TRAb titers.

Case 2: He was diagnosed as GD in 1986, but had had three histories of discontinuation of ATD. Two months after starting ATD in April 2001, he developed muscle weakness of lower extremities and blepharoptosis. Although his thyroid function improved after radioactive iodine (RAI) therapy, his myasthenic symptoms were aggravated. Generalized type MG (type II) was diagnosed because AchRAB and Tensilon test were both positive. Pyridostigmine (Py) and PSL were administered and a thymectomy was performed; there was neither thymoma nor thymic hyperplasia. Although TRAb titers increased transiently after RAI therapy, his symptoms improved and both AchRAB and TRAb titers gradually decreased concomitantly.

Case 3: She was diagnosed as GD in 1997, but had had two histories of discontinuation of ATD. Because of

blepharoptosis, palpitation, and shortness of breath, MMI was restarted before one week prior to admission, when she was admitted for severe MG crisis (acute fulminating type), which necessitated artificial ventilation for a while. Py, inorganic iodine, and PSL were administered and plasmapheresis was also performed. Her condition improved as both AchRAB and TRAb titers gradually decreased. She had a thymectomy; neither thymoma nor thymic hyperplasia was found. She is currently stable with Py, MMI and PSL.

Case 4: She visited our hospital for thyrotoxic symptoms, exophthalmos and diplopia and was diagnosed as GD. Steroid pulse therapy was performed for the treatment of malignant exophthalmos and MMI was also started at the same time. Although her thyrotoxic symptoms were improved gradually, diplopia and proptosis appeared 6 months later. Then ocular type MG (type 1) was diagnosed by positive AchRAB and Tensilon test. Thymoma was not detected by routine computed tomography. Her myasthenic symptoms improved transiently with Am, but worsened after discontinuation of MMI and Am, when both AchRAB and TRAb titers markedly increased. Her condition is currently stable with MMI and Am.

Case 5: He visited our hospital for thyrotoxic symptoms and slight muscle weakness in October 2002. MMI was started as the diagnosis of GD was made. Thymoma was suspected by chest x-ray and computed tomography. Diagnosis of generalized type MG (type II) was established by positive AchRAB and Tensilon test. Thymectomy was performed, although neither thymoma nor thymic hyperplasia was found. Both thyrotoxic and myasthenic symptoms improved as both TRAb and AchRAB titers decreased.

Results

Relationships between clinical and immunological activities

As shown in Fig. 1, there was a significant ($p < 0.05$) positive correlation between the clinical activities of GD and MG in all five cases. Except for Case 1 with no detectable AchRAB and Case 2 with few sample number, there were significant ($p < 0.05$) correlations between two auto-antibodies (TRAb and AchRAB) in the remaining three cases (Cases 3, 4, 5) (Fig. 2). Likewise, there were significant positive ($p < 0.05$) correlations between TRAb and GD, and AchRAB and MG (Fig. 3), as well as those between the clinical and the immunological activities (AchRAB and GD, TRAb and

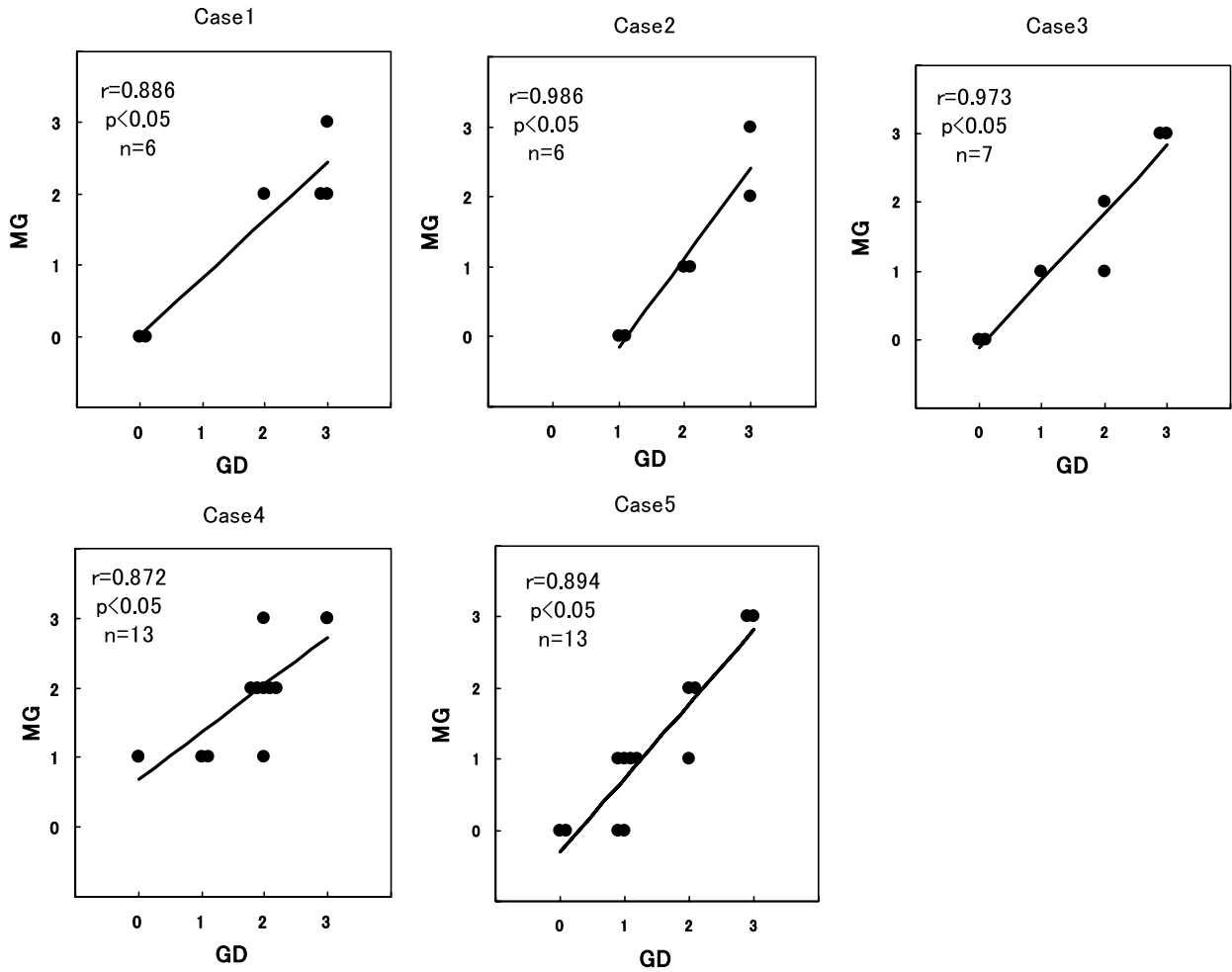


Fig. 1. Correlations between the changes of clinical activities in patients with GD and MG. Correlations between clinical activities of GD and MG in Cases 1-5 are shown.

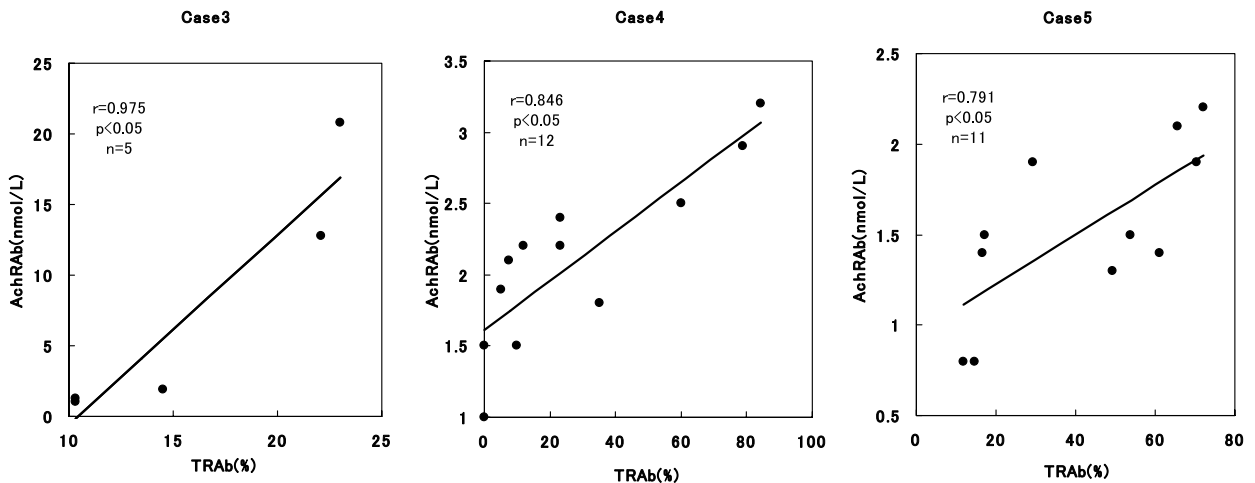


Fig. 2. Correlations between the changes of immunological activities in patients with GD and MG. Correlations between TRAb and AchRAB in Cases 3-5 are shown.

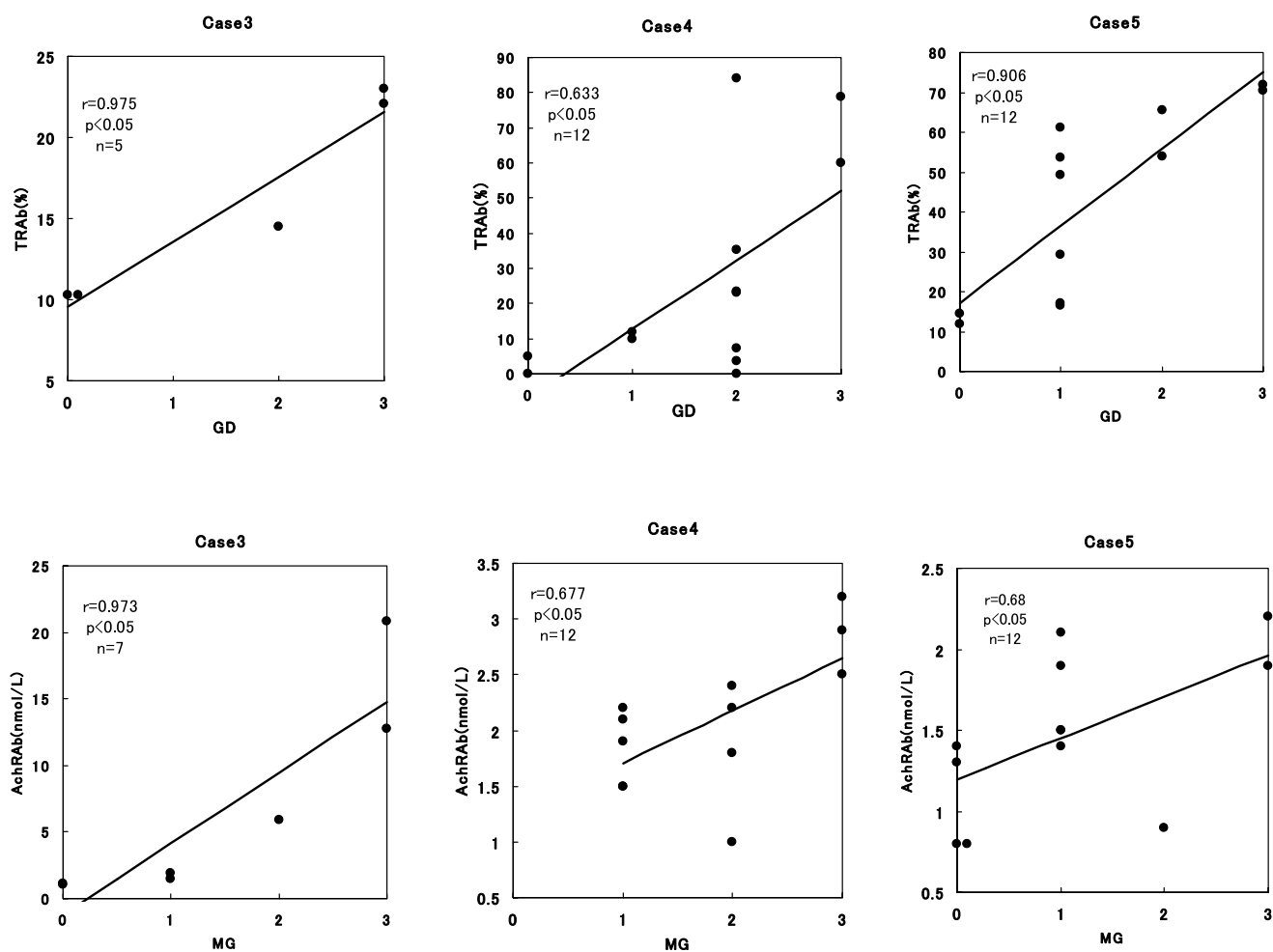


Fig. 3. Correlations between the changes of auto-antibodies against receptors and the corresponding clinical activities in patients with GD and MG. Correlations of TRAb vs. clinical activity of GD (upper panel), and AchRAB vs. clinical activity of MG (lower panel), in Cases 3-5 are shown.

Table 2. HLA typing of 5 cases with Graves' Disease and Myasthenia Gravis

Case	Class I			Class II			
1	A11	A24	B60. B61	Cw3	DR4	DR9	DQ3 DQ4
2		A26 B35		B62 Cw3		DR11	DQ7
3	A2	A26	B55	B61	Cw3 Cw1		DQ1 DQ3
4	A2	A24	B46	B60	Cw1	DR9	DQ3
5	A2	A24	B46 B48		Cw3 Cw1	DR4 DR8	DQ1 DQ3

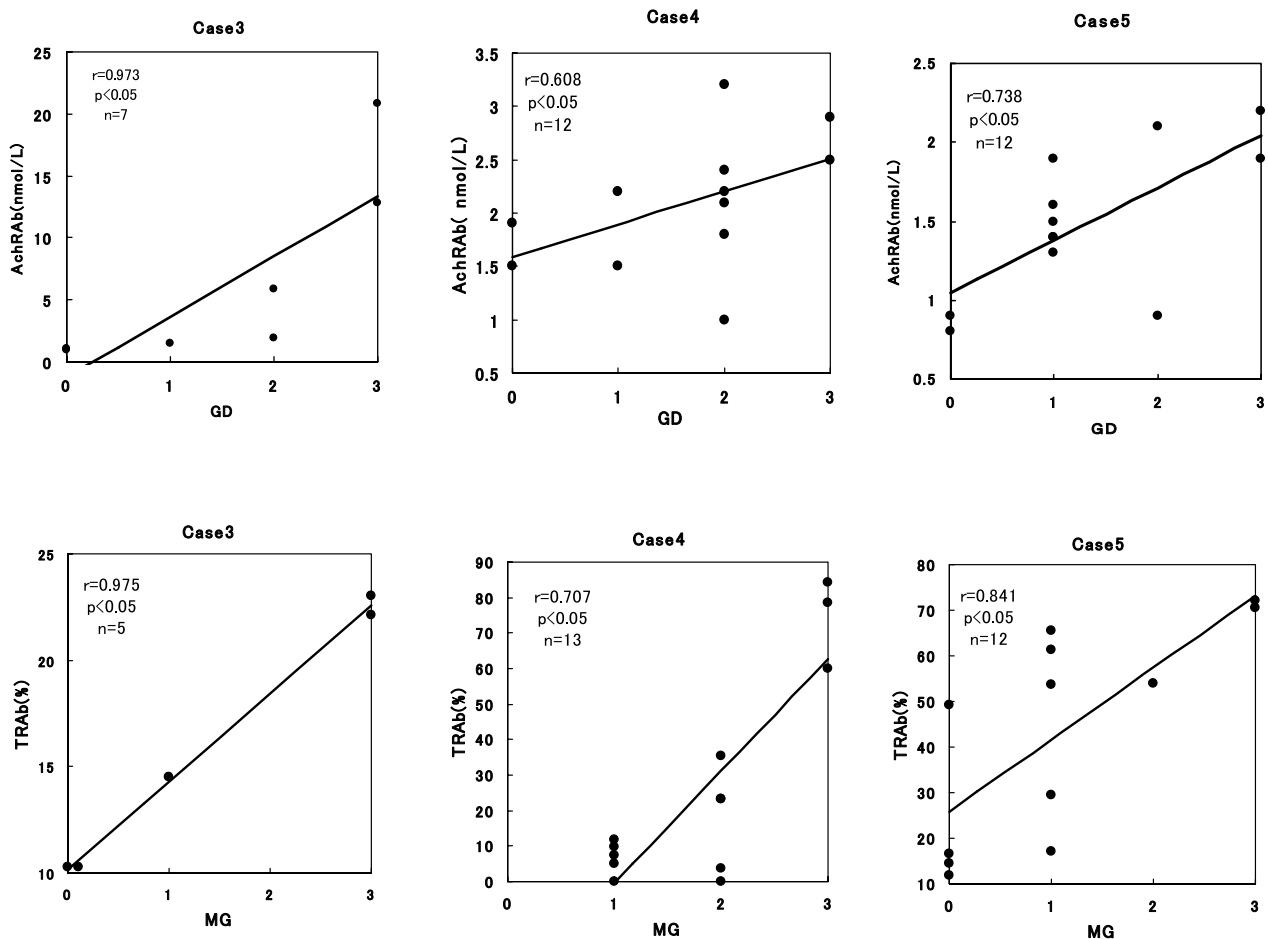


Fig. 4. Correlations between the changes of auto-antibodies against receptors and the clinical activities in patients with GD and MG. Correlations of AchRAb vs. clinical activity of GD (upper panel), and TRAb vs. clinical activity of MG (lower panel), in Cases 3-5 are shown.

MG) in these 3 cases (Fig. 4). However, T3 did not show any significant correlations with GD, MG, TRAb or AchRAb (data not shown).

HLA typing

Although there were no specific class I antigens in 5 cases, among class II antigens, HLA-DQ3 was positive in 4 out of 5 cases, and one (Case 2) had HLA-DQ7, a split antigen of HLA-DQ3 (Table 2). Since the present serological test could not distinguish between DQ8 and DQ9, both split antigens of DQ3, these 4 cases with positive HLA-DQ3 should have either HLA-DQ8 or HLA-DQ9. Thus all of 5 cases appeared to share HLA-DQ3 antigen in common.

Discussion

Both GD and MG are organ-specific autoimmune dis-

eases, but their association is rare; the prevalence of MG in GD patients is only 0.14% in Japan⁴. We described herein the clinical and immunological features of 5 cases with concomitant association of GD and MG encountered during the past five years, and studied clinical and immunological relationships between the two diseases.

It was originally proposed that there was a negative, the so-called 'see-saw', relationship between the clinical activities of GD and MG³. However, we could not find such a negative or 'see-saw' relationship, but we clearly demonstrated a positive relationship between the clinical activities of two disorders in all 5 cases. Our results are consistent with those of others^{8,9}, showing that MG tends to occur when GD is aggravated; a patient with MG became worse after taking an overdose of T₄⁸. Furthermore, we also demonstrated a positive relationship between the immunological activities of the two auto-antibodies against TSH receptor and

Ach receptor in three out of 5 cases. Except for two cases, one (Case 1) with undetectable AchRAb and another (Case 2) with few sample number, we could observe significant positive correlations of clinical activities of GD with TRAb and AchRAb as well as those of MG with AchRAb and TRAb in three out of 5 cases, respectively. However, the possibility that the treatment for GD and/or MG may have affected their clinical and immunological activities during the entire clinical course could not be excluded from our study.

Since our study was designed for retrospective clinical study dealing with only 5 cases with relatively rare association of MG and GD, the statistical analysis was limited by the small numbers of the patients studied and the sample data obtained simultaneously. In fact, two out of 5 cases could not provide enough sample data due to the lack of AchRAb (Case 1) and the very few sample number (Case 2). Thus, it is necessary to study sufficient number of patients and sample data for the appropriate statistical analysis in the future.

In 4 out of 5 of our cases, MG occurred after the development of GD, and in one (Case 5) simultaneously with GD. They were all hyperthyroid when MG occurred; three were hyperthyroid for a long time as a consequence of repeated drug withdrawals and two were during the aggravation of GD. Thus, hyperthyroidism per se may contribute directly to the development of MG.

Two possible mechanisms can be speculated; one is the direct action of thyroid hormone on the immune system, and the other is its indirect action through the increased adrenergic nervous system to augment innate immunity. First, hyperthyroidism triggered by certain stress could enhance the immune response and precipitate or perpetuate autoimmune diseases in the predisposed individuals^{10,11}, suggesting that thyroid hormone itself may partly contribute to the development of MG. The present study, however, showed that circulating T₃ levels did not show any relationships to clinical and immunological activities of MG, raising the possibility of a direct effect of thyroid hormone on the occurrence of MG remote. Second, the hyperactivity of adrenergic nervous system may play a role in the pathogenesis of autoimmune disorders¹². It has been shown that thyroid hormones increase β -adrenergic receptor number and activate receptor-coupled adenylate cyclase-cyclic AMP system^{13,14}, suggesting the possible contribution of thyroid hormone-mediated catecholamines hypersensitivity to activation of innate immunity. Consistent with this assumption, the clinical activity of GD, such as tachycardia, finger tremor,

hyper-hydrosis, mostly resulting from hyperactive adrenergic nervous system, positively correlated with the clinical and immunological activities of MG as shown in this study. However, it remains unknown how the hyperactive sympathetic nervous system associated with hyperthyroidism leads to activation of innate immunity to trigger the occurrence of MG.

The present serological analysis of HLA typing revealed that there were no specific class I antigens in our 5 cases, but among class II antigens, HLA-DQ3 was positive in 4 of 5, and HLA-DQ7 was positive in one. The phenotype frequencies of HLA-DQ3 and HLA-DQ7 in general Japanese population were 28.1% and 34.1%, respectively, while that of HLA-DQ1 (70.1%) is most popular¹⁵. Since the original broad serological specificity of HLA-DQ3 has been shown to arise as clear-cut splits of HLA-DQ7, HLA-DQ8, and HLA-DQ9¹⁶, HLA-DQ7 is considered as a split antigen of HLA-DQ3. However, the present serological analysis failed to discriminate two split antigens (DQ8, DQ9) of DQ3 in 4 cases with positive HLA-DQ3 due to the technical limitation, such that DNA genotype analysis of HLA typing should be more reasonable. Thus, it appears appropriate to consider that HLA-DQ3 was positive in all of our 5 cases as examined by serological analysis.

HLA analysis of GD in Japanese population demonstrated a strong association with HLA-A2, HLA-DR5, and HLA-DPB1*0501^{17,18}. In contrast, HLA analysis of MG in Japanese population revealed a strong association with HLA-DR9, HLA-DRw13, HLA-DQw1, and HLA-DQw3, in childhood-onset MG, but no specific antigens in adult-onset MG¹⁹. However, there have been no studies thus far reported on HLA typing in patients with concomitant association of GD and MG. Therefore, the existence of HLA-DQ3 in all of our 5 cases with adult-onset MG and GD, although its exact split antigens remain to be determined, may play a key role in the concomitant development of both diseases in Japanese patients. Further HLA analysis among more patient's population as well as other races with concomitant GD and MG is needed.

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