Younger Age at Onset and Sex Predict Celiac Disease in Children and Adolescents With Type 1 Diabetes

An Italian multicenter study

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OBJECTIVE — To estimate the prevalence of biopsy-confirmed celiac disease in Italian children and adolescents with type 1 diabetes and to assess whether age at onset of type 1 diabetes is independently associated with diagnosis of celiac disease.

RESEARCH DESIGN AND METHODS — The study group was a clinic-based cohort of children and adolescents with type 1 diabetes cared for in 25 Italian centers for childhood diabetes. Yearly screening for celiac disease was performed using IgA/IgG anti-gliadin and IgA anti-endomysium antibodies.

RESULTS — Of the 4,322 children and adolescents (age 11.8 ± 4.2 years) identified with type 1 diabetes, biopsy-confirmed celiac disease was diagnosed in 292 (prevalence 6.8%, 95% confidence interval [CI] 6.0–7.6), with a higher risk seen in girls than in boys (odds ratio [OR] 1.93, 1.51–2.47). In 89% of these, diabetes was diagnosed before celiac disease. In logistic regression analyses, being younger at onset of diabetes, being female, and having a diagnosis of a thyroid disorder were independently associated with the risk of having diabetes and celiac disease. In comparison with subjects who were older than 9 years at onset of diabetes, subjects who were younger than 4 years at onset had an OR of 3.27 (2.20–4.85).

CONCLUSIONS — We have provided evidence that *1*) the prevalence of biopsy-confirmed celiac disease in children and adolescents with type 1 diabetes is high (6.8%); *2*) the risk of having both diseases is threefold higher in children diagnosed with type 1 diabetes at age <4 years than in those age >9 years; and *3*) girls have a higher risk of having both diseases than boys.

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The association between celiac disease and other autoimmune diseases, particularly type 1 diabetes and thyroid-related autoimmune disorders, has been documented in several studies (1). In a comparison of 40 surveys conducted mainly in European countries (1), the median prevalence of celiac disease with regard to type 1 diabetes has been estimated at 4.1%. A possible explanation for this association could be that the same susceptibility genotypes are involved in the etiopathogenesis of diabetes and celiac disease. In both diseases, genetic susceptibility is associated with the HLA-DQ α1*0501, β1*0201 heterodimer, which preferentially presents gluten-derived gliadin peptides on its antigen-presenting groove to stimulate intestinal mucosal T-cells (2). Moreover, impaired intestinal permeability in subjects with untreated celiac disease could increase the absorption of dietary antigens, which in turn could induce an autoimmune reaction in subjects with genetic susceptibility to autoimmune diseases (3).

Recently, some (4,5), but not all (6,7) studies have suggested an association between the age at diagnosis of celiac disease and autoimmune disorders with patients who were older at diagnosis of celiac disease and, presumably having had longer exposure to dietary gluten, being at higher risk for developing autoimmune disease. Studies have suggested a heterogeneity of determinants of type 1 diabetes by age at onset (8). To date, however, no study has had the statistical power to assess whether the diagnosis of celiac disease is associated with age at onset of type 1 diabetes, with most of the studies recruiting only small numbers of children with diabetes. Evidence for this association would be consistent with the hypothesis of common determinants for both diseases, at least in subgroups of patients.

The present large, multicenter survey was designed to estimate the prevalence of celiac disease in Italian children and adolescents with type 1 diabetes. To test whether there is heterogeneity by age at onset of type 1 diabetes, using multivariate analyses we also assessed whether age at onset of type 1 diabetes is independently associated with diagnosis of celiac disease.

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Abbreviations: EMA, anti-endomysium antibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Figure 1—*Geographic distribution of the 25 Italian collaborative centers for childhood diabetes.*

RESEARCH DESIGN AND METHODS

— The study base of this report was a clinic-based cohort of children and adolescents with type 1 diabetes on the prevalence date (31 December 2001) cared for in 25 Italian centers for childhood diabetes. In Italy, >95% of diabetic children and adolescents are followed in diabetes clinics through the national health system. As shown in Fig. 1, recruited centers were spread out all over Italy (11 in the north, 5 in the center, and 9 in the south) and represented 60% of Italian clinics for childhood diabetes.

Since 1995, the Italian Society of Pediatric Endocrinology and Diabetes has recommended yearly screening for celiac disease in all diabetic children and adolescents after diabetes diagnosis using IgA/IgG anti-gliadin antibodies and/or anti-endomysium antibodies (EMAs), according to local availability (9). Diabetic children and adolescents are diagnosed as having celiac disease when an intestinal biopsy shows small bowel mucosal villous atrophy and crypt hyperplasia that reverts to normal on a gluten-free diet (10–11). All centers agreed to complete a standardized questionnaire in which clinical data of all patients cared for by the clinic on the prevalence date were reported. Patients were determined to have a thyroid disorder on the basis of clinical and laboratory abnormalities (persistent positivity of thyroid autoantibodies at high titers, Hashimoto thyroiditis or Graves disease confirmed by hormonal assessment, thyroid ultrasonography, and/or fine needle aspiration biopsy) (12).

Data are shown as frequencies, means ± SD, and interquartile range of distribution. ANOVA and χ^2 tests were applied whenever appropriate. To assess variables independently associated with the prevalence of celiac disease, we performed logistic regression analyses, with the prevalence of celiac disease as the dependent variable and age, sex, age at diagnosis of diabetes (quartiles defined by age <4 years, 4–6 years, 7–9 years, and >9 years), and presence or absence of a thyroid disorder as covariates. The −2 log likelihood ratio test was used to test the significance of variables. All analyses were performed using Stata (Stata Release 8.0; Stata, College Station, TX).

RESULTS

— On the prevalence date (31 December 2001), 4,322 children and adolescents (2,388 boys and 1,934 girls) with type 1 diabetes were followed in 25 pediatric diabetes clinics spread out all over Italy (Fig. 1). The ages at recruitment and diabetes diagnosis were 11.8 ± 4.2 and 7.4 ± 3.9 years, respectively. Out of this cohort, biopsy-confirmed celiac disease was diagnosed in 292 subjects, giving a prevalence of 6.8% (95% confidence interval [CI] 6.0–7.6), with higher risk in girls than in boys (odds ratio [OR] 1.93, 95% CI 1.51–2.47).

As shown in Tables 1 and 2, the onset of type 1 diabetes preceded the diagnosis of celiac disease in most subjects (258 of 292, 88.4%), whereas the diagnosis of celiac disease preceded that of diabetes in 34 of 292 (11.6%). In 31 of the 258 subjects (12.0%), the diseases were diagnosed simultaneously as a result of screening. Out of 258 patients having diabetes onset before celiac disease diagnosis, 14.5% displayed symptoms of celiac disease, with 13% having gastrointestinal symptoms and 1.5% having atypical symptoms (short stature and/or anemia). Among them, the median lag between diagnoses was 1.3 years (interquartile range 0.25–3.58), whereas in those for whom diabetes was diagnosed after celiac disease, the median lag between diagnoses was 4.93 years (interquartile range 3.21–8.96 years). After excluding patients with celiac disease diagnosed at onset of diabetes, 75 and 95% of patients had celiac disease diagnosed by 4.2 years and 9.4 years of duration of diabetes, respectively. Ta-

Table 1—*Prevalence of biopsy-confirmed celiac disease in Italian multicenter cohort of children and adolescents with type 1 diabetes on the prevalence date (31 December 2001)*

	Boys	Girls	All
n	2,388	1,934	4,322
Celiac disease diagnosed before diabetes (*n* = 34)	0.5 (0.3–0.9)	1.1 (0.7–1.7)	0.8 (0.5–1.1)
Celiac disease diagnosed after diabetes (*n* = 258)	4.3 (3.5–5.2)	8.0 (6.9–9.4)	6.0 (5.3–6.7)
Celiac disease + type 1 diabetes (*n* = 292)	4.8 (4.0–5.8)	9.2 (7.9–10.6)	6.8 (6.0–7.6)

Data are percent (95% CI).

Table 2—*Characteristics of the Italian multicenter cohort of children and adolescents with type 1 diabetes on the prevalence date (31 December 2001)*

	Type 1 diabetes only	Type 1 diabetes diagnosed before celiac disease	Type 1 diabetes diagnosed after celiac disease
n	4,013	258*	34
Age (years)	11.9 ± 4.1	10.7 ± 4.6†‡	13.4 ± 6.8
Age at diagnosis of type 1 diabetes (years)	7.3 ± 3.8	5.5 ± 3.7†	9.8 ± 4.5†
Mean age (years)			
<4	1,004 (25.0)	105 (40.7)	3 (8.8)
4–6	934 (23.3)	81 (31.5)	8 (23.5)
7–9	1,004 (25.0)	36 (13.9)	2 (5.9)
>9	1,072 (26.7)	36 (13.9)	21 (61.8)
Boys (%)	56.4	39.5	38.2
Age at diagnosis of celiac disease (years)	—	7.2 ± 4.3	4.2 ± 3.8
Thyroid disorder (%)	11.3	19.6	38.2

Data are *n* (%) or means ± SD, unless otherwise noted. *In 31 of 258 (12.0%) of subjects, celiac disease was diagnosed at the onset of diabetes; †$P < 0.001$ vs. type 1 diabetes only; ‡$P < 0.001$ vs. type 1 diabetes diagnosed after celiac disease.

ble 3 shows the number of diabetic patients screened per year since 1995 and prevalence of biopsy-confirmed celiac disease by strata of duration of diabetes, after excluding subjects with celiac disease diagnosed before diabetes onset ($n = 34$) and those diagnosed before implementation of the yearly screening program ($n = 55$). A decreasing trend after the onset of diabetes was found; in subjects with diabetes duration >9 years, the upper CI limit was 1.2%, thus providing evidence that using a conservative approach, celiac disease is rarely found after 10 years' duration of diabetes.

Table 3—*Number of type 1 diabetic patients screened per year since 1995 and prevalence of biopsy-confirmed celiac disease, by duration of diabetes*

Duration of type 1 diabetes (years)	No. of subjects screened for celiac disease	Confirmed celiac disease
≤1.0	2,280	3.3 (2.6–4.1)
1.1–2.0	2,387	1.5 (1.0–2.0)
2.1–3.0	2,595	0.9 (0.6–1.4)
3.1–4.0	2,020	0.7 (0.4–1.2)
4.1–5.0	1,766	0.8 (0.5–1.4)
5.1–6.0	1,477	0.7 (0.3–1.2)
6.1–7.0	1,186	0.5 (0.2–1.1)
7.1–8.0	981	0.7 (0.3–1.5)
8.1–9.0	753	0.8 (0.3–1.7)
9.1–10.0	587	0.3 (0.04–1.2)
>10.0	1,207	0.6 (0.2–1.2)

Data are % (95% CI), unless otherwise noted. Only biopsy-proven cases of celiac disease diagnosed since 1995 ($n = 203$), when yearly screening program was implemented in Italy, are included.

Multiple logistic regression analyses were then performed to assess variables independently associated with the risk of having celiac disease and diabetes, compared with the risk of having diabetes alone. As shown in Table 4, age at onset of diabetes, sex, and presence of a thyroid disorder were independently associated with the risk of having both diseases. A significant negative trend of risk with increasing age at onset was evident ($P < 0.001$). In comparison with age at onset of diabetes being >9 years, age at onset <4 years conferred an OR of 3.27 (95% CI 2.20–4.85). Current age was not associated with risk of having celiac disease.

CONCLUSIONS —This study showed a high prevalence (6.8%) of biopsy-confirmed celiac disease in a large Italian cohort of children and adolescents with type 1 diabetes cared for in pediatric outpatient diabetes clinics distributed all over Italy. In addition, it provided the first evidence that age at onset of type 1 diabetes is negatively associated with risk for celiac disease, even after adjusting for potential confounders such as actual age, sex, and presence of thyroid disorders. Indeed, a threefold higher risk for celiac disease was evident in children age <4 years at the onset of diabetes than in those age >9 years. This is an original finding, with previous studies having recruited only small numbers of diabetic patients lacking the statistical power to detect any association. Our finding is consistent with the hypothesis that there are common genetic or environmental factors in the etiopathogenesis of these diseases in younger children.

The possibility that gluten has a role in β-cell autoimmunity has been suggested by reports showing that diabetes-related antibodies disappear in BB rats fed a gluten-free diet (13). In humans, a small prospective study of 11 children confirmed that observation; however, all patients had single autoantibody positivity and very low risk of progression to diabetes (4). In another study conducted in high-risk relatives of type 1 diabetic patients, elimination of dietary gluten did not modify either levels of autoantibodies or progression to diabetes (14). Recently, a clinic-based cohort of nondiabetic adults with celiac disease was shown to have a fourfold increased prevalence of type 1 diabetes–related autoantibodies in patients with >9 years' duration of gluten withdrawal than in those with a duration <5 years (95% CI 1.51–10.6), even after adjusting for age, sex, presence of other autoimmune diseases, and compliance with diet (15). Altogether, these observa-

Table 4—*Results of logistic regression analyses of variables independently associated with presence of both celiac disease and type 1 diabetes in the Italian multicentre cohort of children and adolescents with type 1 diabetes on the prevalence date (31 December 2001)*

	OR (95% CI)	*P*
Boys	1.00	
Girls	1.75 (1.35–2.29)	<0.0001
Age at diagnosis of diabetes (years)		
<4	3.27 (2.20–4.85)	<0.0001
4–6	2.61 (1.73–3.92)	
7–9	1.04 (0.65–1.67)	
>9	1.00	
Thyroid disorder		
No	1.00	0.001
Yes	1.82 (1.29–2.55)	

tions suggest that the association between celiac disease and type 1 diabetes is more complex. Dietary gluten could act as a modifier rather than a determinant of the etiopathogenesis of type 1 diabetes, facilitating the progression of other dietary factors to the lamina propria, where they activate the autoimmune response against β-cells in subjects with genetic susceptibility to diabetes. This mechanism would be consistent with the finding that few cases of type 1 diabetes occur in subjects with celiac disease after gluten is eliminated from the diet. It is interesting that an upregulation of zonulin, a human protein analogous to the *Vibrio cholerae*–derived zonula occludens toxin, which is involved in tight junction regulation, has been found in the early phase of celiac disease (16–18). Genetically determined variations in factors involved in intestinal permeability, such as zonulin, could be involved in the association between celiac disease and type 1 diabetes, particularly in younger children. Well-conducted case-control studies have shown that various dietary factors are associated with risk for type 1 diabetes, such as protein intake, nitrosamines, dairy products, and cow's milk (19–22). These unspecific observations would suggest a role for abnormal intestinal permeability, which, in genetically susceptible individuals, would activate the autoimmune reaction against β-cells.

As recently discussed, the high prevalence of celiac disease in children with type 1 diabetes and the importance of preventing gastrointestinal malignancy and of protecting bone mineral in children and adolescents support a recommendation to screen all incident cases of type 1 diabetes for celiac disease (23). The appropriate lag time between reexaminations, however, has not been defined. Using a large cohort, we have provided evidence that, apart from patients diagnosed at onset of diabetes, 75% of children and adolescents had a diagnosis of celiac disease by 4 years' duration of diabetes. We observed a decreasing trend of prevalence of celiac disease by duration of diabetes; in subjects with diabetes duration >9 years, the upper 95% CI limit was 1.2%, thus providing evidence that even using a conservative approach, celiac disease is rarely found after 10 years' duration of diabetes. Therefore, our findings support the recommendation to annually screen asymptomatic children and adolescents at least by 4 years' duration of diabetes, particularly children with age at onset of diabetes <4 years and girls. Given the cost of tests (approximately $12 per sample for EMA measurement), it is advisable to continue screening asymptomatic children to at least up to 10 years' duration of diabetes, with intervals depending on local economic resources (23).

Another original finding of our survey was the observation of a sex difference in the prevalence of celiac disease, with an almost twofold higher risk seen in girls than in boys, independent of age or presence of other autoimmune diseases. This finding would suggest that sex-linked genetic susceptibility is involved in the etiopathogenesis of disease.

Unlike previous studies in which small clinic-based cohorts of diabetic children were studied, we extended our observations to a large cohort of diabetic children and adolescents, representative of Italian patients. Our rates are similar to those recently obtained in a long-term follow-up study based in one Italian pediatric clinic (24). Lower prevalence estimates provided by other studies are likely due to the less sensitive methods used for screening.

In conclusion, this multicenter national survey provided evidence that *1*) the prevalence of biopsy-confirmed celiac disease in diabetic children and adolescents is high (6.8%); *2*) the risk of celiac disease is negatively and independently associated with age at onset of diabetes, with a threefold higher risk being seen in children age <4 years than in those age >9 years; and *3*) girls have a higher risk of having both diseases.

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