

## CLINICAL STUDY

# Clustering of additional autoimmunity behaves differently in Hashimoto's patients compared with Graves' patients

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## Abstract

**Objective:** It is unclear whether Hashimoto's thyroiditis and Graves' disease (often referred to as autoimmune thyroid disease, AITD) cluster to the same extent with other autoimmune disorders.

**Methods:** We assessed adrenal,  $\beta$ -cell, celiac and gastric antibodies in a cohort of 523 adult patients with Graves' disease and 359 patients with Hashimoto's disease and compared their clustering.

**Results:** Adrenal autoimmunity associated more often with Hashimoto's disease (9.0%) than with Graves' disease (3.3%,  $P=0.001$ ).  $\beta$ -cell autoimmunity was seen more frequently in Hashimoto's disease (25.4%) than in Graves' disease (15.6%,  $P=0.001$ ) patients. We found low prevalences of celiac autoimmunity (1.2% for Graves' and 1.2% for Hashimoto's disease). Celiac and gastric autoimmunity were not statistically different in Hashimoto's and Graves' disease patients. Although gastric autoimmunity itself was equally prevalent (around 20%), Hashimoto's disease often showed significantly more clustering of adrenal autoimmunity with gastric autoimmunity (5.3%) than Graves' disease (1.2%,  $P=0.001$ ). Similarly, clustering of adrenal autoimmunity was seen with  $\beta$ -cell autoimmunity in Hashimoto's patients (3.2%), while such clustering was much less encountered in 359 Graves' patients (0.9%,  $P=0.029$ ).

**Conclusion:** In conclusion, Hashimoto's disease shows a markedly higher clustering of additional autoimmunity, especially with adrenal and  $\beta$ -cell autoimmunity. Combined clustering of gastric and adrenal autoimmunity and combined clustering of adrenal and  $\beta$ -cell autoimmunity were both seen more often in Hashimoto's patients. Clustering with celiac disease appears to be low. These findings indicate that Hashimoto's and Graves' disease differ in their clinical expression regarding additional autoimmunity, which argues against the indiscriminate use of AITD as an entity.

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## Introduction

Autoimmune thyroid disease (AITD) includes Hashimoto's thyroiditis (goitrous or atrophic autoimmune thyroiditis) and Graves' disease. AITD has been proposed to be part of the autoimmune polyglandular syndromes (APS). In the original case report of 1926, Schmidt described the autopsy reports of two patients with Addison's disease and Hashimoto's thyroiditis. Later, Neufeld *et al.* (1) classified the APS into distinct syndromes, e.g. APS-1 consisting of primary adrenal failure with chronic mucocutaneous candidiasis and/or acquired hypoparathyroidism, APS-2 comprising primary adrenal failure, AITD and/or type 1 diabetes. By then, Graves' disease was also included into the classification by introducing the term 'AITD'. In a study of 107 patients with Addison's disease, Betterle & Zanchetta (2) reported the occurrence of AITD in 82% of patients, 50% of whom had Addison's and Hashimoto's, 21% had Addison's and Graves' and 11% had Addison's, AITD and type 1 diabetes.

This difference in clustering frequencies may indicate that Hashimoto's and Graves' disease do not have the same pathogenetic basis.

Although Graves' and Hashimoto's disease share several gene polymorphisms in *CTLA4* (3), other *CTLA4*-gene polymorphisms tend to be more associated with Graves' disease (4). Furthermore, a recent study on Hashimoto's thyroiditis has demonstrated differences in HLA class II associations compared with Graves' disease (5).

Additional definitions of APS have been suggested since APS encompasses a wide clinical spectrum of disease with a highly variable presentation of the first manifestation. Also, the age of first presentation can be anywhere between infancy and late adult life. Neufeld *et al.* (1) defined APS-3 as the association between AITD with one of the following: DM1, atrophic gastritis or vitiligo, alopecia, myasthenia gravis or other organ-specific autoimmune disorders. Currently, relationship of autoimmune thyroid disorders with

other organ-specific autoimmune disorders, such as Addison's disease, type 1 diabetes mellitus, pernicious anemia, celiac disease, dermatitis herpetiformis, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic sclerosis and vitiligo, is well established (6, 7). Disease association with, for example, autoimmune hepatitis, primary biliary sclerosis and Sjögren's syndrome seems possible but require further confirmation (6). Similarly, type 1 diabetes predisposes to other organ-specific autoimmune disorders, such as AITD, Addison's disease, celiac disease and atrophic gastritis (8). APS-4 has been proposed to include all the clinical combinations which could not be allocated to one of the other three APS syndromes. Various authors (8–10), as well as the NICE guidelines, have concluded that preclinical screening for autoantibodies should be performed not only in patients with type 1 diabetes but also in patients with AITD (11, 12).

We asked the question whether differences exist in association with other autoimmune antibodies and/or disorders between Graves' disease and Hashimoto's disease. We undertook to compare adrenal,  $\beta$ -cell, celiac and gastric antibodies in a large cohort of adult patients with Hashimoto's or Graves' disease, in whom various autoantibodies were screened. We report here that patients with Hashimoto's disease show more often adrenal and  $\beta$ -cell autoimmunity than Graves' disease patients. Similarly, our patients with Hashimoto's thyroiditis show a clear clustering of adrenal autoimmunity with gastric autoimmunity, which was not apparent in Graves' disease patients. Surprisingly, both patient groups had a low prevalence of celiac autoimmunity.

The differences in clustering of additional autoimmune phenomena between these disorders may indicate that they should not be taken as to possess identical predisposition to other autoimmune disorders.

## Subjects and methods

### Subjects

Since 2005, 882 Caucasian patients treated for AITD at the outpatient clinic of the Department of Endocrinology-Diabetology of the University Medical Centre in Utrecht (UMCU), The Netherlands, have been screened routinely for the presence of other autoimmune antibodies. AITD included Hashimoto's disease (goitrous or atrophic autoimmune hypothyroidism), defined as (sub)clinical hypothyroidism in combination with thyroid peroxidase (TPO)-antibodies, and Graves' disease (autoimmune hyperthyroidism), defined as clinical hyperthyroidism in combination with positive thyroid-stimulating immunoglobulins. From the same outpatient clinic of the Department of Endocrinology-Diabetology, we included Addison's disease patients

only if the diagnosis of AITD was made prior to or together with the diagnosis of Addison's disease in order to rule out selection bias; our department has no specific Addison's outpatient clinic. Patients with Down syndrome or Turner's syndrome were excluded.

At the time of diagnosis or during follow-up, patients were routinely screened for the presence of autoimmune antibodies: antibodies against TPO, parietal cell antibodies (APC), intrinsic factor (IF) antibodies, tissue transglutaminase antibodies (TTG), endomysium antibodies (Em), adrenal cortex (AC) antibodies and glutamic acid decarboxylase (GAD). IgA immunoglobulin levels were measured to rule out IgA deficiency. Also, plasma vitamin B12 level, homocystein, free thyroxine and TSH levels were routinely determined.

### Methods

A luminescence immunoassay (human LIA, BRAHMS, Henningsdorf, Germany) was used for the quantitative determination of antibodies against TPO and the human TSH receptor. Values of more than 60 U/ml and 1.5 IU/l respectively, were regarded as positive. The sensitivity and specificity for anti-TPO assessments were 89 and 90% respectively, and 98.8 and 99.6% respectively, for the anti-TSH assessment.

ELISA (Euroimmun, Lübeck, Germany) was applied for anti-IF (positive value  $\geq 20$  U/ml), sera dilution: 1:100 sample buffer, anti-TTG (positive value  $\geq 10$  U/ml), sera dilution: 1:100 sample buffer and anti-GAD (positive value  $\geq 10$  IU/l), undiluted. The sensitivity and specificity for these tests were 100 and 100% for the anti-IF test, 96.2 and 99.4% for anti-TTG and 92 and 98% for anti-GAD. All antibody parameters were reported as present or absent. Dubious or weak diagnostic findings were considered as absent antibodies.

An indirect immunofluorescence assay (IFA) was used for screening for circulating antibodies to parietal cells, endomysium (Euroimmun) and the AC (The Binding Site Ltd, Birmingham, UK), with a sensitivity and specificity of 95 and 98% for APC antibodies, 91 and 98% for anti-Em and 95 and 95% for anti-AC. Substrates used were monkey esophagus and monkey adrenal. All antibody parameters were reported as present or absent. Dubious or weak diagnostic findings were considered as absent antibodies, to rule out uncertainty.

### Assessment

At the time of diagnosis and during follow-up, patients were assessed regarding the presence of other autoimmune diseases, primarily for Addison's disease, type 1 diabetes mellitus, celiac disease, atrophic gastritis, pernicious anemia or vitamin B12 deficiency (without specific cause such as use of metformin, alcohol or celiac disease). In the absence of clinical disease, patients with positive antibodies were screened for the presence of

(sub) clinical disease once every year; for example, in patients with adrenal antibodies, basal ACTH levels were measured and an ACTH stimulation test was performed. For patients with positive anti-GAD antibodies, a fasting plasma glucose was measured. In patients with positive APC and/or anti-IF, vitamin B12 levels were measured yearly. The decision to perform gastroscopy in patients with positive APC or with positive Em and TTG antibodies was left to the discretion of the attending physician.

We defined patients as being prone to autoimmunity when they had either another clinical autoimmune disease or had an associated antibody. We defined four categories:

- i. Adrenal autoimmunity was defined as presence of Addison's disease and/or AC antibodies. Addison's disease (adrenal insufficiency) was diagnosed on the basis of a combination of low serum cortisol levels and strong elevations in plasma ACTH or on the basis of an abnormal ACTH stimulation test.
- ii.  $\beta$ -cell autoimmunity was defined as type 1 diabetes mellitus or positivity for anti-GAD antibodies in patients without type 1 diabetes. Celiac autoimmunity was considered to be present in patients who had biopsy-proven celiac disease or who were positive for both Em and TTG antibodies.
- iii. Gastric autoimmunity was considered to be present if patients were positive for at least one of the following: APC antibodies, anti-IF antibodies, signs of atrophic gastritis at gastroscopy, pernicious anemia or low vitamin B12 level ( $<130$  pmol/l), without a specific cause such as use of metformin, alcohol, or celiac disease. Patients in whom a specific cause for vitamin B12 deficiency other than gastric autoimmunity was suspected were not included as having a low vitamin B12 level.
- iv. Other additional autoimmune diseases were looked for in the patients' medical files; vitiligo, alopecia, psoriasis, myasthenia gravis, multiple sclerosis, Crohn's disease, ulcerative colitis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, SLE, rheumatoid arthritis, Sjogren's disease, scleroderma (dermato) myositis, idiopathic thrombocytopenia, premature ovarian failure and autoimmune hypoparathyroidism. If one or more of these disorders were present, the cases were defined as Other autoimmune disease.

### Statistical analysis

For statistical analysis, SPSS, version 15 (IBM Company, Chicago, IL, USA) was used. Group comparisons were performed by the use of the  $\chi^2$  test. Odds ratio (OR) and the 95% confidence interval (95% CI) were used to investigate differences between the groups and clustering of the various autoimmune antibodies. When

sample sizes were small (i.e. below 5), Fisher's exact test was used.  $P < 0.05$  was considered statistically significant.

## Results

A total of 882 Caucasian patients consisted of 523 patients with Graves' disease and 359 patients with Hashimoto's thyroiditis (Table 1).

### Adrenal autoimmunity

Addison's disease was significantly more prevalent in Hashimoto's disease than in Graves' disease (5.3 and 1.7% respectively,  $P = 0.03$ ; OR 3.19, 95% CI 1.43–7.14) (Table 2). There was no difference in gender distribution. All together, adrenal autoimmunity was three times more prevalent in Hashimoto's disease than in Graves' disease ( $P = 0.001$ , Tables 2 and 3).

### $\beta$ -cell autoimmunity

Type 1 diabetes was more prevalent in Hashimoto's disease than in Graves' disease (15.9 and 9.2% respectively,  $P = 0.03$ ). In Hashimoto's disease, 6.6% of patients without type 1 diabetes were GAD-positive compared with 4.6% of non-diabetic patients with Graves' disease ( $P = 0.29$ ).  $\beta$ -Cell autoimmunity was significantly more prevalent in Hashimoto's disease (25.4%) than in Graves' disease (15.6%,  $P = 0.001$ ; OR 1.84, 95% CI 1.26–2.68).

### Celiac autoimmunity

There was no difference in celiac disease, anti-TTG and anti-Em antibodies or celiac autoimmunity between Hashimoto's and Graves' disease. Remarkably, none of the 60 men with Graves' disease showed celiac autoimmunity.

### Gastric autoimmunity

Vitamin B12 deficiency (i.e. plasma vitamin B12 below 130 pmol/l, without a specific cause other than gastric autoimmunity) was encountered in a substantial number of subjects (66 in total). Patients positive for antibodies against parietal cells or IF, without B12 deficiency, were

**Table 1** Biographics of subjects with AITD (mean  $\pm$  s.d.).

	Graves' disease	Hashimoto's disease
<i>n</i>	523	359
Gender (F/M)	444/79	307/52
Age (years)	50.0 $\pm$ 14.0	49.0 $\pm$ 15.0
Duration of disease (years)	12.0 $\pm$ 10.0	10.0 $\pm$ 8.0

**Table 2** Additional autoimmunity (adrenal,  $\beta$ -cell, celiac, gastric and 'other') in Graves' ( $n=523$ ) and Hashimoto's ( $n=359$ ) disease patients. For each entity, 'total' cases is given, followed by subdivision into categories of patients with clinical disease and patients with autoantibodies without clinical disease.

	Graves' No. of cases/ total cases measured (%)	Hashimoto's No. of cases/ total cases measured (%)
Adrenal autoimmunity (total)	14/421 (3.3)	25/278 (9.0)
Addison's disease	9/523 (1.7)	19/359 (5.3)
Adrenal antibodies without Addison's	5/412 (1.2)	6/259 (2.3)
$\beta$ -Cell autoimmunity (total)	65/417 (15.6)	72/284 (25.4)
Diabetes type 1	48/523 (9.2)	57/359 (15.9)
GAD antibodies without diabetes	17/369 (4.6)	15/227 (6.6)
Celiac autoimmunity (total)	5/411 (1.2)	3/260 (1.2)
Celiac disease	2/523 (0.4)	2/359 (0.6)
Anti-TTG and anti-Em without histology	3/414 (0.7)	1/263 (0.4)
Gastric autoimmunity (total)	75/391 (19.2)	64/273 (23.4)
Vitamin B12 deficiency or atrophic gastritis	36/436 (8.3)	30/310 (9.7)
APC or IF without vitamin B12 deficiency	39/355 (11.0)	34/243 (14.0)
Other autoimmune diseases	34/523 (6.5)	43/359 (12.0)

often seen in patients with Graves' (10.2%) and Hashimoto's disease (13.5%). There were no significant differences in gastric autoimmunity (23.4 and 19.2%) between Hashimoto's and Graves' disease patients.

### Other autoimmune diseases

Combined, these diseases were seen more often in Hashimoto's disease than in Graves' disease (12.0 and 6.5%,  $P=0.05$ ; OR 1.96, 95% CI 1.22–3.14). Since these disorders were each present in <1%, we did not look for clustering of each particular disease with Hashimoto's or Graves' disease.

### Clustering of additional autoimmunity

Since clustering of autoimmunity is the hallmark of APS, we looked into detail at the various forms of clusters in Hashimoto's disease and Graves' disease patients.

In Table 4, the prevalences of the various clusters of additional autoimmunity are shown. The combination of adrenal and  $\beta$ -cell autoimmunity was seen more often (OR 3.47, 95% CI 1.06–11.37;  $P=0.029$ ) in patients with Hashimoto's disease (3.2%), compared with patients with Graves' disease (0.9%). If type 1 diabetes patients were excluded, 5 of 234 Hashimoto's disease patients were found adrenal and GAD positive and none of 383 patients with Graves' disease, with even more stringent statistical significance ( $P=0.008$ , Fisher's exact test). Furthermore, the combination of adrenal and gastric autoimmunity was also more often

present in Hashimoto's disease than in Graves' disease (OR 4.70, 95% CI 1.69–13.09;  $P=0.001$ ). Within the Hashimoto's subgroup, patients positive for gastric autoimmunity showed over five times more adrenal autoimmunity than gastric autoimmunity-negative patients (28.6 vs 5.1%;  $\chi^2=21.60$ ;  $P<0.0005$ ). Within the Graves' disease patients, this relationship was not seen (5.6 vs 2.1% respectively,  $P=0.16$ , Fisher's exact test). All other potential cluster combinations showed no significant differences. Notably, among our small group of celiac autoimmunity-positive patients, no (concurrent) adrenal autoimmunity was found and only three patients had concomitantly gastric and celiac autoimmunity.

### Discussion

We undertook to compare adrenal,  $\beta$ -cell, celiac and gastric antibodies in a cohort of adult patients with Hashimoto's or Graves' disease in order to compare clustering of autoimmune manifestations between these thyroid diseases. This study shows that patients with Hashimoto's disease are two- to three-fold more at risk for adrenal antibodies and adrenal autoimmunity than patients with Graves' disease. Although gastric autoimmunity itself was equally prevalent in Hashimoto's and Graves' disease, Hashimoto's disease showed a clear clustering of adrenal autoimmunity with gastric autoimmunity, which was much less apparent (and was not statistically significant) in Graves' disease. Similarly, clustering of adrenal autoimmunity with  $\beta$ -cell autoimmunity was seen in diabetic (9/281) as well as in non-diabetic (5/234) Hashimoto's disease patients, while such clustering was not encountered in 383 non-diabetic Graves' disease patients. Finally, we found relatively low prevalence of celiac autoimmunity in each of the two thyroid disorders.

In thyroid patients (Graves' disease or Hashimoto's disease), the frequency of adrenal antibodies has been reported to be around 2–3% (13–15). Studies comparing additional autoimmunity in Graves' and Hashimoto's disease in sufficient numbers of patients

**Table 3** Adrenal,  $\beta$ -cell, celiac and gastric autoimmunity in Hashimoto's vs Graves' disease.

Auto-immunity	Hashimoto's vs Graves'		
	(No. of cases/ total cases assessed)	OR (95% CI)	P value
Adrenal	25/278 vs 14/421 (9.0 vs 3.3%)	2.87 (1.47–5.63)	0.001
$\beta$ -Cell	72/284 vs 65/417 (25.4 vs 15.6%)	1.84 (1.26–2.68)	0.001
Celiac	3/260 vs 5/411 (1.2 vs 1.2%)	0.95 (0.23–4.00)	NS
Gastric	64/273 vs 75/391 (23.4 vs 19.2%)	1.27 (0.88–1.85)	NS

OR, odds ratio; 95% CI, 95% confidence interval; NS, not significant.

**Table 4** Clustering of combinations of adrenal,  $\beta$ -cell, celiac and gastric autoimmunity in Hashimoto's vs Graves' disease.

	Adrenal and $\beta$ -cell	Adrenal and celiac	Adrenal and gastric	$\beta$ -cell and celiac	$\beta$ -cell and gastric	Celiac and gastric
Hashimoto's	9/281 (3.2%)	0/285 (0%)	15/283 (5.3%)	1/281 (0.4%)	21/282 (7.4%)	2/279 (0.7%)
Graves'	4/423 (0.9%)	0/431 (0%)	5/425 (1.2%)	0/424 (0%)	18/412 (4.4%)	1/424 (0.2%)
OR (95% CI)	3.47 (1.06–11.37)	NA	4.70 (1.69–13.09)	NA	1.76 (0.92–3.37)	3.05 (0.28–33.84)
P value	0.029	NA	0.001	NS	NS	NS

NA, not applicable due to zero events; NS, not significant.

are clearly very scarce. We report here prevalences of adrenal autoimmunity (AC antibodies and/or Addison's disease) in two large groups of Graves' disease and Hashimoto's patients separately. We found an almost threefold higher occurrence of adrenal autoimmunity in Hashimoto's disease than in Graves' disease. Although the study by Boelaert *et al.* (16) also reported that Addison disease was more prevalently found in Hashimoto's disease (1.4%) than in Graves' disease (0.11%), the authors reported only clinical data without clarifying in what proportion of subjects (if any) antibodies were actually assessed. Their data may therefore represent a clear underestimation.

In our patients without type 1 diabetes, positive anti-GAD antibodies were present in 6.6% of patients with Hashimoto's thyroiditis and in 4.6% of patients with Graves' disease, which was not significantly different. These results are comparable with studies previously reported (17–19). A Turkish study reported a prevalence of 5.1% GAD-positivity in 215 patients with Hashimoto's thyroiditis, which is a similar percentage as our finding of 6.6% (17). In Japanese studies, around 4% of Graves' patients and around 3–6% of Hashimoto's patients were positive for GAD-antibodies (18, 19).

In the present cohort, type 1 diabetes was significantly more prevalent in Hashimoto's disease than in Graves' disease (15.9 vs 9.2%), which is in line with the findings of other authors (6, 16).

We found equally low prevalences of celiac disease of 1.2% in Hashimoto's and Graves' disease. This is somewhat lower than recent (smaller) studies which reported percentages between 2.7 and 4.8 (20–23). We ruled out IgA-deficiency since Em and TTG test are an IgA-class test. Since most authors report around 0.5–1.0% celiac disease in the general population, this low prevalence would imply that the association of Graves' or Hashimoto's disease with celiac autoimmunity is at the most relatively weak.

In our study, 20% of both Hashimoto's and Graves' disease patients showed gastric autoimmunity, comparable to previous reports in adults (24), while in children, even higher percentages have been found (25). Moreover, we found a prevalence of vitamin B12 deficiency of 9.7 and 8.3% in Hashimoto's and Graves' disease respectively. This is lower than the overall prevalence of 28% found in a previous study in a mix of 94 thyroid disease patients (26). Although the authors used the same reference range, unfortunately, they did

not mention whether other causes (metformin, alcohol or celiac disease) had been excluded. In a study of 63 adult patients concordant for AITD and DM1, the prevalence of vitamin B12 deficiency due to atrophic gastritis was 6.3% (27), while in our present cohort, this percentage was 9.8% (10/102 patients).

A hallmark of the various APS entities is the clustering of a number of autoimmune disorders. Often, AITD is included in this clustering without differentiation between Hashimoto's and Graves' disease. We therefore wanted to further explore the various clustering in our Hashimoto's and Graves' disease patients. Remarkably, adrenal–gastric autoimmunity clustering was seen in 5.3% of our Hashimoto's disease patients and in only 1.2% of Graves' disease patients. Similarly, clustering of adrenal– $\beta$ -cell autoimmunity was more often encountered in Hashimoto's disease (3.2%) than in Graves' disease (0.9%). Together, this may point to a predisposing effect of adrenal autoimmunity towards additional ( $\beta$ -cell and/or gastric) autoimmunity mainly in Hashimoto's disease.

In summary, we assessed additional autoimmunity in a cohort of Graves' and Hashimoto's disease patients. Patients with Hashimoto's disease showed a two- to three-fold greater risk for adrenal antibodies and adrenal autoimmunity than patients with Graves' disease. Gastric autoimmunity was comparable in both disorders. Interestingly, Hashimoto's disease patients had a greater risk of both adrenal– $\beta$ -cell and adrenal–gastric autoimmunity clustering. Finally, celiac disease was encountered in only about 1% of patients. In conclusion, although Hashimoto's and Graves' disease share a tendency toward gastric autoimmunity and a low prevalence of celiac disease, Hashimoto's disease shows a markedly higher clustering of additional autoimmunity, namely adrenal– $\beta$ -cell and adrenal–gastric autoimmunity. These differences argue against the indiscriminate use of the term AITD, for example, in the delineation of APS.

## Declaration of interest

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

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