

## Coeliac disease in Dutch patients with Hashimoto's thyroiditis and vice versa

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Received: 2006-11-24 Accepted: 2007-03-15

hypothyroidism in seven (3.8%; 95% CI 1.8-7.6), and overt hypothyroidism (Hashimoto's thyroiditis) in 22 (12%; 95% CI 8-16). Moreover, four patients with coeliac disease had Graves' disease (2%; 95% CI 0.8-5) and one patient had post-partum thyroiditis.

**CONCLUSION:** The data from a Dutch population confirm the association between Hashimoto's thyroiditis and coeliac disease. Screening patients with Hashimoto's thyroiditis for coeliac disease and vice versa is recommended.

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**Key words:** Autoimmune disease; Coeliac disease; Hashimoto's thyroiditis

Hadithi M, de Boer H, Meijer JWR, Willekens F, Kerckhaert JA, Heijmans R, Peña AS, Stehouwer CDA, Mulder CJJ. Coeliac disease in Dutch patients with Hashimoto's thyroiditis and vice versa. *World J Gastroenterol* 2007; 13(11): 1715-1722

<http://www.wjgnet.com/1007-9327/13/1715.asp>

### Abstract

**AIM:** To define the association between Hashimoto's thyroiditis and coeliac disease in Dutch patients.

**METHODS:** A total of 104 consecutive patients with Hashimoto's thyroiditis underwent coeliac serological tests (antigliadins, transglutaminase and endomysium antibodies) and HLA-DQ typing. Small intestinal biopsy was performed when any of coeliac serological tests was positive. On the other hand, 184 patients with coeliac disease were subjected to thyroid biochemical (thyroid stimulating hormone and free thyroxine) and thyroid serological tests (thyroglobulin and thyroid peroxidase antibodies).

**RESULTS:** Of 104 patients with Hashimoto's thyroiditis, sixteen (15%) were positive for coeliac serology and five patients with documented villous atrophy were diagnosed with coeliac disease (4.8%; 95% CI 0.7-8.9). HLA-DQ2 (and/or -DQ8) was present in all the five and 53 patients with Hashimoto's thyroiditis (50%; 95% CI 43-62). Of 184 patients with coeliac disease, 39 (21%) were positive for thyroid serology. Based on thyroid biochemistry, the 39 patients were subclassified into euthyroidism in ten (5%; 95% CI 2-9), subclinical

### INTRODUCTION

Coeliac disease, an immune-mediated enteropathy that develops in susceptible individuals upon ingestion of gluten containing diet, is closely associated with other autoimmune endocrine disorders, particularly autoimmune thyroid disease<sup>[1]</sup>. The relationship between coeliac disease and autoimmune thyroid disease was first described three decades ago<sup>[2]</sup>. Since then, coeliac disease has been found to be more prevalent in patients with autoimmune thyroid disease in general and especially in Hashimoto's thyroiditis than in the general population, ranging between 3.3% and 8%<sup>[3-6]</sup>.

The term autoimmune thyroid disease encompasses a number of different entities characterized by varying degrees of thyroid dysfunction and the presence of serum autoantibodies against thyroid tissue-specific components, such as thyroglobulin (TG) and thyroid peroxidase (TPO)<sup>[7]</sup>. Hashimoto's thyroiditis is defined by the presence of high serum thyroid antibody concentrations (TG and/or TPO) accompanied by hypothyroidism<sup>[8]</sup> or goiter<sup>[9]</sup>. Likewise, several studies reported prevalences ranging from 10% to

30%<sup>[10-13]</sup> for autoimmune thyroid disease, and from 4 to 19% for Hashimoto's thyroiditis in patients with coeliac disease<sup>[11-14]</sup>.

Screening patients with autoimmune thyroid disease for coeliac disease, and vice versa, can give an accurate perception to this association<sup>[1]</sup>. However, apart from a report from Italy<sup>[10]</sup>, studies in this field are scarce in the literature. We designed a study to examine the association between Hashimoto's thyroiditis and coeliac disease from a Dutch population. Both patient groups attended the same clinic. Moreover, in view of the apparently conflicting linkage and association between human leukocyte antigen (HLA) and Hashimoto's thyroiditis<sup>[5,15]</sup>, we determined the prevalence of the coeliac specific HLA-DQ2 and -DQ8 in patients with Hashimoto's thyroiditis.

## MATERIALS AND METHODS

### Screening individuals with Hashimoto's thyroiditis for coeliac disease

Between January 2001 and January 2003, 104 consecutive adults with Hashimoto's thyroiditis (43 newly diagnosed and 61 had been on thyroid hormonal replacement therapy) attending the Outpatient Department of Endocrinology in Rijnstate Hospital were included in the study. Hashimoto's thyroiditis was defined by the presence of thyroid antibodies and hypothyroidism<sup>[8]</sup>. This strict criterion was chosen to exclude patients who could be falsely positive to thyroid antibodies. Thirty-six patients (35%) with Hashimoto's thyroiditis had symptoms suggestive of coeliac disease, including diarrhoea ( $n = 17$ ), abdominal pain ( $n = 10$ ), iron deficiency anaemia ( $n = 3$ ), and osteoporosis ( $n = 6$ ), and the remaining 68 (65%) did not have any of these symptoms. The general characteristics of patients with Hashimoto's thyroiditis are described in Table 1.

Coeliac serological tests [serum IgA gliadin antibodies (AGA-IgA), serum IgG gliadin antibodies (AGA-IgG), serum IgA transglutaminase antibodies (TGA), and serum IgA endomysium antibodies (EMA)], and coeliac heterodimers (HLA-DQ2 and -DQ8) were determined in all patients. Patients with Hashimoto's thyroiditis who were positive for any of coeliac serological tests were advised to take a small intestinal endoscopy for histological examination<sup>[16,17]</sup>.

### Screening individuals with coeliac disease for thyroid dysfunction

Between May 1998 and May 2005, 184 adult patients with coeliac disease attending the Outpatient Department of Gastroenterology of Rijnstate Hospital were included in the study. All patients fulfilled the diagnostic criteria of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)<sup>[18]</sup>. During this period, coeliac disease was newly diagnosed in 77 patients (42%) while the other 107 patients (58%) had been already on gluten free diet for a mean period of 6.3 years (range 1-29 years). The general characteristics of patients with coeliac disease are also described in Table 1. At time of inclusion, coeliac serological tests, HLA-DQ typing and

Table 1 General characteristics of patients with Hashimoto's thyroiditis and patients with coeliac disease

Characteristics	Patients with Hashimoto's thyroiditis $n = 104$	Patients with coeliac disease $n = 184$
Mean (range) age, yr	46 (20-74)	53 (18-82)
Mean (range) age at diagnosis, yr	42 (12-72)	43 (3-76)
Men, $n$ (%)	13 (13%)	46 (25%)
mean $\pm$ SD BMI	25.9 $\pm$ 5.3	22.2 $\pm$ 2.8
Caucasians, $n$ (%)	90 (86)	168 (92)
Family history of Hashimoto's thyroiditis or coeliac disease	19%	21%

small intestinal biopsy were performed.

Thyroid biochemistry [thyroid stimulating hormone (TSH) and free thyroxine (FT<sub>4</sub>)] and thyroid antibodies [TG, TPO and if required thyroid stimulating immunoglobulin (TSI)] were determined in patients with coeliac disease. Patients with positive thyroid serology were further classified according to the American Thyroid Association Guidelines into overt hypothyroidism (elevated TSH and decreased FT<sub>4</sub>) and subclinical hypothyroidism (elevated TSH and normal FT<sub>4</sub>)<sup>[19]</sup>. Patients with positive thyroid serology and normal TSH were considered to have euthyroid autoimmune thyroiditis<sup>[7]</sup>. Patients with positive thyroid serology and hypothyroidism were considered to have Hashimoto's thyroiditis according to the study design. Patients were diagnosed with Grave's disease when thyroid biochemistry (low TSH, high serum FT<sub>4</sub>), and radioactive iodine <sup>131</sup>I thyroid scan were compatible with the diagnosis of Graves' disease with or without the presence of TSI<sup>[20]</sup>.

### Thyroid biochemistry

Serum TSHs were determined by a Sandwich immunoassay using an electrochemiluminescence detection method (ECLIA, reference range: 0.30-4.0 mU/L)<sup>[21]</sup>. FT<sub>4</sub> was determined by a competitive immunoassay with the same detection technique<sup>[22,23]</sup>. Reference range for FT<sub>4</sub> was 11-22 pmol/L<sup>[24]</sup>. TSH and FT<sub>4</sub> were measured using a dedicated automatic analyser (Elecsys 2010, Roche Diagnostics, Mannheim, Germany).

### Serum antibodies

Serum thyroid antibodies including TG and TPO were detected by the automated immunoassays Immulite 2000 anti-TG Ab and Immulite 2000 anti-TPO Ab (Euro DPC Ltd, Llanberis, Gwynedd, UK)<sup>[25]</sup>. And 50 IU/mL and 45 IU/mL cut-off values for TG and TPO were employed respectively. TSI was detected by the LUMI test (TRAK human of BRAHMS, Berlin) with a cut-off value of 1.5 IU/L<sup>[26]</sup>. Serum AGA-IgA and AGA-IgG were measured by enzyme-linked immunosorbent assay (ELISA)<sup>[27]</sup>, serum was diluted 1:100 and the results were expressed in Dutch units per millilitre (DU/mL) (CLB Amsterdam). Titres > 4 DU/mL and > 12 DU/mL were considered elevated for AGA-IgA and AGA-IgG, respectively. Serum TGA was determined by ELISA, as described by Dieterich *et al*<sup>[28]</sup> and was expressed as DU/mL using a Dutch reference serum

(100 DU TGA/mL) for calibration with a cut-off value of  $> 5$  DU/mL<sup>[29]</sup>. Serum EMA was determined by means of indirect immunofluorescence on frozen sections of commercial slides of primate ileum (EuroIMMUN) with a cut-off titre of  $> 8$ <sup>[27]</sup>. Seropositivity was defined when one or more of measured antibody tests were positive.

### HLA-DQ typing

Whole blood was obtained for HLA-DQA1 and DQB1 genotyping. PCR-amplified exon 2 amplicons were generated for low to medium resolution typing in a combined single-stranded conformation polymorphism (SSCP)/heteroduplex assay by a semi-automated electrophoresis and gel staining method on the Phastsystem™ (Amersham-Pharmacia-Biotech, Sweden). Alleles DQA1\*05 and DQB1\*02 (encoding the HLA-DQ2 heterodimer) and alleles DQA1\*03 and DQB1\*0302 (encoding the HLA-DQ8 heterodimer) could be reliably characterized in homozygous and heterozygous states. The method has been validated using a panel of reference DNA against the Dynall Allset<sup>+TM</sup> SSP high-resolution typing kits (DynaL AS Oslo, Norway)<sup>[30]</sup>.

### Histopathology

Small intestinal biopsies (at least 3-4) were obtained during upper gastrointestinal endoscopy from the third part of the duodenum with a spike forceps for histology<sup>[16]</sup>. An experienced pathologist (JWRM) did the evaluation of all biopsied material according to the modified Marsh classification<sup>[16]</sup>.

A normal small intestinal mucosa is characterized by normal villous architecture. The villous: crypt ratio is 4:1, and intra-epithelial lymphocyte count is less than 30/100 enterocytes. The first lesion, Marsh I, is characterized by intra-epithelial lymphocytosis defined as the presence of more than 30 intra-epithelial lymphocytes per 100 enterocytes. Marsh II lesion is characterized by crypt hyperplasia (elongation and branching of crypts) next to intra-epithelial lymphocytosis. A more severe stage is Marsh IIIa (also called partial villous atrophy) characterized by intra-epithelial lymphocytosis, crypt hyperplasia, and a reduced villous/crypt ratio below 1:1. Marsh IIIb lesion (subtotal villous atrophy) is characterized by clearly atrophic villi, but still recognizable. When villi are absent or rudimentary, the lesion is described as Marsh IIIc or total villous atrophy<sup>[31,32]</sup>. Marsh III with all subtypes of villous atrophy was considered compatible with coeliac disease.

The Ethical Committee approved the study protocol and all patients had given informed consent.

### Statistical analysis

Continuous data having normal distribution are presented in means  $\pm$  SD, and categorical data are presented in frequency rate and percentage. Comparison between groups was performed by two-tailed *t* test for continuous data, and Pearson Chi-Square ( $\chi^2$ ) test with Yate's correction in cross-tabulations for categorical data. Data not having a normal distribution were transformed (log) and analyzed subsequently. For all statistical analyses, a two-tailed *P* value  $< 0.05$  was considered significant. The correlation coefficient for the age at diagnosis of coeliac disease and

Hashimoto's thyroiditis in the subgroup with both autoimmune diseases was calculated by the Pearson method.

## RESULTS

### Coeliac disease in patients with Hashimoto's thyroiditis

Sixteen (15%) of 104 patients with Hashimoto's thyroiditis were positive for one or a combination of four coeliac serological tests. Eight patients, including three with coeliac disease, were positive in TGA, while six, including five with coeliac disease were positive in EMA. Except one patient who declined endoscopy, small intestinal biopsy from the other 15 patients was normal in nine, characterized by intraepithelial lymphocytosis (Marsh I) in one, and showed crypt hyperplasia and villous atrophy in five patients. These five patients with villous atrophy had gastrointestinal complaints (diarrhoea in four, and abdominal pain in one) that were resolved upon gluten free diet. Therefore, coeliac disease was newly diagnosed in five of 104 patients (4.8%; 95% CI 0.7-8.9) with Hashimoto's thyroiditis (Figure 1). The coeliac specific HLA-DQ heterodimers were present in all five patients who had villous atrophy and in 50 patients with Hashimoto's thyroiditis (50%; 95% CI 43-62).

### Thyroid disorders in patients with coeliac disease

High serum thyroid antibody concentrations (TG and/or TPO) were found in 39 of 184 patients (21%; 95% CI 15-27) with coeliac disease. Of these thyroid seropositive patients, 10 (5%; 95% CI 2-9) had normal thyroid biochemistry and were considered to have euthyroid autoimmune thyroiditis. Seven patients (3.8%; 95% CI 1.8-7.6) had subclinical hypothyroidism. The other 22 patients (12%; 95% CI 8-16) had overt hypothyroidism in combination with positive thyroid serology. They were considered to have Hashimoto's thyroiditis (Figure 1). All 22 patients were treated with thyroid hormone replacement therapy at a median dose of 50  $\mu$ g/d (range 25-200  $\mu$ g/d). Moreover, patients (2%; 95% CI 0.8-5) with coeliac disease had Graves' disease, and one patient with coeliac disease had post-partum thyroiditis.

As a result, abnormal thyroid biochemistry was found in 34 of 184 patients (18%; 95% CI 13-24) with coeliac disease, and clinically relevant thyroid disorders (overt hypothyroidism, 22 and Graves' disease, 4) that necessitated medical treatment, were found in 26 patients (14%; 95% CI 9-19).

### Patients with both Hashimoto's thyroiditis and coeliac disease

Twenty-seven patients (22 from the coeliac group and 5 from the Hashimoto's thyroiditis group) were recognized to have both Hashimoto's thyroiditis and coeliac disease. Coeliac disease was diagnosed at a median age of 46 years (range 18-74 years) and Hashimoto's thyroiditis was diagnosed at a median age of 48 years (range 20-76 years). The median time interval between the diagnoses of both diseases was 5 years (range 0-26 years). The correlation coefficient in the age of diagnosis of coeliac disease and Hashimoto's thyroiditis was 0.778 ( $P < 0.0001$ ). The frequency of recognized associated autoimmune disorders,

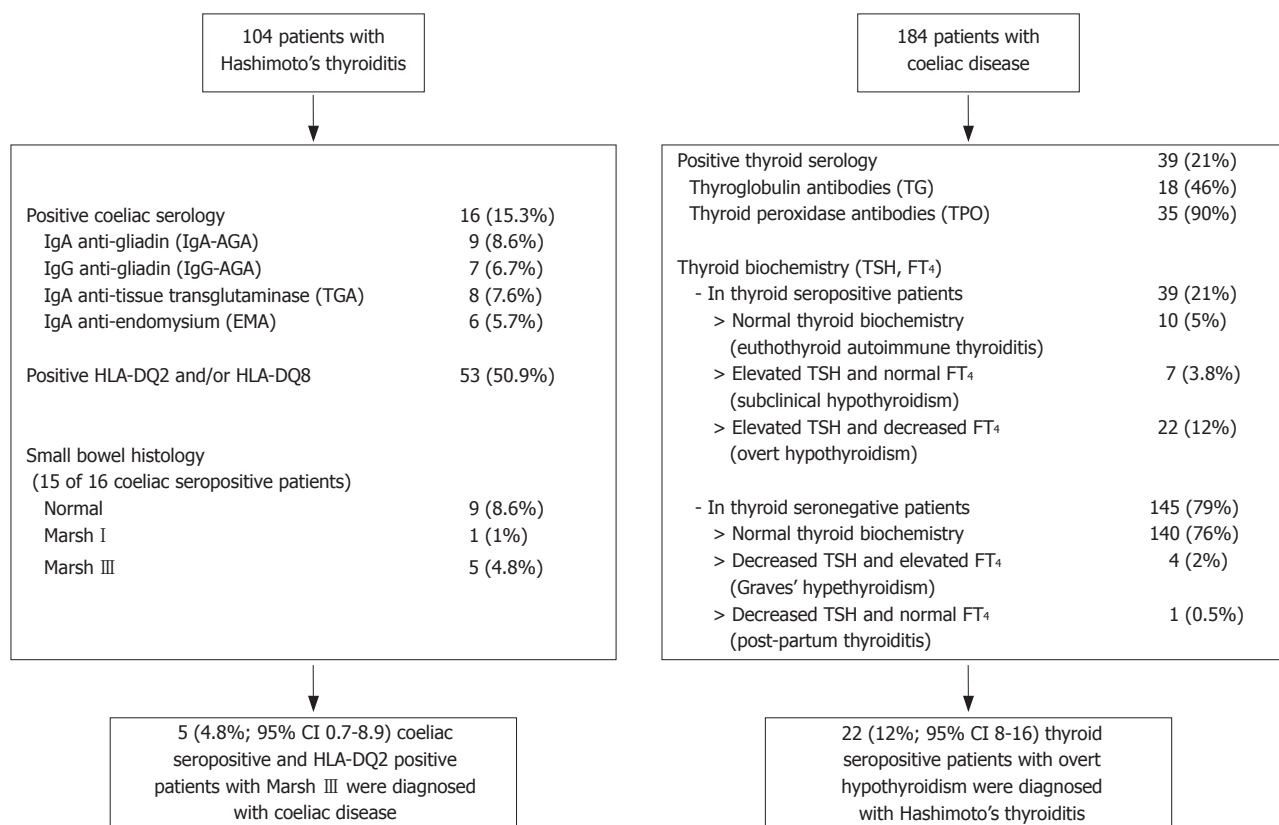


Figure 1 Flow diagram of patient recruitment and examination.

Table 2 Clinical characteristics, thyroid biochemistry, thyroid serology, and HLA-DQ data of patients with both Hashimoto's thyroiditis and coeliac disease compared to patients with Hashimoto's thyroiditis only

Characteristics	Patients with only Hashimoto's thyroiditis <i>n</i> = 99	Patients with both coeliac disease and Hashimoto's thyroiditis <i>n</i> = 27	<i>P</i>
Presence of goiter, <i>n</i> (%)	19 (19)	4 (15)	> 0.05
Presence of autoimmune diseases, <i>n</i> (%)	11 (11)	7 (26)	< 0.01
Thyroid function test			
mean ± SD TSH (normal 0.30-4.00 mU/L)	24 (± 7.6)	16 (± 5.8)	< 0.05
mean ± SD Free T <sub>4</sub> (normal 11-22 pmol/L)	10 (± 4.1)	12 (± 5.2)	> 0.05
Positive thyroid serological tests, <i>n</i> (%)			
Thyroglobulin (TG)	35 (36)	11 (41)	> 0.05
Thyroid peroxidase (TPO)	93 (94)	25 (92)	> 0.05
HLA-DQ 2 and/or -DQ8, <i>n</i> (%)	53 (53)	27 (100)	< 0.01
DQ2 heterozygote	30 (30)	20 (74)	< 0.01
DQ2 homozygote	1 (1)	3 (11)	< 0.01
DQ8 heterozygote	22 (22)	-	-
Combined DQ2 & DQ8	10 (10)	4 (15)	> 0.05

including diabetes mellitus type I, Sjögren's syndrome, pernicious anaemia and autoimmune endocrinopathy, was significantly higher in patients with both diseases (26%; 95% CI 13-44) than in those with only coeliac disease (4%; 95% CI 1-8; *P* < 0.001) or only Hashimoto's thyroiditis (11%; 95% CI 6-18; *P* = 0.006).

Mean serum TSH levels were higher in 99 patients with Hashimoto's thyroiditis only than in 27 patients with both coeliac disease and Hashimoto's thyroiditis (Table 2). Table 3 summarizes the coeliac disease make-up of 140 patients with coeliac disease only and 27 patients with both autoimmune diseases.

Finally, genetic studies revealed a higher frequency of HLA-DQ2 heterodimers in patients with both autoimmune diseases than in those with coeliac disease or Hashimoto's thyroiditis only. HLA-DQ8 was carried more often by patients with Hashimoto's thyroiditis and to a lesser extent by patients with coeliac disease only, but was absent in those with both autoimmune diseases.

## DISCUSSION

In this study, 4.8% (95% CI = 0.7-8.9) of Dutch patients with Hashimoto's thyroiditis had coeliac disease according

**Table 3** Comparison between patients with both Hashimoto's thyroiditis and coeliac disease compared to patients with coeliac disease only

Characteristics	Patients with only coeliac disease <i>n</i> = 140	Patients with both coeliac disease and Hashimoto's thyroiditis <i>n</i> = 27	<i>P</i>
Positive any or combinations of coeliac serology, <i>n</i> (%)	60 (43)	16 (59)	< 0.05
IgA anti-gliadin	43 (31)	12 (44)	< 0.05
IgG anti-gliadin	41 (29)	16 (59)	< 0.01
IgA anti-tissue transglutaminase	43 (31)	14 (50)	< 0.01
IgA anti-endomysium	46 (33)	14 (52)	< 0.01
Positive for HLA-DQ 2 and/or 8, <i>n</i> (%)			
DQ2 heterozygote	83 (59)	20 (74)	< 0.05
DQ2 homozygote	38 (27)	3 (11)	< 0.01
DQ8 heterozygote	9 (6)	-	-
DQ8 homozygote	3 (2)	-	-
Combined DQ2 & DQ8	7 (5)	4 (15)	< 0.05
Small bowel histology, <i>n</i> (%)			
Marsh 0	13 (9)	-	-
Marsh I	13 (9)	2 (7)	> 0.05
Marsh II	6 (4)	2 (7)	> 0.05
Marsh IIIa	49 (35)	12 (44)	> 0.05
Marsh IIIb	42 (30)	6 (22)	> 0.05
Marsh IIIc	17 (12)	5 (19)	> 0.05
Villous atrophy	108 (77)	23 (85)	> 0.05
Presence of autoimmune diseases, <i>n</i> (%)	5 (4)	7 (26)	< 0.01

to the ESPGAN criteria<sup>[18]</sup>, and 12% (95% CI 8-16) of patients with coeliac disease had Hashimoto's thyroiditis according to the American Thyroid Association guidelines<sup>[8]</sup>. Positive disease specific antibodies were more common in our patients than in the corresponding autoimmune disease. Indirect supportive indications to the association between coeliac disease and Hashimoto's thyroiditis included female sex predominance and the high prevalence of other autoimmune disorders in the subgroup of patients with both coeliac disease and Hashimoto's thyroiditis<sup>[33]</sup>. Furthermore, 2% of patients with coeliac disease had Graves' disease. Because the frequency of coeliac disease in patients with Hashimoto's thyroiditis is higher than in patients with Graves' disease (3.4%-6.4% versus 0%-3.8%)<sup>[4,34]</sup>, we limited the screening for coeliac disease to patients with Hashimoto's thyroiditis. Moreover, we restricted the definition of Hashimoto's thyroiditis with hypothyroidism to avoid overestimates.

Studies designed to screen patients with autoimmune thyroid disease for coeliac disease, and vice versa, are scarce in literature. A report from Italy described the association between 152 patients with autoimmune thyroid disease and 185 patients with coeliac disease<sup>[10]</sup>. Autoimmune thyroid disease group in this study consisted of 100 patients with Graves' disease and 52 patients with autoimmune thyroiditis. Of 52 patients with autoimmune thyroiditis, only 26 patients had overt hypothyroidism. We examined the association between 104 patients with Hashimoto's thyroiditis and 184 patients with coeliac disease. We recruited patients with either coeliac disease or Hashimoto's thyroiditis first. Moreover, we restricted the definition of Hashimoto's thyroiditis with hypothyroidism to avoid overestimates. The high prevalence of coeliac disease in Dutch patients with Hashimoto's thyroiditis and of Hashimoto's thyroiditis in patients with coeliac disease was in agreement with previous reports<sup>[4,13,14,34]</sup>. This

supported the evidence of the association between the two autoimmune diseases. The prevalence of Graves' disease in patients with coeliac disease is comparable to that of the general population, but the prevalence of Hashimoto's thyroiditis was at least ten fold higher<sup>[35]</sup>. In this study, patients with both autoimmune diseases were older than those with coeliac disease only (data not shown)<sup>[6]</sup>. Because older age at diagnosis of coeliac disease indirectly reflects the duration of gluten exposure, this finding is supportive to the presumption that prolonged duration of gluten exposure in unrecognized patients with coeliac disease might predispose to other autoimmune diseases such as diabetes mellitus type I, autoimmune thyroiditis, and alopecia<sup>[36]</sup>.

The pooled sensitivity of TGA and EMA in adults is around 90% and 97%, respectively and the pooled specificity is around 95% and 100%, respectively<sup>[37]</sup>. It is indicated that patients negative to TGA or EMA could have been overlooked in the screening process. This outcome results from the inherent pitfall in the serological screening, and the difference between actual and the presumptive prevalence of coeliac disease among patients with Hashimoto's thyroiditis (4.8% *vs* 5.5%). This difference can hypothetically be reflected by a presumptive number of 5.5 patients instead of actual number of 5 patients with coeliac disease in this study. Our findings, in agreement with pooled analysis<sup>[37]</sup>, showed that EMA is better than TGA in screening for coeliac disease and is associated with less false positive cases as verified by small bowel histology, although others showed the opposite with marginal differences<sup>[38,39]</sup>. The difference in the diagnosis by serum antibody tests however, can be explained by the study design and other factors like different commercial kits with different performances<sup>[39]</sup>.

The HLA-DQ2 heterodimer that confers coeliac disease susceptibility is formed by a  $\beta$  chain encoded by

the allele DQB1\*02 (either DQB1\*0201 or \*0202) and an  $\alpha$  chain encoded by the allele DQA1\*05. HLA-DQ2 is present in approximately 90-95% or more of coeliac disease patients. The HLA-DQ8 heterodimer is formed by a  $\beta$  chain and  $\alpha$  chain encoded by DQB1\*0302 and DQA1\*03, respectively, and is present in the remaining 5-10% of patients with coeliac disease<sup>[40-42]</sup>. HLA-DQ2 is common in the healthy population and is carried by approximately 30% of Caucasians<sup>[43]</sup>. HLA-DQ2 and HLA-DQ8 are present in 91% and 13%, respectively of our patients with coeliac disease, which are in agreement with previous rates. This frequency included patients who were heterozygous or homozygous to the heterodimer, and those with combined heterodimers (HLA-DQ2/DQ8). While HLA-DQ2 and -DQ8 are frequently found in patients with diabetes mellitus I or Graves' disease<sup>[44]</sup>, HLA studies in autoimmune thyroid disease, specifically in Hashimoto's thyroiditis have not been consistent. Linkage studies of HLA in autoimmune thyroid disease showed no relationship among populations of different ethnic backgrounds, including Caucasians<sup>[15]</sup> or Japanese<sup>[45]</sup>. On the other hand, DR3<sup>[46,47]</sup>, DR4<sup>[46]</sup> and DR5<sup>[48]</sup> have been reported to be associated with Hashimoto's thyroiditis in Caucasians. With respect to the coeliac specific HLA-DQ heterodimers, 34% were positive to HLA-DQ2<sup>[5]</sup> in a study of 52 children with Hashimoto's thyroiditis. In another study of 10 adults with Hashimoto's thyroiditis, 30% were DQ2 positive and 40% were DQ8 positive<sup>[49]</sup>. According to our knowledge, this is the largest study to investigate the prevalence of the coeliac specific HLA-DQ2/-DQ8 heterodimers in patients with Hashimoto's thyroiditis. Either HLA-DQ2 or HLA-DQ8 was present in 53% of patients with Hashimoto's thyroiditis. In view of the apparently conflicting linkage and association between HLA and autoimmune thyroid disease, the significance of HLA in the etiology of Hashimoto's thyroiditis remains unclear. Apart from the HLA of an individual with an autoimmune disease, additional genetic factors or genetically based immunological disorders are probably responsible for development of other autoimmune diseases in the same individual.

A recent guideline recommended screening adults with osteoporosis or irritable bowel syndrome for coeliac disease regardless of the presence of symptoms. However, the same guideline recommended to screen adults with autoimmune thyroid disorder only when symptoms suggestive of coeliac disease are present<sup>[50]</sup>. With respect to the prevalence, coeliac disease was confirmed by biopsy examination in 0.9%-3% of patients with osteoporosis<sup>[51,52]</sup> and 5% of those fulfilling Rome II criteria for a diagnosis of irritable bowel syndrome<sup>[53]</sup>. These prevalences are comparable to that of coeliac disease in patients with Hashimoto's thyroiditis. Moreover, coeliac disease is related to thyroid dysfunction clinically. Both diseases can present with non-specific symptoms like lethargy, bowel disturbance, and anaemia<sup>[47]</sup>. Thus, it is necessary to identify and treat a coexisting autoimmune disorder in order to adequately manage the primary disorder. Finally, the availability of serological screening tools and the possibility to prevent complications like osteoporosis or lymphoma in unrecognized patients with coeliac disease, favour the

screening of patients with Hashimoto's thyroiditis for coeliac disease even in absence of symptoms. One can argue that screening once in a lifetime is not enough to detect coeliac disease in patients with high risk like Hashimoto's thyroiditis. A strategy was proposed earlier based on selecting individuals with potential to develop coeliac disease by HLA-DQ typing and longitudinal serologic coeliac disease screening<sup>[30,54]</sup>.

In patients with coeliac disease, it is recommended to perform thyroid function tests at diagnosis and repeated later if necessary. Thyroid serological tests are helpful when thyroid function tests are abnormal. Another potential role of thyroid serological tests might be to select individuals for regular surveillance of thyroid function. It must be emphasized however, that positive thyroid and coeliac specific serological tests might represent an epiphenomenon<sup>[55]</sup> because serum autoantibodies generally do not reflect per se a clinical autoimmune disease as demonstrated in this study. Therefore, caution must be taken to avoid misdiagnosis and unnecessary treatments.

This study was limited by its cross-sectional nature that could not provide data about the effect of gluten-free diet on thyroid biochemistry or thyroid serology in patients with Hashimoto's thyroiditis and those newly diagnosed with coeliac disease. Although it is preferable to determine thyroid microsome antibodies rather than TG antibodies because the latter is non-specific<sup>[56]</sup>. In our study, we determined both TG and TPO to improve the serological tests.

In summary, current data confirm the association between Hashimoto's thyroiditis and coeliac disease and screening patients with Hashimoto's thyroiditis for coeliac disease and vice versa is recommended.

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