Prevalença global de la malaltia celíaca a Catalunya. Impacte del cribratge poblacional en població en edat laboral

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PREVALENÇA GLOBAL DE LA MALALTIA CELÍACA A CATALUNYA. IMPACTE DEL CRIBRATGE POBLACIONAL EN POBLACIÓ EN EDAT LABORAL

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The prevalence of coeliac disease is significantly higher in children compared with adults

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SUMMARY

Background
Some limited studies of coeliac disease have shown higher frequency of coeliac disease in infancy and adolescence than in adulthood. This finding has remained unnoticed and not adequately demonstrated.

Aim
To assess whether there are age and gender differences in coeliac disease prevalence.

Methods
A total of 4230 subjects were included consecutively (1 to ≥80 years old) reproducing the reference population by age and gender. Sample size was calculated assuming a population-based coeliac disease prevalence of 1:250. After an interim analysis, the paediatric sample was expanded (2010 children) due to high prevalence in this group. Anti-transglutaminase and anti-endomysial antibodies were determined and duodenal biopsy was performed if positive. Log-linear models were fitted to coeliac disease prevalence by age allowing calculation of percentage change of prevalence. Differences between groups were compared using Chi-squared test.

Results
Twenty-one subjects had coeliac disease (male/female 1:2.5). Coeliac disease prevalence in the total population was 1:204. Coeliac disease prevalence was higher in children (1:71) than in adults (1:357) ($P = 0.00005$). A significant decrease of prevalence in older generations was observed [change of prevalence by age of −5% (95% CI: −7.58 to −2.42%)]. In the paediatric expanded group (1–14 years), a decrease of coeliac disease prevalence was also observed [prevalence change: −17% (95% CI: −25.02 to −6.10)].

Conclusions
The prevalence of coeliac disease in childhood was five times higher than in adults. Whether this difference is due to environmental factors influencing infancy, or latency of coeliac disease in adulthood, remains to be demonstrated in prospective longitudinal studies.

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INTRODUCTION

A number of epidemiological studies using serological methods for coeliac disease (CD) detection have shown that CD is distributed worldwide. However, prevalences ranging from 1:100 to more than 1:500[1–9] have been reported using identical analytical methods for CD screening [human anti-transglutaminase (tTGA) and/or antiendomysial antibodies (EmA)]. These geographical patterns may be attributable to differing exposure to gluten-containing cereals in different time periods, genetic differences and/or changes in environmental triggering risk factors. For example, dietary trends based on national feeding recommendations in Sweden were assumed to be in part responsible for epidemic peaks of CD in this country.[10]

Nevertheless, differences in CD prevalence between studies may also be due to bias in the age and gender of individuals included. In fact, the predominance of CD in female subjects is clearly established,[11–14] and some studies have shown higher frequency of CD in infancy[15] and adolescence[16] than in adulthood. This latter finding was unexpected in a disease considered long lasting and it remained unnoticed and not adequately demonstrated. If confirmed, important questions could be raised such as environmental factors (lifestyle, infections) affecting the youngest cohorts or the possibility of frequent evolution towards latency in CD detected by mass screening. The only way to demonstrate unequivocally the existence of gender- and age-related differences in CD prevalence is by performing a cross-sectional study in which the sample represents the structure of a reference population according to gender and age. And as far as we know, this methodological approach has not been used to date.

The aim of the present study was to assess whether there is an age- and gender-related difference in the prevalence of CD in Catalonia (autonomous region in the northeast of Spain).

MATERIAL AND METHODS

Subjects and study design

The inclusion period was divided into two phases. In the first one, from January 2004 to December 2007, 4230 subjects from 1 to more than 80 years of age (2076 male; 2154 female subjects) were consecutively recruited in the participating centres. None of these subjects declined participation in the study. A large proportion of subjects in the middle age of life (from 20 to 55 years) were recruited in a workplace health surveillance department, whereas individuals in extreme ages of life were recruited in ambulatory minor surgery departments of the paediatric and general tertiary referral hospitals in the region. The predominant types of surgery in children were phimosis, circumcision, adenoidectomy and ophthalmology surgery, whereas in adults they were cataract surgery, varicose vein surgery and arthroscopy. To avoid a bias in the inclusion, only those individuals coming from the catchment areas attended by the hospitals were included.

The sample size was calculated assuming a CD prevalence of 1:250 ($\alpha = 0.05$; $\delta = 0.25$) based on previous epidemiological studies performed in Spain with CD prevalence ranging from 1:118 to 1:389.[2, 17, 18] Subject inclusion exactly reproduced the distribution of the population of Catalonia, regarding gender and age, in the year 2003 according to data from the Catalonia Statistics Institute (available at: http://www.idescat.cat/territ/BasicTerr?TC=5&V0=3&V1=3&V3=669&V4=498&P=N&PARENT=1&CTX=B&ALLINFO=TRUE&ANYS=2003&x=10&y=5). Subjects in the whole sample were classified into 18 age groups of 5 years each (from 1–4 to ≥85 years) for each gender. The consecutive inclusion of subjects was concluded when the calculated number of subjects was achieved in each age and gender group.

In the second phase, from January 2006 to February 2007, the paediatric sample was expanded as an interim analysis at the half point of the recruitment period (December 2005) showed a high CD prevalence in children. The sample size was recalculated based on 1:100 CD prevalence in subjects from 1 to 14 years of age ($\alpha = 0.05$; $\delta = 0.25$). A total of 1230 additional children were recruited in the department of ambulatory minor surgery of the paediatric hospital. Thus, the paediatric group consisted finally of 2010 children (780 recruited in the first phase plus 1230 added in the second phase; 1042 male, 968 female subjects).

All participants were asked about previous diagnosis of CD and about the possibility of intake of gluten-free diet. In affirmative cases, the CD diagnosis was carefully confirmed by reviewing the serology and duodenal histology at the time of diagnosis as well as the response to a gluten-free diet. This confirmation occurred in three of the 4230 cases of the whole population and in seven cases of the expanded paediatric group.

After written informed consent was obtained from all subjects of the whole population and expanded paediatric one, spare serum from the workplace health or preoperative profile was used for CD antibody detection (EmA

and tTGA, see below); this recruitment facilitated 100% acceptance of the serological analysis. When one or both serological markers were positive, the diagnostic work-up of CD (duodenal biopsy and genetic study) was proposed. The duodenal biopsy was accepted in 91% and 95% of subjects of the whole and paediatric samples respectively.

The study protocol was approved by the ethics committees of the participating hospitals.

Antibody detection

Serum IgA-EmA was determined by indirect immunofluorescence (IFI) assay in serum samples at 1/5 dilution, as previously described.[19] Commercial sections of monkey distal oesophagus (BioMedical Diagnostics, Marnela-Vallée, France) were used as IFI substrate. IgA-class tTGA was analysed in serum using a quantitative automated ELISA method by means of a commercially available detection kit (Varelisa CelikeyTM; Phadia AB, Freiburg, Germany) using recombinant human tTG as antigen.[20] As recommended by the manufacturer, titres of EmA >1/5 and tTGA ≥8 U/mL were considered positive. Nevertheless, as >98% of individuals had tTGA <2 U/mL, subjects with values ≥2 U/mL were encouraged to adopt the same diagnostic approach to CD as subjects with unequivocal positive serology. This strategy was applied to identify the maximum range of the gluten sensitivity spectrum. Total serum IgA was measured using rate nephelometry [BN II, Siemens Healthcare Diagnostics (Former Dade Behring), Frankfurt, Germany]. In cases of IgA deficiency, IgG-class EmA was measured.

Genetic markers

Standard techniques for DNA extraction, PCR amplification and product detection were used. To purify genomic DNA from whole blood, a commercial reagent Generation Capture Column Kit (Gentra Systems, Minneapolis, MN, USA) was used. HLA-DQ2 (DQA1*0501 and DQB1*0201 alleles) and HLA-DQ8 (DQA1*0301 and DQB1*0302 alleles) genotyping was performed by PCR amplification using sequence-specific primers (PCR–SSP)[21] on a GeneAmp PCR 2400 System (Perkin Elmer, Norwalk, Connecticut, USA). PCR products were detected by electrophoresis on 2% agarose gel and were visualised under UV light. Analysis of HLA-DQ8 haplotype was performed only on those patients with negative DQ2.

Duodenal biopsy and diagnostic criteria for CD

Four endoscopic biopsies from the second and third portions of the duodenum in adults and Watson-Crosby capsule biopsy in children were processed using haematoxylin/eosin staining and CD3 immunophenotyping, and the biopsies were blindly evaluated by two expert gastrointestinal pathologists (A.S. and V.C.). Histopathological findings were staged according to the Marsh criteria,[22] as revised by Rostami *et al.*[23]: 'Infiltrative' lesions with intraepithelial lymphocytosis are defined as Marsh type I, 'infiltrative/hyperplastic' lesions are defined as Marsh II, and 'partial (A) subtotal (B) and total (C) villous atrophy' as Marsh III. We assumed that intraepithelial lymphocytosis was present when more than 25 IEL/100 epithelial cells were observed.[24]

A possible diagnosis of CD was considered when some degree of histological abnormality of the gluten-sensitive enteropathy spectrum was found. However, as appropriate clinical, histological and/or serological assessment after gluten-free diet was not available for all patients with mild enteropathy, the diagnosis of CD was considered sure in patients with atrophy and unequivocal positive serology (titres of EmA >1/5 and/or tTGA ≥8 U/mL). Previously diagnosed CD cases and those identified in the study period by serology (EmA >1/5 and/or tTGA ≥8 IU/mL) that had atrophy-proven biopsy were considered to be CD cases for the purpose of calculating CD prevalence.

Statistical analyses

Coeliac disease prevalence rates were calculated by dividing the number of CD cases by the number of subjects recruited in each 5-year age group and these rates were multiplied by 1000 subjects. Given that a preliminary statistical analysis in the whole sample demonstrated a significant decline in CD prevalence during the first 5 years of life, prevalence rates were computed for 1-year age groups in the expanded paediatric sample.

The 95% confidence intervals (95% CI) of prevalence rates could not be calculated assuming a normal distribution, as certain age groups showed no CD cases. Therefore, a binomial distribution[25] was assumed for the number of CD cases to compute the exact 95% CI for the prevalence rates as well as the percentage change (% change) of prevalence by age group. The % change was estimated by means of a generalised linear model[26] known as log-binomial model.[27, 28] The appropriateness of the model was assumed if the ratio between the residual deviance and the residual degrees of freedom significantly departed from one another.[26] In this analysis, the age group is the slope of this specific log-linear model where the outcome is the prevalence, and therefore, the age-group variable was considered to be a continuous

one. In this model, we should note that the median age for each age group was used as age variable for the whole sample analysis, whereas the specific annual age group was used as age variable in the paediatric sample. Therefore, % change of prevalence was obtained by subtracting 1 from the exponent of the slope of the fitted models and multiplying this quantity by 100.[29] These % changes were considered statistically significant when 95% CI did not include the 0 value. Negative values of the % change were interpreted as a decline in CD prevalence, whereas positive values showed a rise in CD prevalence. Differences between groups were compared using Chi-squared tests.[30] A threshold of 0.05 was set for assuming statistical significance. All statistical analyses were performed using the R statistical package.[31]

RESULTS

CD prevalence in the whole study sample (4230 subjects)

Twenty-one of 4230 subjects had positive serology. Of these, two patients did not accept biopsy (a 1-year-old boy and a 28-year-old man with tTGA values of 8.7 IU/mL and EmA 1/80, and 3.94 IU/mL and EmA 1/20 respectively), and one had normal duodenal histology (an 82-year-old man with confirmed positive serology in two separate samples, tTGA 4 IU/mL and EmA 1/80). The remaining 18 subjects showed villous atrophy at the duodenal biopsy. In addition, three more cases included in the prevalence study had previously been diagnosed with CD. Thus, the total number of CD patients included in the prevalence study was 21 (6 male, 15 female subjects; male/female ratio 1:2.5), giving a CD prevalence of 4.97 per 1000 (95% CI: 3.08–7.58) and ratio of cases to noncases of 1:204. CD prevalence according to age group and 95% CI are shown in Table 1. CD prevalence was clearly higher in children (1–14 years) (14.1 per 1000; 95% CI: 7.0–25.1 or ratio 1:71) than in adults (2.8 per 1000; 95% CI: 1.4–5.3 or ratio 1:357). Significant differences in CD prevalence among the age groups were found ($P = 0.00005$). A significantly decreasing CD prevalence in older subjects vs. younger ones was observed (% change: −5; 95% CI: −7.58 to −2.42; Figure 1).

Age variables were also grouped as 0–14, 15–29, 30–44, 45–59, 60–74 and 75 years and older in order to assess whether the excess of zero cases among older age groups might affect the estimate of CD prevalence by age. This analysis showed that CD prevalence decreased by −5% (95% CI: −7.35 to −2.13, see Table S1 and Figure S1). Therefore, both analyses led to the conclusion that CD prevalence decreased by −5% by year of age, independently of the age group definitions.

Table 1 | Coeliac disease (CD) prevalence in the whole study sample according to distribution of Catalan population

Age (years)	*n*	CD cases	Prevalence × 1000	95% CI
1–4	221	6	27.15	10.03–58.15
5–9	280	3	10.71	2.22–30.99
10–14	279	2	7.17	0.87–25.65
15–19	204	1	4.90	0.12–27.01
20–24	289	4	13.84	3.78–35.06
25–29	364	1	2.75	0.07–15.21
30–34	347	0	0.00	0.00–10.57
35–39	332	2	6.02	0.73–21.59
40–44	301	1	3.32	0.08–18.37
45–49	269	0	0.00	0.00–13.62
50–54	252	0	0.00	0.00–14.53
55–59	237	0	0.00	0.00–15.44
60–64	172	0	0.00	0.00–21.22
65–69	195	0	0.00	0.00–18.74
70–74	180	0	0.00	0.00–20.29
75–79	144	0	0.00	0.00–25.29
80–84	129	1	7.75	0.20–42.43
≥85	35	0	0.00	0.00–100.03
Total	4230	21	4.97	3.08–7.58

Chi-squared test: $P = 0.00005$.

CD cases, CD cases detected in the study; *n*, number of subjects in each age group; 95% CI, 95% confidence interval of CD prevalence.

In Table 2, the 21 CD cases with a degree of histological damage, serology and genetic studies are detailed. Two of the three CD cases detected before the present screening were diagnosed 1 year before inclusion, both due to classic malabsorption syndrome, and the remaining case was diagnosed 5 years earlier, as she belonged to a group at risk of CD (first-degree relative). In the two cases with negative genetic study, a good clinical, serological and histological response confirmed the CD diagnoses.

CD prevalence in the expanded paediatric sample (2010 children)

Twenty of the 2010 children had positive serology. The parents of one of these, a 1-year-old boy, did not accept biopsy. The duodenal histology of the remaining 19 cases

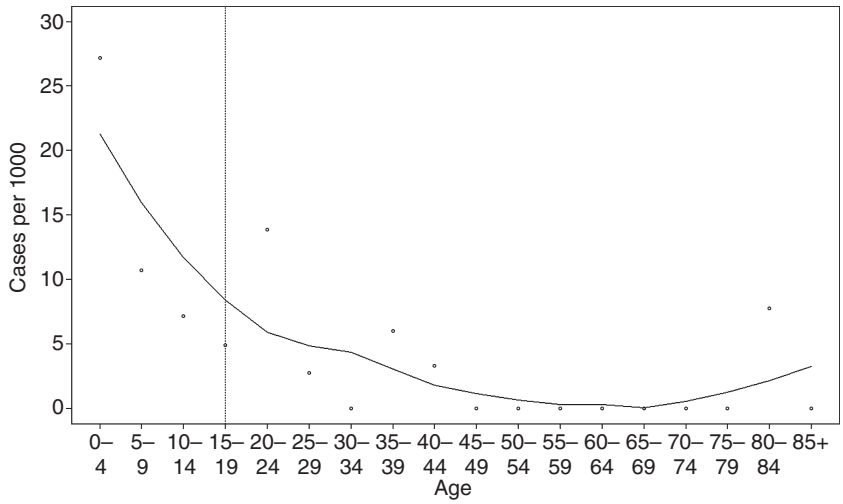

Figure 1 | Decreased coeliac disease prevalence in adulthood compared with childhood.

showed villous atrophy. Seven more patients previously diagnosed with CD before the start of the study (average age at diagnosis 5 years, ranging from 1 to 13) were also included. Thus, the total number of CD patients included in the CD prevalence study of the paediatric sample was 26 (7 male, 19 female subjects; male/female ratio 1:2.7),

Table 2 | Description of coeliac disease (CD) patients identified in whole study sample

Case	Gender	Age at CD diagnosis (years)	EmA (titres)*	tTGA (IU/mL)*	Duodenal biopsy*	Genetic study	CD diagnosed before screening
1	Female	1	1/320	100	Marsh 3C	DQ2+	Yes
2	Male	1	1/320	100	Marsh 3C	DQ2+	Yes
3	Female	2	1/320	100	Marsh 3C	DQ2+	No
4	Female	2	1/80	20.7	Marsh 3A	DQ2+	No
5	Male	3	1/160	51	Marsh 3B	DQ2+	No
6	Female	4	1/320	100	Marsh 3C	DQ2+	No
7	Male	6	1/160	39.8	Marsh 3A	DQ2+	No
8	Female	7	1/320	88.7	Marsh 3C	DQ2+	No
9	Female	8	1/80	31.5	Marsh 3B	DQ2+	No
10	Female	10	1/160	112	Marsh 3C	DQ2+	Yes
11	Female	13	1/80	46.6	Marsh 3A	DQ2+	No
12	Female	15	1/160	77	Marsh 3C	DQ2+	No
13	Male	20	1/40	6.76	Marsh 3B	DQ2+	No
14	Female	21	1/320	159.0	Marsh 3C	DQ2+	No
15	Female	22	1/160	114.0	Marsh 3C	DQ2+	No
16	Female	23	1/10	4.51	Marsh 3B	DQ2+	No
17	Female	29	1/20	5.0	Marsh 3A	DQ2 and DQ8–†	No
18	Female	36	1/20	5.76	Marsh 3A	DQ2 and DQ8–‡	No
19	Male	38	1/320	63.46	Marsh 3C	DQ2+	No
20	Female	44	1/320	62.88	Marsh 3B	DQ2+	No
21	Male	82	1/80	12.25	Marsh 3A	DQ2+	No

* Serological and histological characteristics at the time of diagnosis.

† Both alleles of DQ2 (DQA1*0501, DQB1*0201) and DQ8 (DQA1*0301, DQB1*0302) negative.

‡ DQB1*0201 positive and both alleles of DQ8 (DQA1*0301, DQB1*0302) negative.

Table 3 | Paediatric coeliac disease (CD) prevalence according to Catalan paediatric population

Age (years)	*n*	CD cases	Prevalence × 1000	95% CI
1	157	8	50.96	22.25–97.93
2	152	4	26.32	7.22–66.01
3	155	1	6.45	0.16–35.42
4	147	2	13.61	1.65–48.28
5	142	1	7.04	0.18–38.61
6	139	1	7.19	0.18–39.43
7	136	2	14.71	1.79–52.11
8	140	2	14.29	1.73–50.65
9	137	0	0.00	0.00–26.57
10	144	2	13.89	1.69–49.27
11	140	0	0.00	0.00–26.01
12	142	1	7.04	0.18–38.61
13	135	2	14.81	1.80–52.49
14	144	0	0.00	0.00–25.29
Total	2010	26	12.93	8.47–18.89

Chi-squared test: $P = 0.001465$.

CD cases, CD cases detected in the study; *n*, number of subjects by age; 95% CI, 95% confidence interval of CD prevalence.

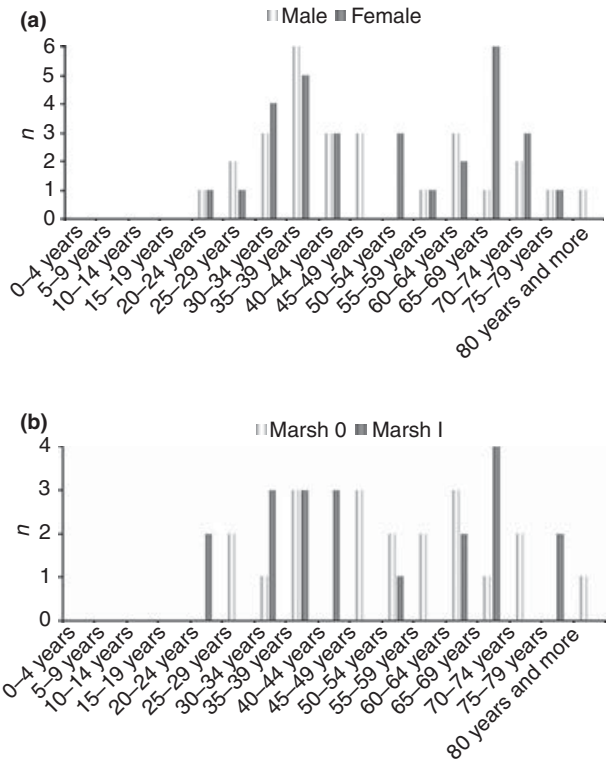

Figure 3 | (a) Gender and age distribution of coeliac disease (CD) cases with borderline serology ($n = 57$). (b) Histological findings related to CD cases with borderline serology, by age groups ($n = 40$).

disclosing CD prevalence in the paediatric group of 12.93 per 1000 (95% CI: 8.47–18.89). Detailed paediatric CD prevalence is shown in Table 3. Therefore, a significant decrease of CD prevalence according to age in children was observed (% change: −17; 95% CI: −25.02 to −6.10), which was particularly marked beyond 3 years of age (Figure 2). There were significant differences when CD prevalence was compared among ages ($P = 0.001465$).

The degree of histological damage of the 26 paediatric CD cases was 3 with Marsh 3A, 9 with Marsh 3B and 14 with Marsh 3C (cases 1–11 of Table 2 plus 15 more cases diagnosed in second phase of recruitment, included in Table S2); all of them were DQ2 positive. Of the seven CD patients diagnosed before screening, two had classic CD, three had atypical clinical presentations and

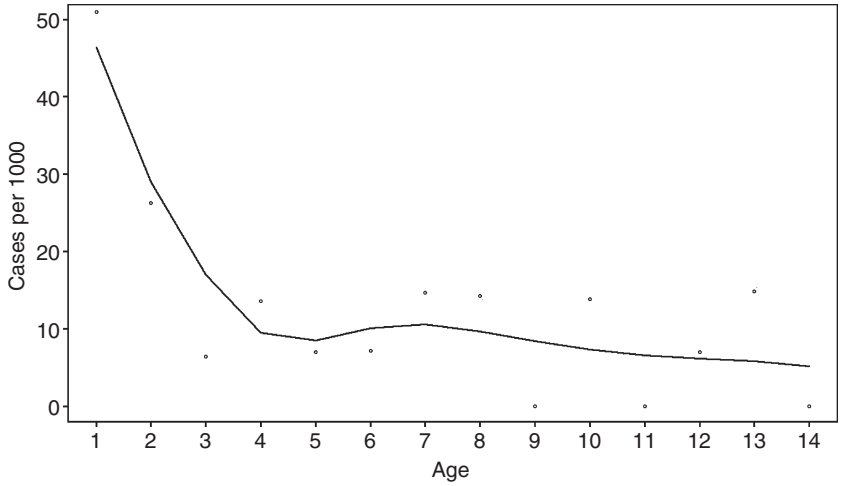

Figure 2 | Decreased coeliac disease prevalence in older children.

two showed silent disease (diagnosed as a result of disease-associated conditions).

Histology and genetics of individuals with borderline serology

Fifty-seven of the 4230 individuals had negative EmA and borderline tTGA values between 2 and 8 U/mL (13.4 per 1000; 1:74). Forty of them accepted further assessment with genetic study and duodenal biopsy, yielding a percentage of participation in the CD diagnostic work-up of 70% individuals with borderline serology. The following histological and genetic findings were found: 20 had duodenal lymphocytosis (Marsh 1) [7 DQ2+ (35%); 6 DQB1*0201+ (30%); 2 DQA1*0501+ (10%); 2 DQ8+ (10%); 3 DQ2 and DQ8 negative (15%)]; and the remaining 20 all showed normal biopsy (Marsh 0) [10 DQ2+ (50%); 6 DQB1*0201+ (30%); 3 DQ8+ (15%); 1 DQ2 and DQ8 negative (5%)]. In Figure 3, the cases with borderline serology according to 5-year age groups are shown for patients with duodenal lymphocytosis and for cases with normal biopsy. Gender and age distribution of these cases (18 male, 22 female subjects, ratio: 1:1.2; mean age: 49, range: 20–86) was completely different from that shown for patients with atrophy (mean age: 18.4 years; range: 1–82). No cases with borderline serology were detected among individuals under the age of 20 years.

DISCUSSION

This work is the first prevalence study of CD in which prevalence has been determined in a sample that exactly reproduces the same gender and age structure as the reference population. CD prevalence in the global population of this study was in the range found in other studies (1:204), and a clear female predominance in CD was confirmed in all age groups. Nevertheless, a fivefold increase in CD prevalence was found in the paediatric group as compared to the adult group (1:71 vs. 1:357). This increase was an unexpected finding in a disease considered lifelong and we propose some possible explanations for this phenomenon. None of them can be demonstrated with the present cross-sectional design but there are hypotheses that could be confirmed in future longitudinal studies.

A similar decrease in CD prevalence related to age was found in a Brazilian study, which included individuals aged 1–60 years, although the authors did not assert that the sample matched the Brazilian population structure.[15] The authors suggested that CD prevalence declined with age, probably due to an increase in mortality associated with CD, and also partly attributable to the deficient healthcare services in some regions of the country. However, the age-related decrease of CD prevalence detected in our study cannot be explained by a high mortality among CD patients. This assertion is supported by several observations: firstly, in contrast to the case of Brazil,[15] life expectancy in the Catalan population is one of the highest in the world, with universal healthcare coverage; secondly, only a mild excess mortality risk related to CD has been reported worldwide,[32, 33] but this fact in itself would not explain the absence of CD in subjects born from 1925 to 1962; and thirdly, the expanded paediatric group of the present study demonstrated a dramatic decrease in CD prevalence beyond 3 years. This drop in CD prevalence in this age group cannot be explained by Catalan childhood mortality, which is as low as 0.16 deaths per 1000 inhabitants from 1 to 4 years of age, 0.08 per 1000 inhabitants from 5 to 9 years of age, and 0.14 per 1000 inhabitants from 10 to 14 years of age.[34] In addition, in the data provided on the analysis of mortality in Catalonia[34] no deaths related to CD-associated conditions or CD comorbidities were registered.

Recent epidemiological studies performed in Finland have suggested that a substantial portion of CD patients are diagnosed after the age of 65 years (21.3×1000 inhabitants). In our study, we found a slight increase in CD prevalence in individuals older than 80 years, probably reflecting the same phenomenon.[35]

It could be argued that the existence of certain environmental factors such as viral infections and changes in feeding policies (recommendations in breast feeding, or the time of gluten introduction) are possible explanations for the high CD prevalence in early infancy. However, we have not been able to identify any effect related to these triggering factors on the appearance of a possible CD epidemic mainly among children under 3 years of age. On the contrary, breast feeding, a factor considered to be protective for CD development,[36] has increased in Catalonia both in frequency and duration. Data provided by the Government of Catalonia at the website http://www.gencat.cat/generalitat/cas/govern/infocatalunya/ show a breast-feeding increase from 39% in 1989 to 62% in 2005 at 3 months of age, and from 6% in 1989 to 31% in 2005 at 6 months of age.

Another proposed triggering factor for CD is the early introduction of dietary gluten. However, in contrast to what might be expected, the cohorts of individuals born before the ESPGAN recommendations introduced during the 1980s,[37] among whom dietary gluten had been intro-

duced very early and abruptly, showed the lowest CD prevalence.

The different sources of recruitment for the study (minor surgery units and a workplace health surveillance department) might theoretically have determined differences in CD prevalence. However, particular attention was paid to avoid inclusion bias in the participating centres, thereby minimising this limitation. This avoidance was achieved mainly by including consecutive cases operated on due to very frequent age-related minor conditions in the minor surgery units (cataract surgery, arthroscopy, etc.). These conditions affect a vast majority of subjects of the general population at some time in life. In addition, the decreasing CD prevalence in older subjects was unrelated to the site of recruitment, allowing us the inclusion of individuals of the entire age groups. This inclusion is in contrast to how a majority of previously published prevalence studies were performed, which generally included only individuals of certain periods of life or from specific contexts (adulthood, school children, blood donors, etc.).[5, 6]

Although we cannot rule out the existence of viral infections or changes in dietary habits acting as a cohort effect, increasing the prevalence in the youngest children, an alternative explanation for the age-related differences in CD prevalence found in this study is a possible evolution towards latency or tolerance of a high proportion of CD lesions, mainly those appearing in early childhood and detected by screening. This hypothesis is further supported by some evidence found in the literature.[38, 39] It has been reported that up to 20% of children diagnosed with atrophy in infancy maintain a preserved villous architecture more than 10 years after gluten challenge, with this being more frequent in those children diagnosed before the age of 3 years,[38] and it has also been reported that CD patients diagnosed in adulthood exhibit an attenuated clinical, serological, and histological picture compared to those diagnosed in infancy.[39] In fact, in this study, a similar trend was observed in adult CD patients, who showed lower values of tTGA with attenuated duodenal lesion as compared to coeliac children. Moreover, in an epidemiological study performed in Turku, Finland, in a cohort of children with HLA-conferred CD risk, spontaneous disappearance of transglutaminase-related auto-immunity without exclusion of gluten from the diet was observed in 49% of cases.[40] This fact suggests that in children diagnosed by mass screening the evolution towards latency may be even greater than in those diagnosed by symptoms. In this sense, the results of our study, showing a marked decrease of CD prevalence beyond 3 years, similar to what was found in other countries such as Italy[16] and Brazil[15] with different gluten intake and/or healthcare conditions, may represent another view of the same phenomenon.

The results of this study have shown that subjects with borderline positive serology and CD patients with atrophy are clearly differentiated populations; the most important factor supporting this assertion is the difference in genetic profile. Individuals with borderline serology had a percentage of positive DQ2 (42%) higher than that found in the Catalan population (18%)[41] but clearly lower than that found in patients with unequivocally positive serology and atrophy (95%). In addition, in contrast to CD patients showing a female predominance, the gender distribution of individuals with borderline serology is similar to that of the general population. These differences in the genetic characteristics between CD patients with atrophy and subjects with borderline serology suggest that a great majority of individuals with borderline serology had probably never had a previous CD with atrophy that evolved to latency.

Nevertheless, 50% of subjects with borderline serology had duodenal lymphocytosis, and we demonstrated in a previous study that some of these individuals had a good clinical and histological response to a gluten-free diet.[42] This fact suggests that some individuals with a certain genetic predisposition (positive DQ2 or DQB1*0201) may have an attenuated form of gluten sensitivity that might remain throughout life.

In conclusion, the observed decreasing CD prevalence in older generations, which is particularly striking beyond 3 years of life, suggests that the development in adult life of latency may be more frequent than previously thought, particularly in CD patients detected by screening. This hypothesis needs to be confirmed in future longitudinal studies to better define the natural history of CD. It cannot be used at present as an argument for stopping GFD in patients with a consistent diagnosis of CD.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Evolution of prevalence by age in age groups of 14 years.

Figure S2. Decreased coeliac disease (CD) prevalence related to older age excluding previously diagnosed CD cases (cases already known to have CD at the time of recruitment).

Figure S3. Decreased coeliac disease (CD) prevalence in older children excluding previously diagnosed CD cases (cases already known to have CD at the time of recruitment).

Table S1. Coeliac disease prevalence provided in age groups of 14 years.

Table S2. Description of coeliac disease patients identified in the paediatric sample.

Table S3. Coeliac disease (CD) prevalence provided in 5-year age groups according to distribution of Catalan population excluding previously diagnosed CD cases.

Table S4. Coeliac disease (CD) prevalence provided in 1-year age groups according to Catalan paediatric population distribution excluding previously diagnosed CD cases.

Appendix S1. The log-binomial model.

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Appendix

1. The log-binomial model

The log-binomial model used here is a generalized linear model [McCullagh] where the link function is the logarithm of the proportion under study and the distribution of the error is binomial.

If we define Y_i as the number of CD cases out of the N_i individuals at risk in the i-th age group, we can assume $Y_i \sim Binomial(N_i, p_i)$, where p_i is the prevalence which can be estimated by maximum likelihood as $\hat{p}_i = \frac{Y_i}{N_i}$. Considering age as a continuous variable, the model is defined as $\log(p_i) = \alpha + \beta X_i$, where α is the intercept term and β is the slope of the model. In this line, $(e^{\alpha}) \cdot 1000$ is the prevalence per 1000 estimate for the reference age group and $(e^{\beta} - 1) \cdot 100$ is the percentage change of prevalence by age group.

2. R-Macro (Note that uses library epitools)

```
age.cat<-function(x)
{
if (x<=4) y<-2.5
if ((x>4)&&(x<=9)) y<-7.5
if ((x>9)&&(x<=14)) y<-12.5
if ((x>14)&&(x<=19)) y<-17.5
if ((x>19)&&(x<=24)) y<-22.5
if ((x>24)&&(x<=29)) y<-27.5
if ((x>29)&&(x<=34)) y<-32.5
if ((x>34)&&(x<=39)) y<-37.5
if ((x>39)&&(x<=44)) y<-42.5
if ((x>44)&&(x<=49)) y<-47.5
if ((x>49)&&(x<=54)) y<-52.5
if ((x>54)&&(x<=59)) y<-57.5
if ((x>59)&&(x<=64)) y<-62.5
if ((x>64)&&(x<=69)) y<-67.5
if ((x>69)&&(x<=74)) y<-72.5
if ((x>74)&&(x<=79)) y<-77.5
if ((x>79)&&(x<=84)) y<-82.5
if (x>84) y<-87.5
y
}

age.6<-function(x)
{
if ((x>=0)&&(x<=14)) y<-1
if ((x>14)&&(x<=29)) y<-2
if ((x>29)&&(x<=44)) y<-3
if ((x>44)&&(x<=59)) y<-4
if ((x>59)&&(x<=64)) y<-5
if ((x>64)) y<-6
y
}


###### Read Data
## Directory: Working directory where files should be
## fitxer: file with individual data
# Note that the process transforms individual data into groups
according to age-groups defined
## in functions age.cat and age.6

library(epitools)
directori<-"C:/2010 - Celiacs Cohort/Definitiva/"
fitxer<-"CeliacsR.txt"
BD<-as.data.frame(read.table(paste(directori,fitxer,sep=""),header=T))
summary(BD)
BD$ED<-as.numeric(lapply(as.numeric(BD$Edat),age.cat))
BD$ED6<-as.numeric(lapply(as.numeric(BD$Edat),age.6))
# Note: in the original dataset Edat is Age variable

###### Analysis 6 Age groups: 0-14, 15-29,30-44,45-59,60-74,75+

out.matrix.6<-as.data.frame(matrix(0,6,7))
names(out.matrix.6)<-
c("Age","N","NCel","Prev","Prev1000","LIPrev","LSPrev")
out.matrix.6$Age<-seq(7,85,15)
```

```
out.matrix.6$N<-as.numeric(table(BD$ED6))
out.matrix.6$NCel<-as.numeric((table(BD$ED6,BD$celiac))[,2])
out.matrix.6$Prev<-out.matrix.6$NCel/out.matrix.6$N
out.matrix.6$Prev1000<-(out.matrix.6$NCel/out.matrix.6$N)*1000
for (i in 1:6)
{
prev.tmp<-binom.exact(out.matrix.6$NCel[i],out.matrix.6$N[i])
out.matrix.6$Prev[i]<-prev.tmp$proportion
out.matrix.6$Prev1000[i]<-prev.tmp$proportion*1000
out.matrix.6$LIPrev[i]<-prev.tmp$lower*1000
out.matrix.6$LSPrev[i]<-prev.tmp$upper*1000
}

plot(out.matrix.6$Age,out.matrix.6$Prev1000,ylim=c(0,30),ylab="Cases
per 1000",xlab="Age",xaxt="n")
model.loess<-loess(out.matrix.6$Prev1000~out.matrix.6$Age)
model.pred<-abs(predict(model.loess))
lines(out.matrix.6$Age,model.pred)
age.grp<-c("0-14","15-29","30-44","45-59","60-64",">64")
axis(side=1,at=out.matrix.6$Age,labels=age.grp)


### Log-Binomial model
### In this model Age refers to Age

model.bin.b<-glm(cbind(NCel,N-
NCel)~Age,family=binomial(link="log"),data=out.matrix.6)
model.bin<- summary(glm(cbind(NCel,N-
NCel)~Age,family=binomial(link="log"),data=out.matrix.6))
lmodel.bin.li<-model.bin$coef[2,1]-1.96*model.bin$coef[2,2]
lmodel.bin.ls<-model.bin$coef[2,1]+1.96*model.bin$coef[2,2]
lmodel.bin.med<-model.bin$coef[2,1]
model.bin.li<-(exp(lmodel.bin.li)-1)*100
model.bin.ls<-(exp(lmodel.bin.ls)-1)*100
model.bin.mean<-(exp(lmodel.bin.med)-1)*100

#### Note that model.bin.li is the lower limit of the percentage
change of prevalence confidence interval,
##### model.bin.ls is the upper limit and model.bin.med is the mean
value

print(paste("Percent Change of PRevalence:",round(model.bin.mean,2),"
95% CI(",round(model.bin.li,2),";",round(model.bin.ls,2),")"))

################################
### Trend test of prevalence: Another look at the significance to the
percent change of prevalence
prop.trend.test(out.matrix.6$NCel,out.matrix.6$N)


################# Analysis 5-year Age groups

out.matrix<-as.data.frame(matrix(0,18,7))
names(out.matrix)<-
c("Age","N","NCel","Prev","Prev1000","LIPrev","LSPrev")
out.matrix$Age<-seq(2.5,87.5,5)
out.matrix$N<-as.numeric(table(BD$ED))
out.matrix$NCel<-as.numeric((table(BD$ED,BD$celiac))[,2])
out.matrix$Prev<-out.matrix$NCel/out.matrix$N
out.matrix$Prev1000<-(out.matrix$NCel/out.matrix$N)*1000
for (i in 1:18)
```

```
{
prev.tmp<-binom.exact(out.matrix$NCel[i],out.matrix$N[i])
out.matrix$Prev[i]<-prev.tmp$proportion
out.matrix$Prev1000[i]<-prev.tmp$proportion*1000
out.matrix$LIPrev[i]<-prev.tmp$lower*1000
out.matrix$LSPrev[i]<-prev.tmp$upper*1000
}

#Global prevalence and its 95% Confidence interval: 21 CD among 4230
individuals

binom.exact(21,4230)

plot(out.matrix$Age,out.matrix$Prev1000,ylim=c(0,30),ylab="Cases per
1000",xlab="Age",xaxt="n")
model.loess<-loess(out.matrix$Prev1000~out.matrix$Age)
model.pred<-abs(predict(model.loess))
lines(out.matrix$Age,model.pred)
age.grp<-c("0-4","5-9","10-14","15-19","20-24","25-29","30-34","35-
39","40-44","45-49","50-54","55-59","60-64","65-69","70-74","75-
79","80-84","85+")
axis(side=1,at=out.matrix$Age,labels=age.grp)
abline(v=17.5,lty=3)


### Log-Binomial model
### In this model Age refers to Age

model.bin.b<-glm(cbind(NCel,N-
NCel)~Age,family=binomial(link="log"),data=out.matrix)
model.bin<- summary(glm(cbind(NCel,N-
NCel)~Age,family=binomial(link="log"),data=out.matrix))
lmodel.bin.li<-model.bin$coef[2,1]-1.96*model.bin$coef[2,2]
lmodel.bin.ls<-model.bin$coef[2,1]+1.96*model.bin$coef[2,2]
lmodel.bin.med<-model.bin$coef[2,1]
model.bin.li<-(exp(lmodel.bin.li)-1)*100
model.bin.ls<-(exp(lmodel.bin.ls)-1)*100
model.bin.mean<-(exp(lmodel.bin.med)-1)*100


#### Note that model.bin.li is the lower limit of the percentage
change of prevalence confidence interval,
##### model.bin.ls is the upper limit and model.bin.med is the mean
value

print(paste("Percent Change of PRevalence:",round(model.bin.mean,2),"
95% CI(",round(model.bin.li,2),";",round(model.bin.ls,2),")"))

################################
### Trend test of prevalence: Another look at the significance to the
percent change of prevalence
prop.trend.test(out.matrix$NCel,out.matrix$N)



####### PAEDIATRIC ################################

library(epitools)
directori<-"C:/2010 - Celiacs Cohort/Definitiva/"
fitxer<-"PediatricR.txt"
```

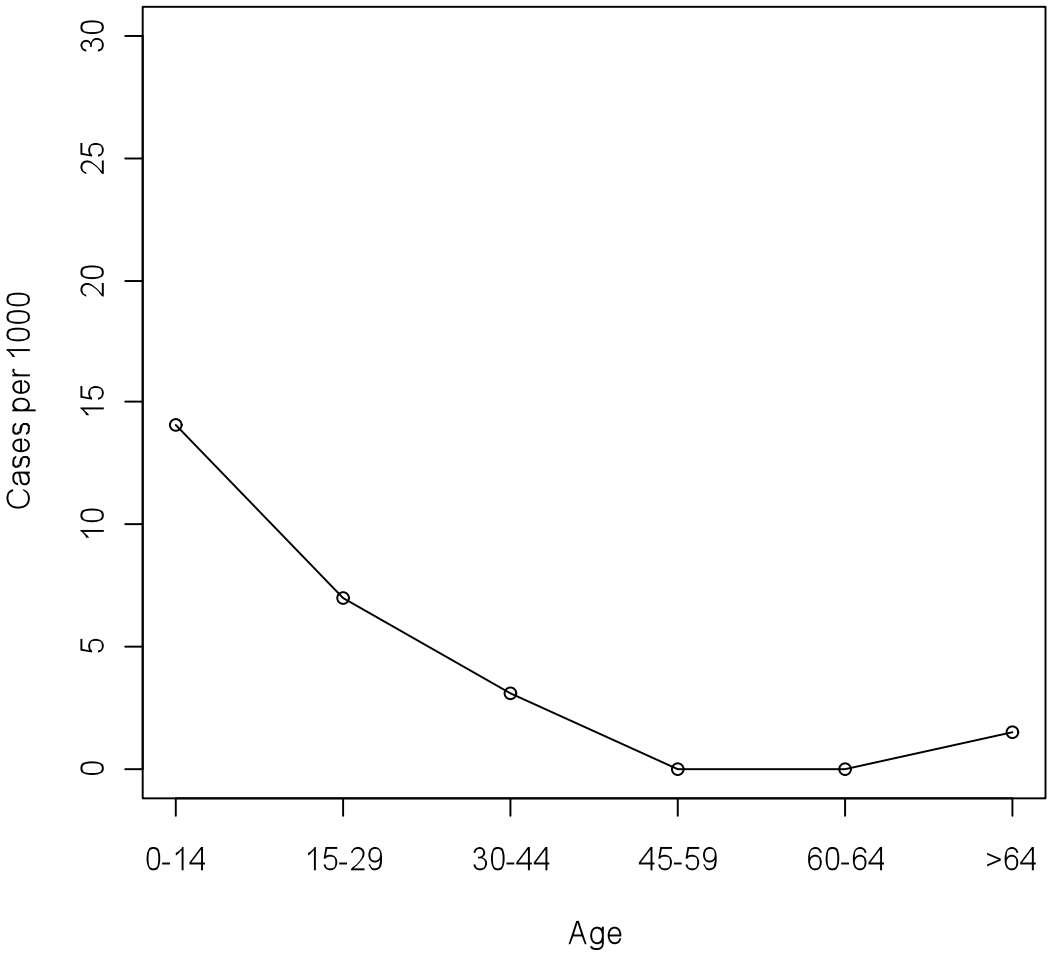
```
BD.Nen<-
as.data.frame(read.table(paste(directori,fitxer,sep=""),header=T))
BD.Nen$ED3<-as.numeric(lapply(as.numeric(BD.Nen$Age),age.3.Nen))
summary(BD.Nen)
out.matrix.Nen<-as.data.frame(matrix(0,14,7))
names(out.matrix.Nen)<-
c("Age","N","NCel","Prev","Prev1000","LIPrev","LSPrev")
out.matrix.Nen$Age<-1:14
out.matrix.Nen$N<-as.numeric(table(BD.Nen$Edat))
out.matrix.Nen$NCel<-
as.numeric((table(BD.Nen$Edat,BD.Nen$celiac))[,2])
out.matrix.Nen$Prev<-out.matrix.Nen$NCel/out.matrix.Nen$N
out.matrix.Nen$Prev1000<-(out.matrix.Nen$NCel/out.matrix.Nen$N)*1000
for (i in 1:14)
{
prev.tmp<-binom.exact(out.matrix.Nen$NCel[i],out.matrix.Nen$N[i])
out.matrix.Nen$Prev[i]<-prev.tmp$proportion
out.matrix.Nen$Prev1000[i]<-prev.tmp$proportion*1000
out.matrix.Nen$LIPrev[i]<-prev.tmp$lower*1000
out.matrix.Nen$LSPrev[i]<-prev.tmp$upper*1000
}

### GLOBAL Prevalence
binom.exact(25,2010)

plot(out.matrix.Nen$Age,out.matrix.Nen$Prev1000,ylim=c(0,50),ylab="Cas
es per 1000",xlab="Age",xaxt="n")
model.loess<-loess(out.matrix.Nen$Prev1000~out.matrix.Nen$Age)
model.pred<-abs(predict(model.loess))
lines(out.matrix.Nen$Age,model.pred)
ed.grp<-c(1:14)
axis(side=1,at=out.matrix.Nen$Age,labels=ed.grp)

### Log-Binomial model
### In this model Age refers to Age

model.bin.Nen<-summary(glm(cbind(NCel, N-
NCel)~Age,family=binomial(link="log"),data=out.matrix.Nen))
lmodel.bin.Nen.li<-model.bin.Nen$coef[2,1]-
1.96*model.bin.Nen$coef[2,2]
lmodel.bin.Nen.ls<-
model.bin.Nen$coef[2,1]+1.96*model.bin.Nen$coef[2,2]
lmodel.bin.Nen.med<-model.bin.Nen$coef[2,1]
model.bin.Nen.li<-(exp(lmodel.bin.Nen.li)-1)*100
model.bin.Nen.ls<-(exp(lmodel.bin.Nen.ls)-1)*100
model.bin.mean.Nen<-(exp(lmodel.bin.Nen.med)-1)*100

#### Note that model.bin.li is the lower limit of the percentage
change of prevalence confidence interval,
##### model.bin.ls is the upper limit and model.bin.med is the mean
value

print(paste("Percent Change of
PRevalence:",round(model.bin.mean.Nen,2)," 95%
CI(",round(model.bin.Nen.li,2),";",round(model.bin.Nen.ls,2),")"))


################################
### Trend test of prevalence: Another look at the significance to the
percent change of prevalence
prop.trend.test(out.matrix.Nen$NCel,out.matrix.Nen$N)
```

CD prevalence provided in age groups of 14 years.

Age (years)	N	CD cases	Prevalence X 1000	CI (95%)
0-14	11	780	14.11	7.06 – 25.09
15-29	6	857	7.01	2.57 – 15.17
30-44	3	980	3.06	0.63 – 8.91
45-59	0	758	0	0 – 4.85
60-74	0	172	0	0- 21.22
75+	1	683	1.46	0.04 – 8.13

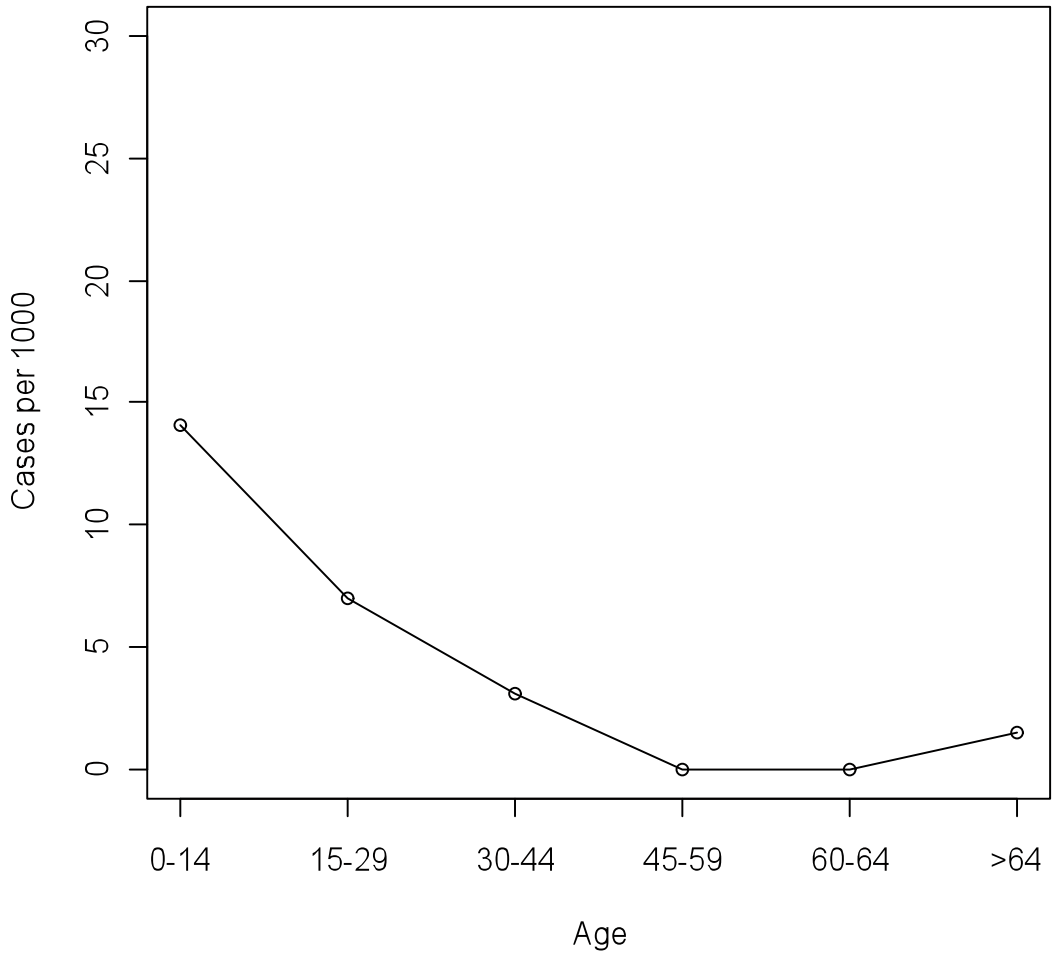
Chi-Square test: p=0.001243; CI= Confidence interval

Evolution of prevalence by age in age groups of 14 years



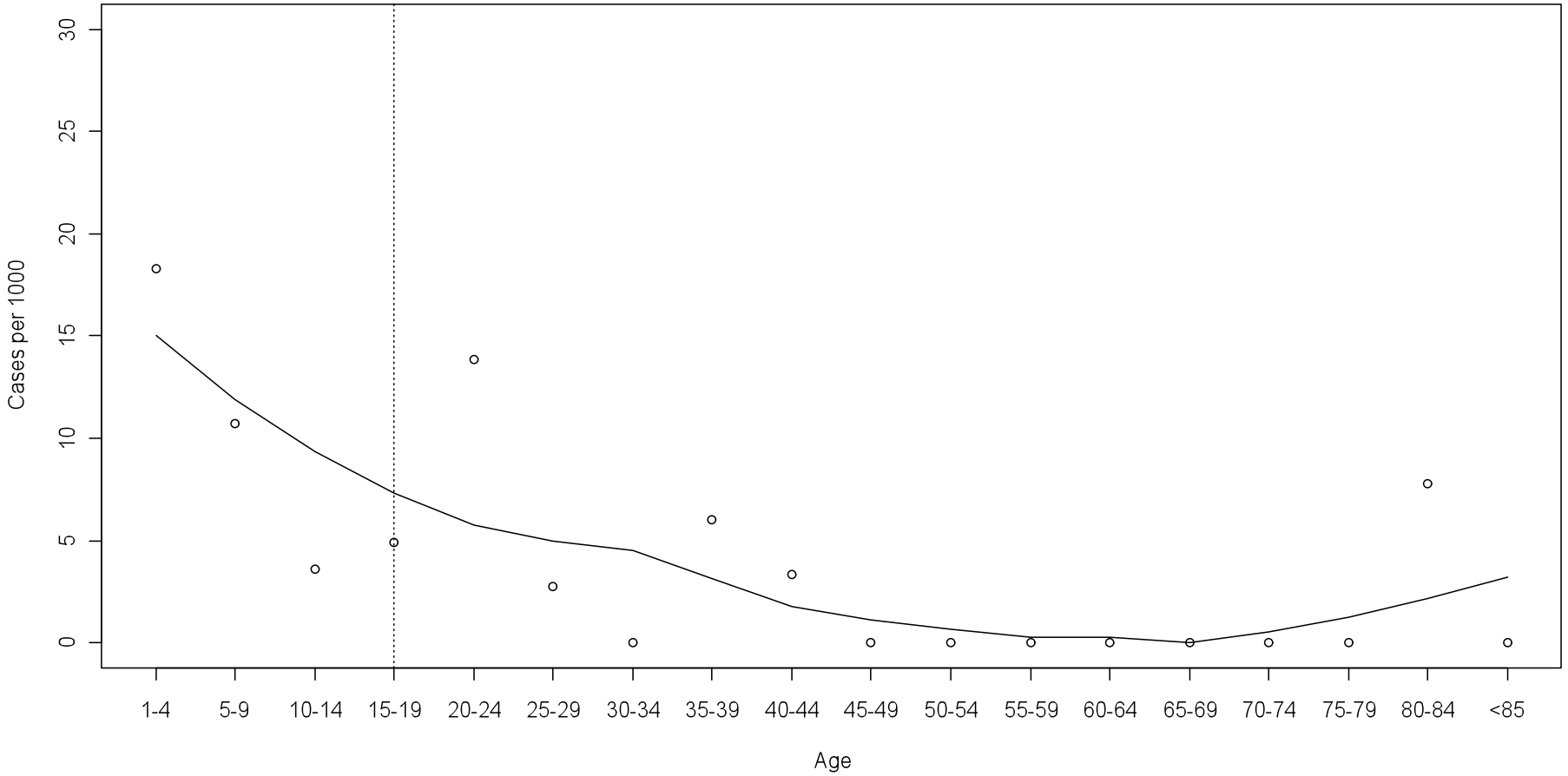
Percent change of prevalence by age -4.78% (95% CI: -7.36; -2.14)

Supplementary Figure S1. Evolution of prevalence by age in age groups of 14 years

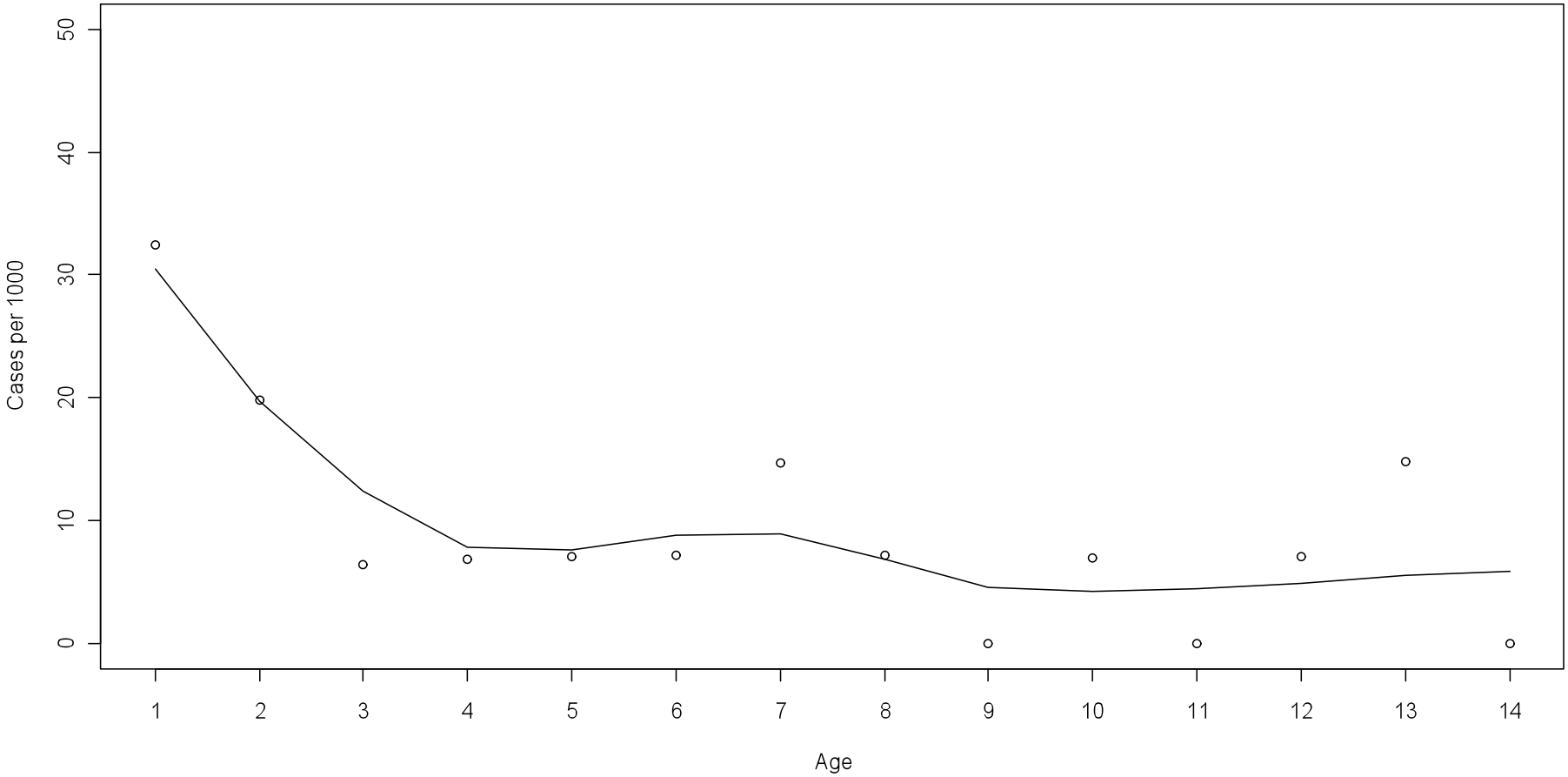


Percent change of prevalence by age -4.78% (95% CI: -7.36; -2.14)

Supplementary Figure S2. Decreased CD prevalence related to older age excluding previously diagnosed CD cases (cases already known to have celiac disease at the time of recruitment). This figure corresponds to data available in Supplementary Table S3.



Supplementary Figure S3. Decreased CD prevalence in older children excluding previously diagnosed CD cases (cases already known to have celiac disease at the time of recruitment). This figure corresponds to data available in Supplementary Table S4.



Supplementary Table S1. CD prevalence provided in age groups of 14 years

Age (years)	N	CD cases	Prevalence X 1000	CI (95%)
0-14	11	780	14.11	7.06 – 25.09
15-29	6	857	7.01	2.57 – 15.17
30-44	3	980	3.06	0.63 – 8.91
45-59	0	758	0	0 – 4.85
60-74	0	172	0	0- 21.22
75+	1	683	1.46	0.04 – 8.13

Chi-Square test: p=0.001243; CI= Confidence interval

Supplementary Table S2. Description of CD patients identified in the paediatric sample

Case	Sex	Age at CD diagnosis (years)	EmA (titters)	t-TGA (IU/mL)	Duodenal biopsy	Genetic study	CD diagnosed before screening
1	Male	1	1/320	100	Marsh 3C	DQ2+	Yes
2	Female	1	1/320	100	Marsh 3C	DQ2+	Yes
3	Male	1	1/320	100	Marsh 3C	DQ2+	No
4	Female	1	1/320	100	Marsh 3B	DQ2+	No
5	Male	1	1/80	2.9	Marsh 3C	DQ2+	No
6	Female	1	1/80	9.2	Marsh 3B	DQ2+	No
7	Female	1	1/20	4.6	Marsh 3B	DQ2+	No
8	Male	1	1/320	100	Marsh 3C	DQ2+	Yes
9	Female	2	1/80	20.7	Marsh 3A	DQ2+	No
10	Female	2	1/320	100	Marsh 3C	DQ2+	No
11	Female	2	1/320	100	Marsh 3C	DQ2+	Yes
12	Female	2	1/320	100	Marsh 3B	DQ2+	No
13	Male	3	1/160	51	Marsh 3B	DQ2+	No
14	Male	4	1/320	100	Marsh 3C	DQ2+	Yes
15	Female	4	1/320	100	Marsh 3C	DQ2+	No
16	Female	5	1/80	7.4	Marsh 3C	DQ2+	No
17	Male	6	1/160	39.8	Marsh 3A	DQ2+	No
18	Female	7	1/320	88.7	Marsh 3C	DQ2+	No
19	Female	7	1/320	100	Marsh 3C	DQ2+	No
20	Female	8	1/80	31.5	Marsh 3B	DQ2+	No
21	Female	8	1/160	24	Marsh 3B	DQ2+	Yes
22	Female	10	1/160	25.8	Marsh 3B	DQ2+	No
23	Female	10	1/160	100	Marsh 3C	DQ2+	Yes
24	Female	12	1/160	34.8	Marsh 3B	DQ2+	No
25	Female	13	1/80	46.6	Marsh 3A	DQ2+	No
26	Female	13	1/320	91.3	Marsh 3C	DQ2+	No

Supplementary Table S3. CD prevalence provided in 5-year age groups according to distribution of Catalan population excluding previously diagnosed CD cases

Age (Years)	N	CD Cases	Prevalence x 1000.00	CI (95%)	
2.5	219	4	18.26	5.00	46.10
7.5	280	3	10.71	2.22	30.99
12.5	278	1	3.60	0.09	19.88
17.5	204	1	4.90	0.12	27.01
22.5	289	4	13.84	3.78	35.06
27.5	364	1	2.75	0.07	15.21
32.5	347	0	0.00	0.00	10.57
37.5	332	2	6.02	0.73	21.59
42.5	301	1	3.32	0.08	18.37
47.5	269	0	0.00	0.00	13.62
52.5	252	0	0.00	0.00	14.53
57.5	237	0	0.00	0.00	15.44
62.5	172	0	0.00	0.00	21.22
67.5	195	0	0.00	0.00	18.74
72.5	180	0	0.00	0.00	20.29
77.5	144	0	0.00	0.00	25.29
82.5	129	1	7.75	0.20	42.43
87.5	35	0	0.00	0.00	100.03
TOTAL	4227	18	4.26	2.52	6.72

Chi-square test: p=0.0008218; CI: Confidence Interval.
Change in prevalence by increment in one year of age -4.28% (95% CI: -6.84%;-1.64%).

Supplementary Table S4. CD prevalence provided in 1-year age groups according to Catalan paediatric population distribution excluding previously diagnosed CD cases

Age (years)	N	CD Cases	Prevalence x 1000.00	CI 95%	
1	154	5	32.47	10.62	74.14
2	151	3	19.87	4.12	56.96
3	155	1	6.45	0.16	35.42
4	146	1	6.85	0.17	37.57
5	142	1	7.04	0.18	38.61
6	139	1	7.19	0.18	39.43
7	136	2	14.71	1.79	52.11
8	139	1	7.19	0.18	39.43
9	137	0	0.00	0.00	26.57
10	143	1	6.99	0.18	38.35
11	140	0	0.00	0.00	26.01
12	142	1	7.04	0.18	38.61
13	135	2	14.81	1.80	52.49
14	144	0	0.00	0.00	25.29
TOTAL	2003	19	9.48	5.72	14.77

Chi-square test: p=0.01936; CI: Confidence Interval.
Change in prevalence by increment in one year of age -13.19% (95% CI: -23.18%;-1.92%).