High Proportions of People With Nonceliac Wheat Sensitivity Have Autoimmune Disease or Antinuclear Antibodies

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BACKGROUND & AIMS: There is much interest in wheat sensitivity among people without celiac disease (CD), but little is known about any risks associated with the condition. We evaluated the prevalence of autoimmune diseases (ADs) among patients with nonceliac wheat sensitivity (NCWS), and investigated whether they carry antinuclear antibodies (ANA). **METHODS:** We performed a retrospective study of 131 patients diagnosed with NCWS (121 female; mean age, 29.1 years) at 2 hospitals in Italy from January 2001 through June 2011. Data were also collected from 151 patients with CD or irritable bowel syndrome (IBS) (controls). Patient medical records were reviewed to identify those with ADs. We also performed a prospective study of 42 patients (38 female; mean age, 34 years) diagnosed with NCWS from July 2011 through March 2014 at 3 hospitals in Italy. One hundred age- and sex-matched subjects with CD or IBS served as controls. Serum samples were collected from all subjects and ANA levels were measured by immunofluorescence analysis. Participants completed a questionnaire and their medical records were reviewed to identify those with ADs. **RESULTS:** In the retrospective analysis, similar portions of subjects with NCWS (29%) and CD (29%) developed ADs (mainly Hashimoto's thyroiditis, 29 cases), compared with a smaller proportion of subjects with IBS (4%) ($P < .001$). In the prospective study, 24% of subjects with NCWS, 20% of subjects with CD, and 2% of subjects with IBS developed ADs ($P < .001$). In the retrospective study, serum samples tested positive for ANA in 46% of subjects with NCWS (median titer, 1:80), 24% of subjects with CD ($P < .001$), and 2% of subjects IBS ($P < .001$); in the prospective study, serum samples were positive for ANA in 28% of subjects with NCWS, 7.5% of subjects with CD ($P = .02$), and 6% of subjects with IBS ($P = .005$ vs patients with NCWS). ANA positivity was associated with the presence of the HLA DQ2/DQ8 haplotypes ($P < .001$). **CONCLUSIONS:** Higher proportions of patients with NCWS or CD develop autoimmune disorders, are ANA positive, and showed DQ2/DQ8 haplotypes compared with patients with IBS.

Keywords: Food Allergy; Gluten Sensitivity; Celiac Disease; Immune Response.

Celiac disease is an immune-based reaction to dietary gluten (storage proteins found in wheat, barley, and rye) that primarily affects the small intestine in genetically predisposed patients and resolves when gluten is eliminated from the diet.[1] Although it is not certain whether CD can be included among the autoimmune diseases (ADs), in CD patients circulating autoantibodies (anti–tissue transglutaminase) are observed and a specific tissue cell type (enterocyte) is destroyed by $CD8^+$ T cells.[2] In addition, other ADs have been reported in association with CD in 20%–30% of cases.[3,4]

In the last few years, a new clinical entity has emerged that appears to include patients who consider themselves to be suffering from problems caused by wheat and/or gluten ingestion, even though they do not have CD or wheat allergy.[5] This clinical condition has been named *nonceliac gluten sensitivity*,[6] although in a recent article, we suggested the more appropriate term *nonceliac wheat sensitivity* (NCWS),[7] because it is not known to date what component of wheat actually causes the symptoms. Other areas of doubt in NCWS regard its pathogenesis, while some papers have reported intestinal immunologic activation,[8–10] others have linked NCWS to the dietary short chain carbohydrate (fermentable oligo-di-monosaccharides and polyols) load,[11] ruling out an immunologic involvement in NCWS. In the current study, we therefore compared the risk of autoimmunity in CD and NCWS patients, evaluating the frequency of ADs and the frequency of serum antinuclear antibody (ANA) positivity in these 2 conditions.

Methods

Study Design and Population

The study was divided into 2 different parts: a retrospective evaluation and a prospective survey. In the first, the clinical charts of NCWS patients attending the outpatient centers of the Department of Internal Medicine at the University Hospital of Palermo and the Department of Internal Medicine of the Hospital of Sciacca were reviewed with a retrospective method. They had all been diagnosed with NCWS between January 2001 and June 2011 and included in a previously published study.[12] These charts included specific sections for associated ADs and

Abbreviations used in this paper: **AD, autoimmune disease; ANA, antinuclear antibody; DBPC, double-blind placebo-controlled; EmA, anti-endomysium; IBS, irritable bowel syndrome; NCWS, nonceliac wheat sensitivity.**

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the presence of serum ANA. Incomplete clinical charts were excluded. Two control groups were selected. The first was composed of 101 celiac disease patients and the second of 50 irritable bowel syndrome (IBS) patients. Both were randomly chosen by a computer-generated method from subjects diagnosed during the same period (2001−2011) and age- (+2 years) and sex-matched (+5%) with the NCWS patients. The IBS controls had been receiving the same elimination diet as the NCWS patients and had not shown any clinical improvement; they belonged to the cohort of subjects we had studied previously.[12]

In the second part of the study, we prospectively surveyed adult patients with an IBS-like clinical presentation, according to the Rome II criteria, and a definitive diagnosis of NCWS. The patients were recruited between July 2011 and March 2014 at 3 centers: the 2 already mentioned and the Gastroenterology Unit of the ARNAS Civico Hospital of Palermo, Italy. Most of the patients had been referred due to intestinal symptoms, the onset of which, they reported, could be related to wheat ingestion. During the study period, the newly diagnosed NCWS patients were randomly assigned to one of several studies that we are currently performing on NCWS. In this way, 42 patients were recruited. Twenty-two of the patients included in the current study also agreed to be included in other as yet unpublished studies. Two control groups were again selected. One included 40 consecutive patients with a new CD diagnosis, sex- (+5%) and age-matched (+2 years) with the NCWS patients, and enrolled during the prospective study period. The second group included 50 subjects with IBS unrelated to NCWS or other types of food intolerance, who were consecutively recruited during the study period and sex- and age-matched with the NCWS patients. IBS diagnosis had been made in accordance with Rome II criteria and none of these subjects improved on an elimination diet without wheat, cow's milk, egg, tomato, or chocolate.

For both the prospective and the retrospective studies, exclusion criteria were positive antiendomysium (EmA) in the culture medium of the duodenal biopsies, even if the villi to crypts ratio in the duodenal mucosa was normal; self-exclusion of wheat from the diet and refusal to reintroduce it before entering the study; and other organic gastrointestinal diseases.

Noncelia Wheat Sensitivity Diagnosis

To diagnose NCWS in both the retrospective and the prospective parts, various criteria were adopted. Firstly, all the patients met the recently proposed criteria[13]: negative serum anti−tissue transglutaminase and EmA IgA and IgG antibodies; absence of intestinal villous atrophy; IgE-mediated immune-allergy tests negative to wheat (skin prick tests and/or serum specific IgE detection). Other criteria adopted in our patients were resolution of the IBS symptoms on a standard elimination diet without wheat, cow's milk, egg, tomato, chocolate, or other food(s) causing self-reported symptoms; symptom reappearance on double-blind placebo-controlled (DBPC) wheat challenge, performed as described previously.[12] As in previous studies, DBPC cow's milk protein challenge and other "open" food challenges were also performed[12] (for details see Supplementary Material). Additional inclusion criteria were age older than 18 years; follow-up duration longer than 9 months after the initial diagnosis; and at least 2 outpatient visits during the follow-up period.

Celiac Disease Diagnosis

Celiac disease was diagnosed in the presence of positive serum anti−tissue transglutaminase and/or EmA antibodies and duodenal villous atrophy at histologic examination, followed by symptom resolution after commencement of the gluten-free diet.

Outcomes

Serum Antinuclear Antibodies. The frequency of serum ANA positivity in NCWS and the control groups was evaluated. ANA was identified by HEp-2 cells, using an indirect immunofluorescence technique; a titer of 1:40 or higher was considered positive and the sera were titered at progressive dilutions until they became negative.

In the patients included in the prospective study, the ANA pattern was also classified as "homogeneous," "fine speckled," or "coarse speckled" and "nucleolar."

Frequency of Associated Autoimmune Diseases. The clinical chart used for the retrospective study listed the ADs and physicians had to fill out the specific fields. The hospital records of all patients diagnosed with an autoimmune disorder were thoroughly examined to ascertain whether the recognized diagnostic criteria for each disorder had been fulfilled.

Questionnaire for Autoimmunity. In the prospective study, the presence of autoimmune disorders in both the study and control groups was evaluated by a structured questionnaire and a review of patients' clinical records. The presence of one of the following was looked for in all subjects: connective tissue diseases (eg, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, scleroderma, mixed connective tissue disease, ankylosing spondylitis, or Sjögren's disease), autoimmune endocrinologic diseases (eg, Hashimoto's thyroiditis, Graves' disease, insulin-dependent diabetes mellitus, or Addison's disease), autoimmune hepatitis (types 1, 2 and 3), primary biliary cirrhosis, epilepsy with cerebral calcification, unexplained cerebellar ataxia, alopecia, psoriasis, atrophic autoimmune gastritis, immune anemia, neutropenia, or thrombocytopenia. Age at diagnosis of the diseases and treatments received were recorded in all cases.

Laboratory Methods

Serology for CD, duodenal histology studies, and HLA-DQ typing were performed in all patients as described previously[12] (see Supplementary Material). Duodenal histology lesions were classified according to Corazza and Villanacci as: normal mucosa (villi to crypts ratio >3 and $CD3^+$ intraepithelial intestinal lymphocytes <25/100 enterocytes), grade A (villi to crypts ratio >3 and $CD3^+$ intraepithelial intestinal lymphocytes >25/100 enterocytes), grade B1 (villi to crypts ratio <3, partial villous atrophy), and grade B2 (total villous atrophy).[14]

Statistical Analysis

Data were expressed as mean ± SD when the distribution was Gaussian and differences were calculated using Student *t* test. Otherwise, data were expressed as median and range and analyzed with the Mann–Whitney U test. Fisher's exact or the χ^2 tests were used where appropriate. The Mantel–Haenszel test was used to compare the severity of the duodenal histology damage in NCWS and CD patients. Multiple logistic regression

analysis was performed to evaluate the association between the presence of serum ANA and the other clinical variables evaluated. $P < .05$ was considered significant. All analyses were performed using the SPSS software package (version 16.0, released 2007, SPSS Inc, Chicago, IL).

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, was approved by the institution's human research committee (University Hospital of Palermo), and registered at clinicaltrials.gov (registration number: NCT02248545).

Results

Patient Clinical Characteristics

After the exclusion of the incomplete clinical records in which some data were lacking and exclusion of the data of the patients who had tested positive for EmA antibodies in the culture of the duodenal mucosa, 131 NCWS patients of the retrospective cohort were included (see Supplementary Material). Tables 1 and 2 show the clinical characteristics of the patients included in the retrospective and in the prospective studies, respectively, in comparison with the control groups. In general, in NCWS patients, there was a higher percentage of self-reported wheat intolerance and coexisting atopic diseases than CD controls ($P < .001$).

Frequency of Positive Serum Antinuclear Antibody

Patients with NCWS were more likely to be ANA positive than both patients with CD and IBS, in both the retrospective and prospective studies (Figure 1). In the retrospective study, NCWS patients showed a very high frequency of serum ANA (46%), significantly higher than in CD (24%) subjects and IBS (2%) controls ($P < .001$, for both). Median values of the ANA titer were 1:80 in both NCWS (range, 1:40−1:640) and CD (range, 1:40−1:1280).

Table 1. Clinical Characteristics of 131 NCWS Patients, 101 CD Controls, and 50 IBS Controls Included in the Retrospective Study

	NCWS (n = 131)	CD (n = 101)	IBS (n = 50)	*P* value
Age, *y*, mean ± SD	39.1 ± 11	40.1 ± 12.3	38.8 ± 10.8	Not applicable, matching factor
Sex, female/male, n	121/10	91/10	44/6	Not applicable, matching factor
Duration of symptoms, *y*, median (range)	6.5 (1−40)	5.5 (1−30)	6 (1−25)	NCWS vs CD, $P = .8$ NCWS vs IBS, $P = .8$ CD vs IBS, $P = .8$
Self-reported wheat intolerance, n (%)	78/131 (59)	20/101 (20)	8/50 (16)	NCWS vs CD, $P < .001$ NCWS vs IBS, $P < .001$ CD vs IBS, $P = .6$
Family history of CD, n (%)	10/131 (8)	17/101 (17)	0/50	NCWS vs CD, $P = .04$ NCWS vs IBS, $P = .06$ CD vs IBS, $P = .01$
Coexisting atopic diseases, n (%)	39/131 (30)	10/101 (10)	3/50 (6)	NCWS vs CD, $P < .001$ NCWS vs IBS, $P < .001$ CD vs IBS, $P = .5$
Body mass index <20 kg/m^2, n (%)	32/131 (24)	26/101 (26)	4/50 (8)	NCWS vs CD, $P = .9$ NCWS vs IBS, $P = .02$ CD vs IBS, $P = .02$
Anemia,[a] n (%)	34/131 (27)	52/101 (52)	3/50 (6)	NCWS vs CD, $P < .001$ NCWS vs IBS, $P < .001$ CD vs IBS, $P < .001$
Multiple food sensitivity, n (%)	90/131 (69)	Not evaluated	Not evaluated	
Haplotypes DQ2 or DQ8, n (%)	65/131 (50)	101/101 (100)	14/50 (28)	NCWS vs CD, $P < .001$ NCWS vs IBS, $P < .001$ CD vs IBS, $P < .001$
Duodenal histology,[b] n (%)			Not evaluated	$P < .001$
Normal	26 (20)	0		
Grade A	105 (80)	0		
Grade B1	0	64 (64)		
Grade B2	0	37 (36)		

NOTE. CD and IBS controls were randomly chosen by a computer-generated method from subjects diagnosed during the same period and age- and sex-matched (+5%) with the NCWS patients. Family history of CD was evaluated in first-degree family members.

[a]Anemia was defined by hemoglobin values <12 g/dL in females and <13 g/dL in males.

[b]Duodenal histology lesions were classified according to Corazza and Villanacci as: normal mucosa (villi to crypts ratio >3 and CD3$^+$ intraepithelial intestinal lymphocytes <25/100 enterocytes), grade A (villi to crypts ratio >3 and CD3$^+$ intraepithelial intestinal lymphocytes >25/100 enterocytes), grade B1 (villi to crypts ratio <3, partial villous atrophy) and grade B2 (total villous atrophy).

Table 2. Clinical Characteristics of 42 NCWS Patients, 40 Celiac Disease Controls, and 50 IBS Controls Included in the Prospective Study

	NCWS (n = 42)	Celiac disease (n = 40)	IBS (n = 50)	*P* value
Age, *y*, mean ± SD	34 ± 12	35.5 ± 11.3	33.1 ± 7.6	Not applicable, matching factor
Sex, female/male, n	38/4	35/5	43/7	Not applicable, matching factor
Duration of symptoms, *y*, median (range)	5.3 (1−35)	5.0 (1−20)	5.4 (1−28)	NCWS vs celiac disease, $P = .77$ NCWS vs IBS, $P = .79$ Celiac disease vs IBS, $P = .81$
Self-reported wheat intolerance, n (%)	30/42 (71)	9/40 (22)	8/50 (16)	NCWS vs celiac disease, $P < .001$ NCWS vs IBS, $P < .001$ Celiac disease vs IBS, $P = .43$
Family history of celiac disease, n (%)	8/42 (19)	6/40 (15)	0/50	NCWS vs celiac disease, $P = .77$ NCWS vs IBS, $P = .01$ Celiac disease vs IBS, $P = .01$
Coexisting atopic diseases, n (%)	14/42 (33)	6/40 (15)	4/50 (8)	NCWS vs celiac disease, $P = .07$ NCWS vs IBS, $P = .01$ Celiac disease vs IBS, $P = .32$
Body mass index <20 kg/m^2, n (%)	10/42 (24)	10/40 (25)	4/50 (8)	NCWS vs celiac disease, $P = 1$ NCWS vs IBS, $P = .05$ Celiac disease vs IBS, $P = .04$
Anemia,[a] n (%)	14/42 (33)	16/40 (40)	3/50 (6)	NCWS vs celiac disease, $P = .6$ NCWS vs IBS, $P < .001$ Celiac disease vs IBS, $P < .001$
Multiple food sensitivity, n (%)	18/42 (43)	Not evaluated	Not evaluated	
Haplotypes DQ2 or DQ8, n (%)	23/42 (55)	40/40 (100)	17/50 (34)	NCWS vs celiac disease, $P < .001$ NCWS vs IBS, $P = .05$ Celiac disease vs IBS, $P < .001$
Duodenal histology,[b] n (%)			Not evaluated	$P < .001$
Normal	17 (41)	0		
Grade A	25 (59)	0		
Grade B1	0	28 (70)		
Grade B2	0	12 (30)		

NOTE. Celiac disease and IBS controls were randomly chosen by a computer-generated method from subjects diagnosed during the same period, and age- and sex-matched (+5%) with the NCWS patients. Family history of celiac disease was evaluated in first-degree family members.

[a]Anemia was defined by hemoglobin values <12 g/dL in females and <13 g/dL in males.

[b]Duodenal histology lesions were classified according to Corazza and Villanacci as: normal mucosa (villi to crypts ratio >3 and CD3$^+$ intraepithelial intestinal lymphocytes <25/100 enterocytes), grade A (villi to crypts ratio >3 and CD3$^+$ intraepithelial intestinal lymphocytes >25/100 enterocytes), grade B1 (villi to crypts ratio <3, partial villous atrophy), and grade B2 (total villous atrophy).

These results were confirmed in the prospective study, where the frequency of ANA positivity was higher in NCWS patients (28%) than in the 2 control groups (7.5% in CD, $P < .02$ and 6% in IBS patients, $P < .005$). The ANA titer ranged between 1:40 and 1:1280 in both groups. The ANA pattern in the prospective study was equally distributed: "homogeneous" in 11 cases, "fine speckled" in 11 cases, "coarse speckled" in 9 cases, and "nucleolar" in 11 cases.

Variables Associated With Serum Antinuclear Antibody Positivity

The presence of serum ANA in NCWS patients correlated with HLA DQ2/DQ8 haplotypes. In detail, in the retrospective study, HLA DQ2/DQ8 haplotypes were present in 49 of 59 ANA-positive vs 16 of 72 ANA-negative patients ($P < .001$). A significant correlation was also observed with a high frequency of duodenal lymphocytosis ($P = .02$) (Figure 2). No correlation was found with sex, age, disease duration, presence of coexisting atopic diseases, multiple food sensitivity, anemia, or body mass index <20 kg/m^2. On multivariate analysis, the only variable significantly associated with ANA positivity was the presence of the HLA DQ2/DQ8 haplotypes (odds ratio = 3.5; 95% confidence interval: 1.6−5.8; $P < .001$).

The prospective study confirmed the association between ANA positivity and HLA status: HLA DQ2/DQ8 haplotypes were present in 10 of 12 ANA-positive vs 13 of 30 ANA-negative patients ($P = .04$). Again, no other

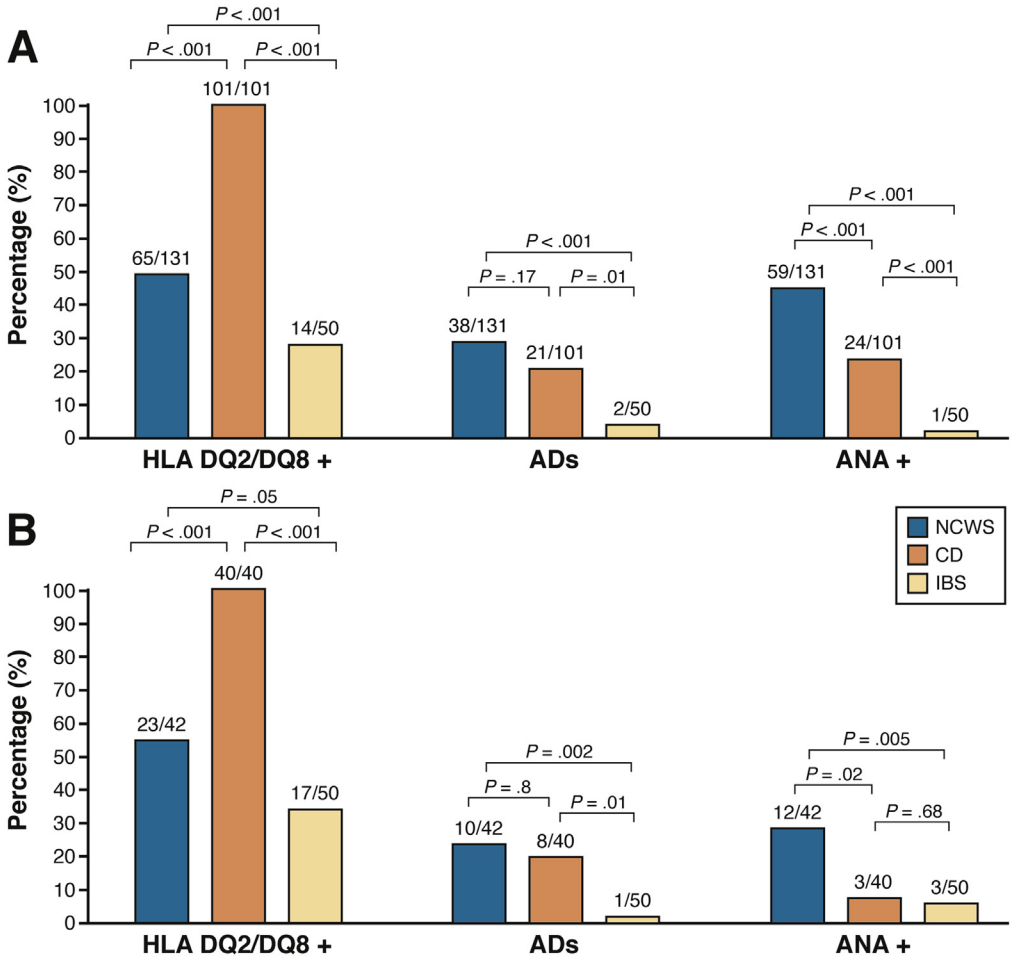

Figure 1. Frequency of HLA haplotypes DQ2 and/or DQ8-positive serum ANAs and ADs in 131 NCWS patients, 101 CD controls, and 50 IBS controls, included in the retrospective study (*A*) and in 42 NCWS patients, 40 CD controls, and 50 IBS controls, included in the prospective study (*B*). (*A*) Serum ANA positivity: NCWS vs CD, $P < .001$; NCWS vs IBS, $P < .001$; CD vs IBS, $P < .001$. Frequency of ADs: NCWS vs CD, $P = .17$; NCWS vs IBS, $P < .001$; CD vs IBS, $P = .01$. (*B*) Serum ANA positivity: NCWS vs CD, $P = .02$; NCWS vs IBS, $P = .005$; CD vs IBS, $P = .68$. Frequency of ADs: NCWS vs CD, $P = .8$; NCWS vs IBS, $P = .002$; CD vs IBS, $P = .01$.

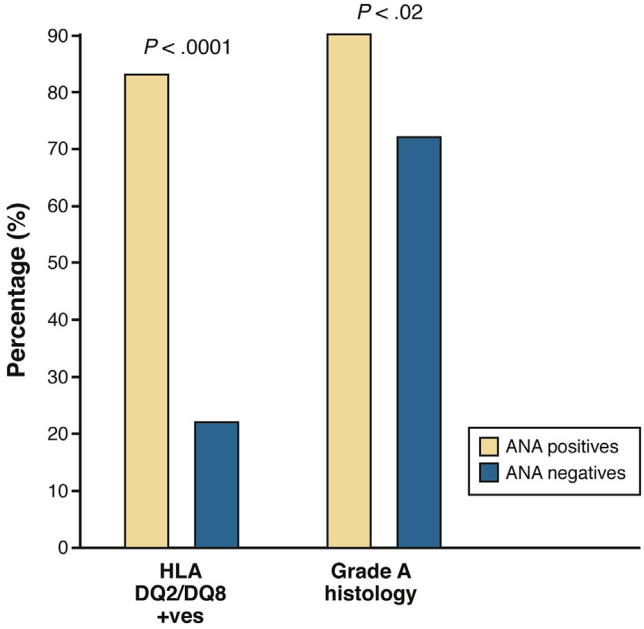

Figure 2. Percentage of DQ2/DQ8-positive patients and grade A duodenal histology (normal villi to crypts ratio with intraepithelial lymphocytes >25/100 enterocytes) in NCWS patients showing serum ANA positivity (59 cases) or serum ANA negativity (72 cases).

variables correlated with ANA positivity, including duodenal lymphocytosis.

Frequency of Associated Autoimmune Diseases

Patients with NCWS showed a frequency of AD similar to CD, but significantly higher than IBS controls, in both the retrospective and prospective studies (Figure 1). In the retrospective study, NCWS patients had an associated AD in 29% of the cases vs 21% in CD patients, and both these groups had a higher frequency than IBS controls (NCWS vs IBS, $P < .001$; CD vs IBS, $P < .01$). In detail, NCWS patients presented one or more of the following diseases: Hashimoto's thyroiditis (29 cases), psoriasis (4 cases), type 1 diabetes (4 cases), mixed connective tissue disease (1 case), and ankylosing spondylitis (1 case).

In the prospective study, the frequency of AD was also virtually identical to that of the retrospective study. Ten NCWS patients showed concomitant autoimmune thyroiditis (24%), but no other ADs were found.

In all NCWS patients, the diagnosis of thyroiditis had been made before that of NCWS (median time, 8 years). Eight of the ten patients with NCWS and thyroiditis presented hypothyroidism and were receiving replacement therapy.

Variables Associated With the Presence of Autoimmune Diseases

Different trends in the presence of ADs were observed in the 2 study parts.

In the retrospective study, the presence of ADs in NCWS patients did not correlate with any of the other variables studied, including the presence of haplotypes DQ2/DQ8, which were present in 19 of 38 patients (50%) with ADs and in 40 of 93 (43%) NCWS patients without an associated AD. However, in the prospective study, there was a trend toward an association with thyroiditis in NCWS patients and the presence of the haplotypes DQ2/DQ8 in 8 of 10 patients (80%) with thyroiditis and in 15 of 32 patients (47%) without thyroiditis, but this association was not statistically significant ($P = .08$).

Regarding the possible association between serum ANA positivity and presence of ADs, different results were also found. In the retrospective study, serum ANAs were present in 20 of 38 (53%) patients with ADs and in 39 of 93 without ADs (42%) ($P = .33$). In contrast, in the prospective study, ANA positivity was associated with thyroiditis: 6 of 10 with thyroiditis vs 6 of 32 without thyroiditis ($P = .02$).

No other variables significantly correlated with the presence of ADs in either of the 2 study parts, including duodenal lymphocytosis, which was present only in 15 of the 38 NCWS patients with AD in the retrospective study and in 4 of the 10 with AD in the prospective study.

Discussion

NCWS is an emerging clinical condition that, in the last few years, has attracted the interest of researchers.[6,13] There are considerable data, including from the current study, that seem to indicate that it is a sex-related disease, with a much higher frequency in females.[9,12,14] However, the lack of a diagnostic marker for NCWS is the main problem in identifying patients. In addition, after basing the diagnosis on DBPC challenge, many clinical characteristics still remain to be defined, including whether NCWS is associated with nutritional problems or ADs, as is the case for CD. Recent data have demonstrated that nutrient deficiency is present in about 18% of NCWS patients,[15] and we showed that a low body mass index and reduced bone mass density are frequent in these patients.[16] Data about autoimmunity in NCWS are very scarce, no past studies have been specifically designed to clarify this aspect and, in particular, no previous investigations have studied the presence of serum autoantibodies.

We therefore retrospectively evaluated the frequency of serum ANA positivity in NCWS patients diagnosed by DBPC challenge in a historical cohort studied in the years 2001−2011. Serum ANA was positive in 46% of the NCWS patients, a percentage significantly higher than those observed in the celiac (24%) and IBS controls (2%). In addition, the findings obtained in the prospective cohort, although collected in a small group of NCWS patients, also confirmed that NCWS is characterized by a higher frequency of positive serum ANA than in CD (28% vs 7.5%). Literature data have reported a frequency of positive autoantibodies in CD patients of about 25%, although the specific frequency of ANA positivity was about 8%.[17,18] The data in healthy controls, using the IF (immunofluorescence) method, have shown ANA positivity in a relatively high proportion of individuals: >10% at 1:80 dilution.[19,20] However, although the reported frequency of positive serum ANA in the general population was high, the frequency we observed in the NCWS was evidently even higher. We also found ANA-positive sera at a low dilution (median, 1:80) and in the absence of specific signs or symptoms of overt ADs, in particular systemic lupus erythematosus.

ANA positivity in our patients was found to be associated with the presence of the DQ2/DQ8 haplotypes and, to a lesser extent, with the presence of duodenal lymphocytosis (grade A histology). Obviously, these associations strongly suggest a celiac condition, but it must be emphasized that all the patients we included were negative for CD-specific antibodies and showed normal intestinal villi when evaluated after a prolonged time on a gluten-containing diet (at least 100 g wheat per day, for a minimum of 4 weeks). In addition, we also excluded patients, both from the retrospective and from the prospective cohorts, who tested positive for EmA antibodies in the culture medium of the duodenal biopsies, a positive result that could precede the onset of overt CD.[21] However, it has been reported that analysis of the intraepithelial intestinal lymphocyte flow cytometric pattern is an accurate method for identifying CD in the initial diagnostic biopsy of seronegative patients presenting with lymphocytic enteritis, and it seems to be better than anti−tissue transglutaminase 2 intestinal deposits.[22] Therefore, a more accurate evaluation of the duodenal histology, using this method, should be recommended in all NCWS patients that show the DQ2 or the DQ8 haplotypes and duodenal lymphocytosis.

We also found a quite high frequency of autoimmune thyroiditis associated with NCWS in both cohorts (22% and 24% in the retrospective and in the prospective cohorts, respectively), although other ADs—more frequently psoriasis and type 1 diabetes mellitus—were reported more rarely in the retrospective cohort. Previous studies have reported conflicting data about the presence of autoimmune comorbidities in NCWS patients. These were not reported in 2 studies involving a relatively low number of patients,[23,24] but a recent multicenter Italian study including nearly 500 patients reported a high prevalence of Hashimoto's thyroiditis,[25] and the same study showed that other autoimmune conditions, such as psoriasis, can coexist in these patients, as reported previously in a clinical setting recalling NCWS.[26] In this respect, our study design with a structured questionnaire and based on a review of patients' clinical records, both dedicated to ascertain the presence of autoimmune comorbidities, showed that NCWS might behave in a similar way to CD.[3,4]

Once again, these findings pose the question of whether there is a real distinction between NCWS and CD, and suggest a possible overlap between these 2 conditions. However, it should be emphasized that the majority of the ADs

were found in patients with absolutely normal duodenal histology and this is hard evidence that NCWS is an immune-mediated condition different from CD.

Another strong immunologic hypothesis to consider is the one suggested by Junker et al, who identified the amylase/trypsin inhibitors as activators of innate immune responses by engaging the Toll-like receptor system.[27] In fact, it has been demonstrated that patients with hereditary angioedema tend to produce autoantibodies and have a propensity to develop immunoregulatory disorders, probably due to the increased activation of B cells, which was found to be associated with a high expression of TLR-9.[28] Whether similar mechanisms have a role in NCWS autoimmunity obviously remains to be demonstrated.

Despite the interesting results, however, the limits of our study must be emphasized. We studied patients referred to tertiary centers with experience in CD and NCWS and this factor clearly led to a selection bias, therefore, our results cannot be extended to the broad population of self-treated or diagnosed NCWS patients. In addition, in this respect, we and others have suggested that NCWS cannot be considered a unique condition, but rather an "umbrella" term that includes various conditions with different types of pathogenesis.[12,29–33] Regarding our previous hypothesis that NCWS could be an allergic disease,[28] it should also be remembered that negative IgE test results cannot exclude food allergy. In the absence of specific biomarkers, we should cautiously indicate that this high frequency of autoimmunity can apply to NCWS patients who react to a challenge with a low amount of wheat (equivalent to about 20 g bread in our experiment). Finally, it is noteworthy that, of the 273 patients included in the original cohort of the retrospective study, 123 (44%) were excluded because the data in their charts concerning a possible associated condition secondary to autoimmunity were incomplete. We cannot rule out the possibility that if the clinical records had been complete, they would have included patients with clinical characteristics pointing to associated autoimmune features.

The strong points of our data, on the other hand, were the patient selection (based on a NCWS diagnosis made by using the DBPC challenge method), the study design (which was specifically constructed to reveal the presence of ADs and the frequency of serum ANA positivity), and the confirmation of the retrospective results by the prospective ones.

In conclusion, our data showed a strong tendency toward autoimmunity in the NCWS patients, characterized by both associated ADs and the presence of serum ANA positivity, which, in turn, was correlated with the HLA DQ2/DQ8 haplotypes.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org. and at http://dx.doi.org/10.1053/j.gastro.2015.05.040.

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Conflicts of interest
The authors disclose no conflicts.

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Supplementary Material

Elimination Diet and Double-Blind Placebo-Controlled Challenge

On entering the study, all patients commenced a standard elimination diet, with the exclusion of wheat, cow's milk, eggs, tomato, and chocolate. Patients self-reporting food hypersensitivity were also asked to avoid ingestion and/or contact with the food(s) causing symptoms. Food diaries were maintained by the patients during the elimination diet period to assess dietary intake and adherence to the diet. After 4 weeks on the elimination diet, they underwent DBPC challenges. The challenges were performed with the reintroduction of a single food at a time. Patients were randomized to receive either the "active food" or the placebo, according to a computer-generated order, determined by an observer not involved in the study.

In the case of wheat, the DBPC challenge was performed with capsules coded A or B containing wheat or xylose, respectively. Capsules A or B were given for 2 consecutive weeks and then after 1 week of washout the patients received the other capsules for another 2 weeks (cross-over design). Wheat challenge was performed administering a daily dose of 13 g flour, equal to about 20 g bread. A total of 12 capsules daily were given 3 times daily, away from meals.

DBPC for cow's milk was performed by administering capsules coded as A or B containing milk proteins (casein from bovine milk, lactoalbumin, lactoglobulin; daily dose 6 g, equal to about 200 mL cow's milk) or xylose, respectively. A total of 6 capsules daily were given 3 times daily, away from meals.

The codes of the capsules were broken only at the end of the study and the investigators did not know the content of the capsules during the study period. Challenges for other foods in patients with suspected multiple food hypersensitivity were performed in an open fashion.

During all phases of the study, including the challenge period, the severity of symptoms was recorded: the patients completed a 100-mm visual analog scale, with 0 representing no symptoms, which assessed overall symptoms and the specific symptoms they each reported.

The challenges were stopped when clinical reactions occurred (increase in visual analog scale score >30) for at least 2 consecutive days (onset of abdominal discomfort or pain, associated with a change in stool frequency and/or stool appearance). The challenges were considered positive if the same symptoms that had been present initially reappeared after their disappearance on elimination diet.

Laboratory Methods for Celiac Disease Diagnosis

On entering the study, all patients underwent serum anti−tissue transglutaminase IgA and IgG, EmA IgA, and anti-gliadin IgA and IgG assays, performed using commercial kits (Eu-tTG IgA, anti-endomysium, and anti-gliadin IgA and IgG; Eurospital Pharma, Trieste, Italy). Patients were also typed for HLA-DQ phenotypes by polymerase chain reaction using sequence-specific primers, with a rapid method (DQ-CD Typing Plus by BioDiaGene, Palermo, Italy).