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**A UNIQUE PREVALENCE STUDY REFLECTING
THE POPULATION OF CATALONIA BY AGE AND
SEX SHOWED A MARKEDLY REDUCED
PREVALENCE OF COELIAC DISEASE IN
ADULTHOOD COMPARED TO CHILDHOOD**

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ORIGINAL MANUSCRIPT: A UNIQUE PREVALENCE STUDY REFLECTING THE POPULATION OF CATALONIA BY AGE AND SEX SHOWED A MARKEDLY REDUCED PREVALENCE OF COELIAC DISEASE IN ADULTHOOD COMPARED TO CHILDHOOD

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Running head: PREVALENCE OF COELIAC DISEASE IN CATALONIA

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Key words: Coeliac disease, anti-transglutaminase antibody, antiendomysial antibody, prevalence.

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ABSTRACT

AIM: To assess whether there are age and sex differences in coeliac disease (CD) prevalence.

METHODS: 4230 subjects were consecutively included (1 to \geq 80-years-old) reproducing the reference population by age and sex. Sample size was calculated assuming a population-based CD 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 antiendomysial antibodies were determined and duodenal biopsy was performed if positive. Log-linear models were fitted to CD prevalence by age allowing calculation of percentage change of prevalence. Differences between groups were compared using Chi-square test.

RESULTS: 21 subjects had CD (male/female 1:2.5). CD prevalence in total population was 1:204. CD 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 -5.04% (95%CI: -7.58 to -2.42%)]. In the paediatric expanded group (1-14 years), a decrease of CD prevalence was also observed [prevalence change: -16.09 %; (95%CI: -25.02 to -6.10)].

CONCLUSION: Prevalence of CD in childhood was 5 times higher than in adults. Whether this difference is due to environmental factors influencing infancy or latency evolution of CD in adulthood remains to be demonstrated in prospective longitudinal studies.

INTRODUCTION

A number of epidemiological studies using serological methods for coeliac disease (CD) detection have shown that CD is distributed world-wide. 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 sex of individuals included. In fact, the predominance of CD in females 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 to be long-lasting and it remained unnoticed and not adequately demonstrated. If confirmed, important questions could be raised such as environmental factors (life-style, infections) affecting the youngest cohorts or the possibility of frequent evolution towards latency in CD detected by mass-screening. The only way to unequivocally demonstrate the existence of sex- and age-related differences in CD prevalence is by performing a cross-sectional study in which the sample represents the structure of the reference population according to sex and age. And as far as we know, this methodological approach has not been used to date.

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3 The aim of the present study was to assess whether there is an age- and sex-related
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5 difference in the prevalence of CD in Catalonia (autonomous region in the northeast of
6
7 Spain).
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10 11 **MATERIAL AND METHODS**

12 13 **Subjects and study design**

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16 The inclusion period was divided into 2 phases. In the first one, from January 2004 to
17
18 December 2007, 4230 subjects from 1 to more than 80 years of age (2076 male; 2154
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20 female) were consecutively recruited in the participating centres. None of these
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22 subjects declined participation in the study. A large proportion of subjects in the
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24 middle age of life (from 20 to 55 years) were recruited in a workplace health
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26 surveillance department, whereas individuals in extreme ages of life were recruited in
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28 ambulatory minor surgery departments of the paediatric and general tertiary referral
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30 hospitals in the region. The predominant types of surgery in children were phimosis
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32 circumcision, adenoidectomy and ophthalmology surgery, while in adults they were
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34 cataract surgery, varicose vein surgery and arthroscopy. To avoid a bias in the
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36 inclusion, only those individuals coming from the catchment areas attended by the
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38 hospitals were included.
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47 The sample size was calculated assuming a CD prevalence of 1:250 ($\alpha=0.05$; $\delta=0.25$)
48
49 based on previous epidemiological studies performed in Spain with CD prevalence
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51 ranging from 1:118 to 1:389 [2,17,18]. Subject inclusion exactly reproduced the
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53 distribution of the population of Catalonia, regarding sex and age, in the year 2003
54
55 according to data from the Catalonia Statistics Institute (available at:
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3 <http://www.idescat.cat/territ/BasicTerr?TC=5&V0=3&V1=3&V3=669&V4=498&P=N&P>
4
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6 [ARENT=1&CTX=B&ALLINFO=TRUE&ANYS=2003&x=10&y=5](#). Subjects in the whole

7
8 sample were classified into 18 age-groups of 5 years each (from 1-4 to ≥ 85 years) for
9
10 each sex. The consecutive inclusion of subjects finished when the calculated number of
11
12 subjects was achieved in each age-sex group.
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14
15 In the second phase, from January 2006 to February 2007, the paediatric sample was
16
17 expanded since an interim analysis at the half point of the recruitment period
18
19 (December 2005) showed a high CD prevalence in children. The sample size was
20
21 recalculated based on 1:100 CD prevalence in subjects from 1 to 14 years of age
22
23 ($\alpha=0.05$; $\delta=0.25$). A total of 1230 additional children were recruited in the department
24
25 of ambulatory minor surgery of the paediatric hospital. Thus, the paediatric group
26
27 consisted finally of 2010 children (780 recruited in the first phase plus 1230 added in
28
29 the second phase; 1042 male, 968 female).
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35 All participants were asked about previous diagnosis of CD and about the possibility of
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37 intake of gluten-free diet. In affirmative cases, the CD diagnosis was carefully
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39 confirmed by reviewing the serology and duodenal histology at the time of diagnosis as
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41 well as the response to a gluten-free diet. This occurred in 3 of the 4230 cases of the
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43 whole population and in 7 cases of the expanded paediatric group.
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49 After written informed consent was obtained from all subjects of the whole population
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51 and expanded paediatric one, spare serum from the workplace health or preoperative
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53 profile was used for CD antibody detection (EmA and t-TGA. See below); this
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55 recruitment facilitated 100% acceptance of the serological analysis. When one or both
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57 serological markers were positive, the diagnostic work-up of CD (duodenal biopsy and
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1
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3 genetic study) was proposed. The duodenal biopsy was accepted in 90.5% and 94.7%
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5
6 of subjects of the whole and paediatric samples respectively.

7
8 The study protocol was approved by the ethics committees of the participating
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10 hospitals.
11

12 13 14 15 16 **Antibody detection**

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18 Serum IgA-EmA was determined by indirect immunofluorescence (IFI) assay in serum
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20 samples at 1/5 dilution, as previously described.[19] Commercial sections of monkey
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22 distal oesophagus (BioMedical Diagnostics, Marne-la-Vallée, France) were used as IFI
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24 substrate. IgA-class tTGA was analyzed in serum using a quantitative automated ELISA
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26 method by means of a commercially available detection kit (Varelisa Celikey™, Phadia
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28 AB, Freiburg, Germany) using recombinant human tTG as antigen.[20] As
29
30 recommended by the manufacturer, titers of EmA > 1/5 and t-TGA ≥ 8 U/mL were
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32 considered positive. Nevertheless, since > 98% of individuals had tTGA < 2 U/mL,
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34 subjects with values ≥ 2 U/mL these subjects were encouraged to adopt the same
35
36 diagnostic approach to CD as subjects with unequivocal positive serology. This strategy
37
38 was applied in order to identify the maximum range of the gluten sensitivity spectrum.
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40 Total serum IgA was measured using rate nephelometry [(BN II, Siemens Healthcare
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42 Diagnostics (Former Dade Behring)]. In cases of IgA deficiency, IgG-class EmA was
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44 measured.
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56 **Genetic markers**

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58 Standard techniques for DNA extraction, PCR amplification and product detection were
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60 used. To purify genomic DNA from whole blood, a commercial reagent Generation®

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3 Capture Column Kit (Gentra Systems. Minnesota, USA) was used. HLA-DQ2 (DQA1*0501
4 and DQB1*0201 alleles) and HLA-DQ8 (DQA1*0301 and DQB1*0302 alleles) genotyping
5 was performed by PCR amplification using sequence-specific primers (PCR-SSP) [21] on
6 a GeneAmp PCR 2400 System (Perkin Elmer). PCR products were detected by
7 electrophoresis on 2% agarose gel and were visualized under UV light. Analysis of HLA-
8 DQ8 haplotype was performed only on those patients with negative DQ2.
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21 **Duodenal biopsy and diagnostic criteria for coeliac disease**

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23 Four endoscopic biopsies from the 2nd-3rd portions of the duodenum in adults and
24 Watson-Crosby capsule biopsy in children were processed using haematoxylin/eosin
25 staining and CD3 immunophenotyping, and the biopsies were blindly evaluated by two
26 expert gastrointestinal pathologists (A.S. and V.C.). Histopathological findings were
27 staged according to the Marsh criteria,[22] as revised by Rostami et al [23]:
28 'infiltrative' lesions with intraepithelial lymphocytosis are defined as Marsh type I,
29 'infiltrative/hyperplastic' lesions are defined as Marsh II, and 'partial (A) subtotal (B)
30 and total (C) villous atrophy' as Marsh III. We assumed that intraepithelial
31 lymphocytosis was present when more than 25 IEL/100 epithelial cells were observed.
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48 A possible diagnosis of CD was considered when some degree of histological
49 abnormality of the gluten-sensitive enteropathy (GSE) spectrum was found. However,
50 since appropriate clinical, histological, and/or serological assessment after gluten-free
51 diet was not available for all patients with mild enteropathy, the diagnosis of CD was
52 considered to be sure in patients with atrophy and unequivocal positive serology
53 (Titters of EmA > 1/5 and / or t-TGA \geq 8 U/mL). Previously diagnosed CD cases and
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3 those identified in the study period by serology ($\text{EmA} > 1/5$ and/or $\text{tTGA} \geq 8$ IU mL)
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6 that had atrophy-proven biopsy were considered to be CD cases for the purpose of
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8 calculating CD prevalence.
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10 11 12 13 **Statistical analysis**

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15 CD prevalence rates were calculated by dividing the number of CD cases by the
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17 number of subjects recruited in each 5-year age-group and these rates were multiplied
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19 by 1000 subjects. Given that a preliminary statistical analysis in the whole sample
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21 demonstrated a significant decline in CD prevalence during the first 5 years of life,
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23 prevalence rates were computed for one-year age groups in the expanded paediatric
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25 sample.
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30 The 95% confidence intervals (95% CI) of prevalence rates could not be calculated
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32 assuming a normal distribution, as certain age groups showed no CD cases. Therefore,
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34 a binomial distribution [25] was assumed for the number of CD cases in order to
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36 compute the exact 95% CI for the prevalence rates as well as the percentage change
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38 (% Change) of prevalence by age group. The % Change was estimated by means of a
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40 generalized linear model [26] known as log-binomial model.[27,28] The
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42 appropriateness of the model was assumed if the ratio between the residual deviance
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44 and the residual degrees of freedom significantly departed from one another.[26] In
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46 this analysis, the age-group is the slope of this specific log-linear model where the
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48 outcome is the prevalence, and therefore, the age-group variable was considered to be
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50 a continuous one. In this model we should note that the median age for each age-
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52 group was used as age variable for the whole sample analysis, whereas the specific
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54 annual age group was used as age variable in the paediatric sample. Therefore, %
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3 Change of prevalence was obtained by subtracting 1 from the exponent of the slope of
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5 the fitted models and multiplying this quantity by 100.[29] These % Changes were
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7 considered statistically significant when 95% CI did not include the 0 value. Negative
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9 values of the % Change were interpreted as a decline in CD prevalence, whereas
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11 positive values showed a rise in CD prevalence. Differences between groups were
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13 compared using Chi-square tests.[30] A threshold of 0.05 was set for assuming
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15 statistical significance. All statistical analysis was performed using the R statistical
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17 package.[31]
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26 RESULTS

27 CD prevalence in the whole study sample (4230 subjects)

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29 Twenty-one of 4230 subjects had positive serology. Of these, 2 cases did not accept
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31 biopsy (a 1-year-old boy and a 28-year-old man with t-TGA values of 8.7 IU/mL and
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33 EmA 1/80, and 3.94 IU/mL and EmA 1/20, respectively), and one had normal duodenal
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35 histology (an 82-year-old man with confirmed positive serology in two separate
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37 samples, t-TGA 4 IU/mL and EmA 1/80). The remaining 18 cases showed villous
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39 atrophy at the duodenal biopsy. In addition, 3 more cases included in the prevalence
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41 study had previously been diagnosed with CD. Thus, the total number of CD patients
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43 included in the prevalence study was 21 (6 males, 15 females; male/female ratio 1:2.5)
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45 giving a CD prevalence of 4.97 per 1000 (95% CI: 3.08 – 7.58) and ratio of cases to non-
46
47 cases of 1:204. CD prevalence according to age-group and 95% CI is shown in Table 1.
48
49 CD prevalence was clearly higher in children (1 to 14 years) (14.1 per 1000; 95% CI: 7.0
50
51 – 25.1 or ratio 1:71) than in adults (2.8 per 1000; 95% CI: 1.4 – 5.3 or ratio 1:357).
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60 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: -5.04; -95%CI: -7.58 to -2.42) (Figure 1).

Table 1: CD prevalence in the whole study sample according to distribution of Catalan population

Age (years)	N	CD cases	Prevalence x 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

CD cases: CD cases detected in the study; N: Number of subjects in each age group.
Chi-Square test: p=0.00005; 95% CI=95% Confidence interval of CD prevalence.

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3 Age variable was also grouped as 0-14, 15-29, 30-44, 45-59, 60-74 and 75 years and
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5 older in order to assess whether the excess of zero cases among older age groups
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7 might affect the estimate of CD prevalence by age. This analysis showed that CD
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9 prevalence decreased by -4.78% (95% CI: -7.35%; -2.13%, see supplementary data-
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11 [supplemental Table 1 and supplemental Figure 1](#)). Therefore, both analyses led to the
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13 conclusion that CD prevalence decreased by -5% by year of age, independently of the
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15 age group definitions.
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21 In Table 2, the 21 CD cases with a degree of histological damage, serology and genetic
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23 studies are detailed. Two of the three CD cases detected before the present screening
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25 were diagnosed 1 year before inclusion, both due to classic malabsorption syndrome,
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27 and the remaining case was diagnosed 5 years earlier, since she belonged to a group at
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29 risk of CD (first-degree relative). In the 2 cases with negative genetic study a good
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31 clinical, serological and histological response confirmed the CD diagnoses.
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Table 2: Description of CD patients identified in whole study sample

Case	Sex	Age at CD diagnosis (years)	EmA (titters) [#]	t-TGA (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 - DQ8 - *	No
18	Female	36	1/20	5.76	Marsh 3A	DQ2 - DQ8 - **	No
19	Male	38	1/320	63.46	Marsh 3C	DQ2+	No
20	Female	44	1/320	62.88	Marsh 3 B	DQ2+	No
21	Male	82	1/80	12.25	Marsh 3A	DQ2+	No

* 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.

[#]Serological and histological characteristics at the time of diagnosis.

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 showed villous atrophy. Seven more cases 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 males, 19 females; male/female ratio 1:2.7), disclosing a 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: -16.09; 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 ($p=0.001465$).

Table 3: Paediatric CD prevalence according to Catalan paediatric population.

Age (years)	N	CD cases	Prevalence X 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

CD cases: CD cases detected in the study; N: Number of subjects by age.

Chi-Square test: $p=0.001465$; 95% CI= 95% Confidence interval of CD prevalence.

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 to 11 of Table 2 plus 15 more cases diagnosed in second phase of recruitment, included in a [supplemental Table 2](#)); all of them were DQ2 positive. Of the 7 CD cases diagnosed before screening, 2 had classic

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3 CD, 3 had atypical clinical presentations, and 2 showed silent disease (diagnosed as a
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5 result of disease-associated conditions).
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10 **Histology and genetics of individuals with border-line serology**

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12 Fifty-seven of the 4230 individuals had negative EmA and borderline tTGA values
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14 between 2-8 U/mL (13.4 per 1000; 1:74). Forty of them accepted further assessment
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16 with genetic study and duodenal biopsy, yielding a percentage of participation in the
17
18 CD diagnostic work-up of 70.1% individuals with border-line serology. The following
19
20 histological and genetic findings were found: 20 had duodenal lymphocytosis (Marsh 1)
21
22 [7 DQ2+ (35%); 6 DQB1*0201+ (30%); 2 DQA1*0501+ (10%); 2 DQ8+ (10%); 3 DQ2 and
23
24 DQ8 negative (15%)]; and the remaining 20 all showed normal biopsy (Marsh 0) [10
25
26 DQ2+ (50%); 6 DQB1*0201+ (30%); 3 DQ8+ (15%); 1 DQ2 and DQ8 negative (5%)]. In
27
28 Figure 3 the cases with borderline serology according to 5-year age groups are shown
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30 for patients with duodenal lymphocytosis and for cases with normal biopsy. Sex and
31
32 age distribution of these cases (18 male, 22 females, ratio: 1:1.2; mean age: 49, range:
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34 20-86) was completely different from that shown for patients with atrophy (mean age:
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36 18.4 years; range: 1 to 82). No cases with borderline serology were detected among
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38 individuals under the age of 20.
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50 **DISCUSSION**

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52 This is the first prevalence study of CD in which prevalence has been determined in a
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54 sample that exactly reproduces the same sex and age structure as the reference
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56 population. CD prevalence in the global population of the present study was in the
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58 range found in other studies (1:204), and a clear female predominance in CD was
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3 confirmed in all age groups. Nevertheless, a 5-fold increase in CD prevalence was
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5 found in the paediatric group as compared to the adult group (1:71 vs. 1:357). This was
6
7 an unexpected finding in a disease considered to be life-long and we propose some
8
9 possible explanations for this phenomenon. None of them can be demonstrated with
10
11 the present cross-sectional design but there are hypotheses that could be confirmed in
12
13 future longitudinal studies.
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18 A similar decrease in CD prevalence related to age was found in a Brazilian study which
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20 included individuals aged 1 to 60, although the authors did not assert that the sample
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22 matched the Brazilian population structure [15]. The authors suggested that CD
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24 prevalence declined with age, probably due to an increase in mortality associated with
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26 CD, and also partly attributable to the deficient health-care services in some regions of
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28 the country. However, the age-related decrease of CD prevalence detected in our
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30 study cannot be explained by a high mortality among CD patients. This assertion is
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32 supported by several observations: firstly, in contrast to the case of Brazil [15], life-
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34 expectancy in the Catalan population is one of the highest in the world, with universal
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36 health-care coverage; secondly, only a mild excess mortality risk related to CD has
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38 been reported worldwide [32,33], but this fact in itself would not explain the absence
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40 of CD in subjects born from 1925 to 1962; and thirdly, the expanded paediatric group
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42 of the present study demonstrated a dramatic decrease in CD prevalence beyond 3
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44 years. This drop in CD prevalence in this age group cannot be explained by Catalan
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46 childhood mortality, which is as low as 0.16 deaths per 1000 inhabitants from 1 to 4
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48 years of age, 0.08 per 1000 inhabitants from 5 to 9 years of age, and 0.14 per 1000
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50 inhabitants from 10 to 14 years of age [34]. In addition, in the data provided on the
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3 analysis of mortality in Catalonia [34] no deaths related to CD-associated conditions or
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5 CD co-morbidities were registered.
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8 Recent epidemiological studies performed in Finland have suggested that a substantial
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10 portion of CD patients are diagnosed after the age of 65 (21.3 x 1000 inhabitants). In
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12 our study we found a slight increase in CD prevalence in individuals older than 80,
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14 probably reflecting the same phenomenon [35].
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18 It could be argued that the existence of certain environmental factors such as viral
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20 infections and changes in feeding policies (recommendations in breast feeding, or the
21
22 time of gluten introduction) are possible explanations for the high CD prevalence in
23
24 early infancy. However, we have not been able to identify any effect related to these
25
26 triggering factors on the appearance of a possible CD epidemic mainly among children
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28 under 3 years of age. On the contrary, breast-feeding, a factor considered to be
29
30 protective for CD development [36], has increased in Catalonia both in frequency and
31
32 duration. Data provided by the Government of Catalonia at the website
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34 <http://www.gencat.cat/generalitat/cas/govern/infocatalunya/> show a breast-feeding
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36 increase from 39.2% in 1989 to 61.9% in 2005 at 3 months of age, and from 6.3% in
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38 1989 to 31.3% in 2005 at 6 months of age.
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46 Another proposed triggering factor for CD is the early introduction of dietary gluten.
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48 But in contrast to what might be expected, the cohorts of individuals born before the
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50 ESPGAN recommendations introduced during the 1980s [37], among whom dietary
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52 gluten had been introduced very early and abruptly, showed the lowest CD prevalence.
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55 The different sources of recruitment for the study (minor surgery units and a
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57 workplace health surveillance department) might theoretically have determined
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59 differences in CD prevalence. However, particular attention was paid to avoiding
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3 inclusion bias in the participating centres, thereby minimizing this limitation. This was
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5 achieved mainly by including consecutive cases operated on due to very frequent age-
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7 related minor conditions in the minor surgery units (cataract surgery, arthroscopy,
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9 etc.). These conditions affect a vast majority of subjects of the general population at
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11 some time in life. In addition, the decreasing CD prevalence in older subjects was
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13 unrelated to the site of recruitment, allowing us the inclusion of individuals of the
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15 entire age groups. This is in contrast to how the majority of previously published
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17 prevalence studies were performed, which generally included only individuals of
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19 certain periods of life or from specific contexts (adulthood, school children, blood
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21 donors, etc.).[5,6]
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28 Though we cannot rule out the existence of viral infections or changes in dietary habits
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30 acting as a cohort effect, increasing the prevalence in the youngest children, an
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32 alternative explanation for the age-related differences in CD prevalence found in the
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34 present study is a possible evolution towards latency or tolerance of a high proportion
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36 of CD lesions, mainly those appearing in early childhood and detected by screening.
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38 This hypothesis is further supported by some evidence found in the literature.[38, 39]
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40 It has been reported that up to 20% of children diagnosed with atrophy in infancy
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42 maintain a preserved villous architecture more than 10 years after gluten challenge,
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44 with this being more frequent in those children diagnosed before the age of 3 [38], and
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46 it has also been reported that CD patients diagnosed in adulthood exhibit an
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48 attenuated clinical, serological, and histological picture compared to those diagnosed
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50 in infancy.[39] In fact, in the present study a similar trend was observed in adult CD
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52 patients, who showed lower values of tTGA with attenuated duodenal lesion as
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54 compared to coeliac children. Moreover, in an epidemiological study performed in
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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 the present 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 sex 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 the great majority of individuals with borderline serology had probably never had a previous CD with atrophy which 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

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3 individuals with a certain genetic predisposition (positive DQ2 or DQB1*0201) may
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6 have an attenuated form of gluten sensitivity that might remain throughout life.
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9 In conclusion, the observed decreasing CD prevalence in older generations, which is
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11 particularly striking beyond 3 years of life, suggests that the CD evolution towards
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13 latency may be more frequent than previously thought, particularly in CD patients
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15 detected by screening. This hypothesis needs to be confirmed in future longitudinal
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17 studies to better define the natural history of CD, nor can it be used at present as an
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19 argument for stopping GFD in patients with a consistent diagnosis of CD.
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3 **Figure Legends**
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8 Figure 1: Decreased CD prevalence in adulthood compared to childhood.
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11 Figure 2: Decreased CD prevalence in older children.
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14 Figure 3-a: Sex and age distribution of CD cases with borderline serology (n= 57).
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17 Figure 3-b: Histological findings related to CD cases with borderline serology, by age-groups
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19 (n=40).
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For Peer Review

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CONFLICT OF INTEREST/STUDY SUPPORT

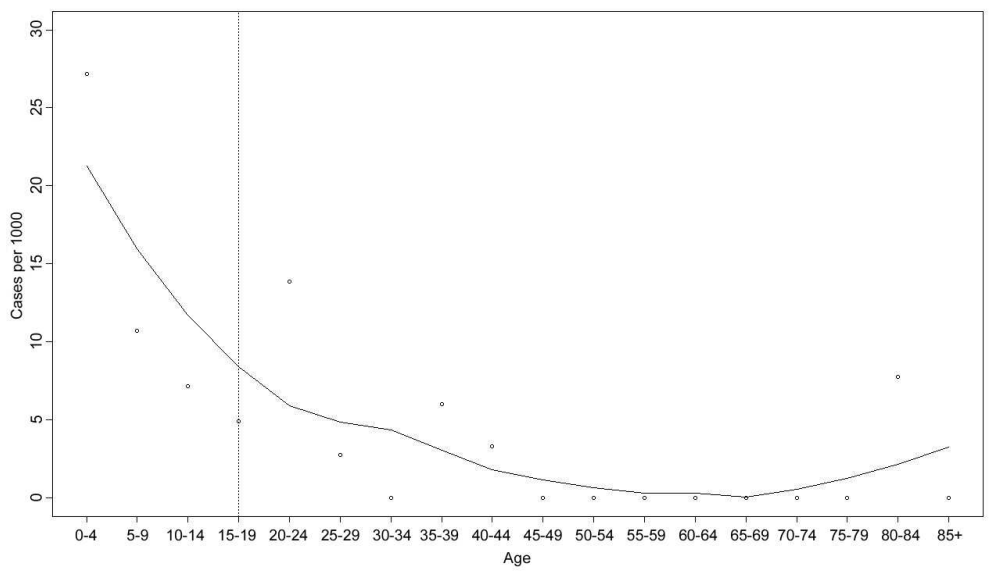
Guarantor of the article: Maria Esteve, MD, PhD.

Specific author contributions: Study concept and design: Meritxell Mariné, Maria Esteve, Carme Farré, Fernando Fernández-Bañares, Josep M Viver. Acquisition of data: Meritxell Mariné, Carme Farré, Montserrat Alsina, Pere Vilar, Montserrat Cortijo, Antonio Salas, Fernando Fernández-Bañares, Mercè Rosinach, Rebeca Santaolalla, Carme Loras, Teresa Marquès, Victòria Cusí, Maribel Hernández, Anna Carrasco, Josep M Viver, Maria Esteve. Analysis and interpretation of data: Carme Loras, Maria Esteve, Fernando Fernández-Bañares, Josepa Ribes. Manuscript writing: Carme Loras, Maria Esteve, Fernando Fernández-Bañares, Josep M Viver, Josepa Ribes. Critical revision of the manuscript for important intellectual content: Meritxell Mariné, Carme Farré, Montserrat Alsina, Pere Vilar, Montserrat Cortijo, Antonio Salas, Fernando Fernández-Bañares, Josepa Ribes, Mercè Rosinach, Rebeca Santaolalla, Carme Loras, Teresa Marquès, Victòria Cusí, Maribel Hernández, Anna Carrasco, Josep M Viver, Maria Esteve. Statistical analysis: Maria Esteve, Meritxell Mariné, Fernando Fernández-Bañares, Carme Farré, Josepa Ribes. Study supervision: Maria Esteve, Fernando Fernández-Bañares, Carme Farré, Meritxell Mariné. Study coordination: Meritxell Mariné, Maria Esteve, Carme Farré.

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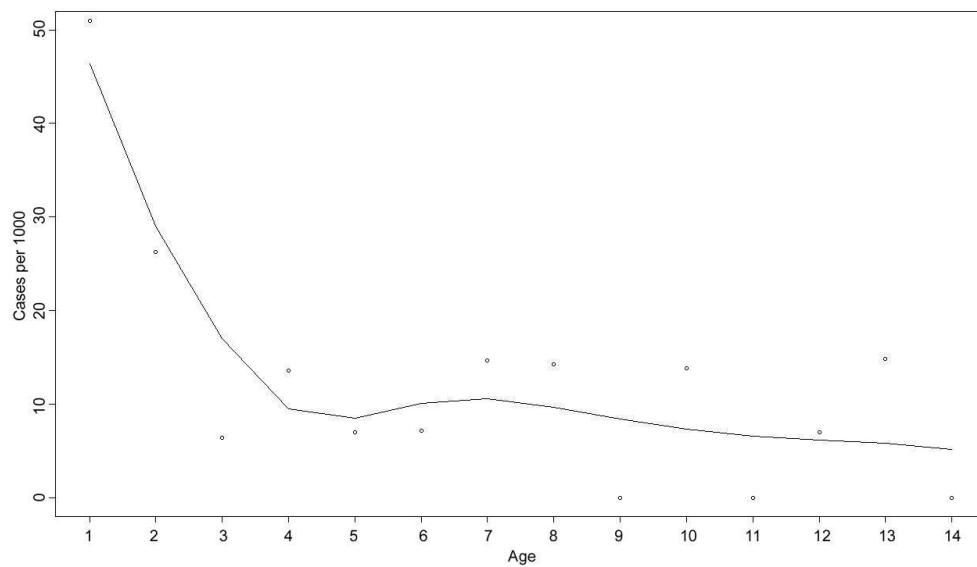
Potential competing interests: None.

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Decreased CD prevalence in adulthood compared to childhood
451x275mm (72 x 72 DPI)

Peer Review



Decreased CD prevalence in older children
451x275mm (72 x 72 DPI)

Peer Review

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Figure 3-A

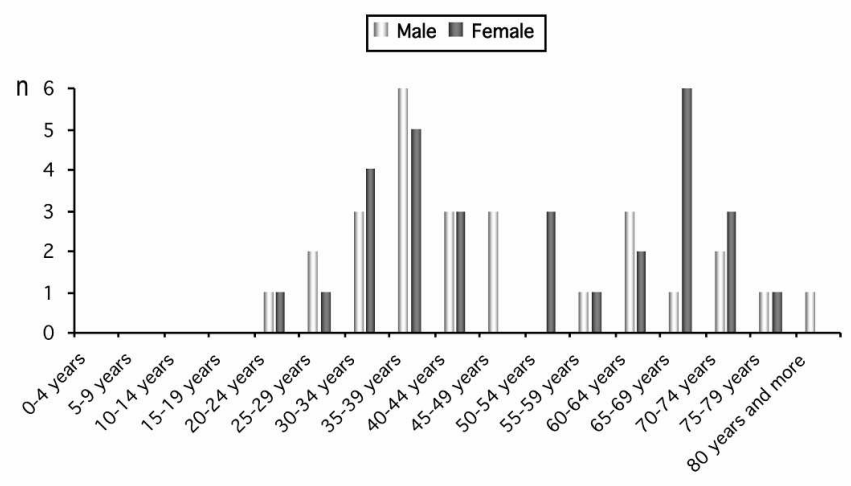


Figure 3-B

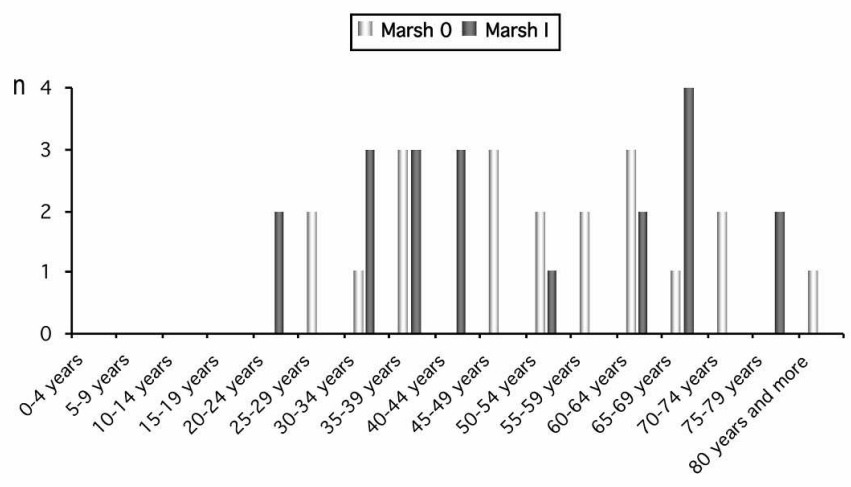


Figure 3-a: Sex and age distribution of CD cases with borderline serology (n= 57).
Figure 3-b: Histological findings related to CD cases with borderline serology, by age-groups (n=40).

46x63mm (600 x 600 DPI)

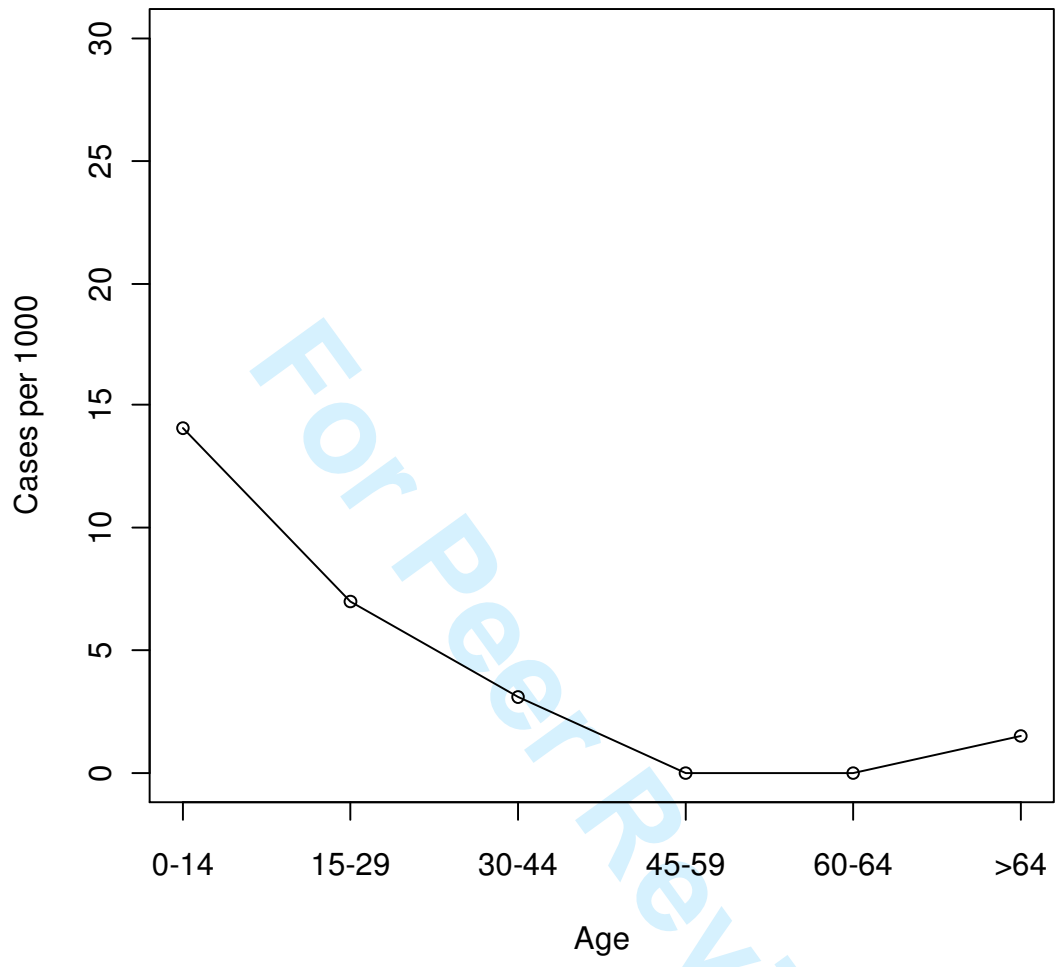
Supplemental Table 1. CD prevalence provided in age groups of 14 years.

Age (years)	N	CD Prevalence X		
		cases	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

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Supplemental Figure 1. Evolution of prevalence by age in age groups of 14 years



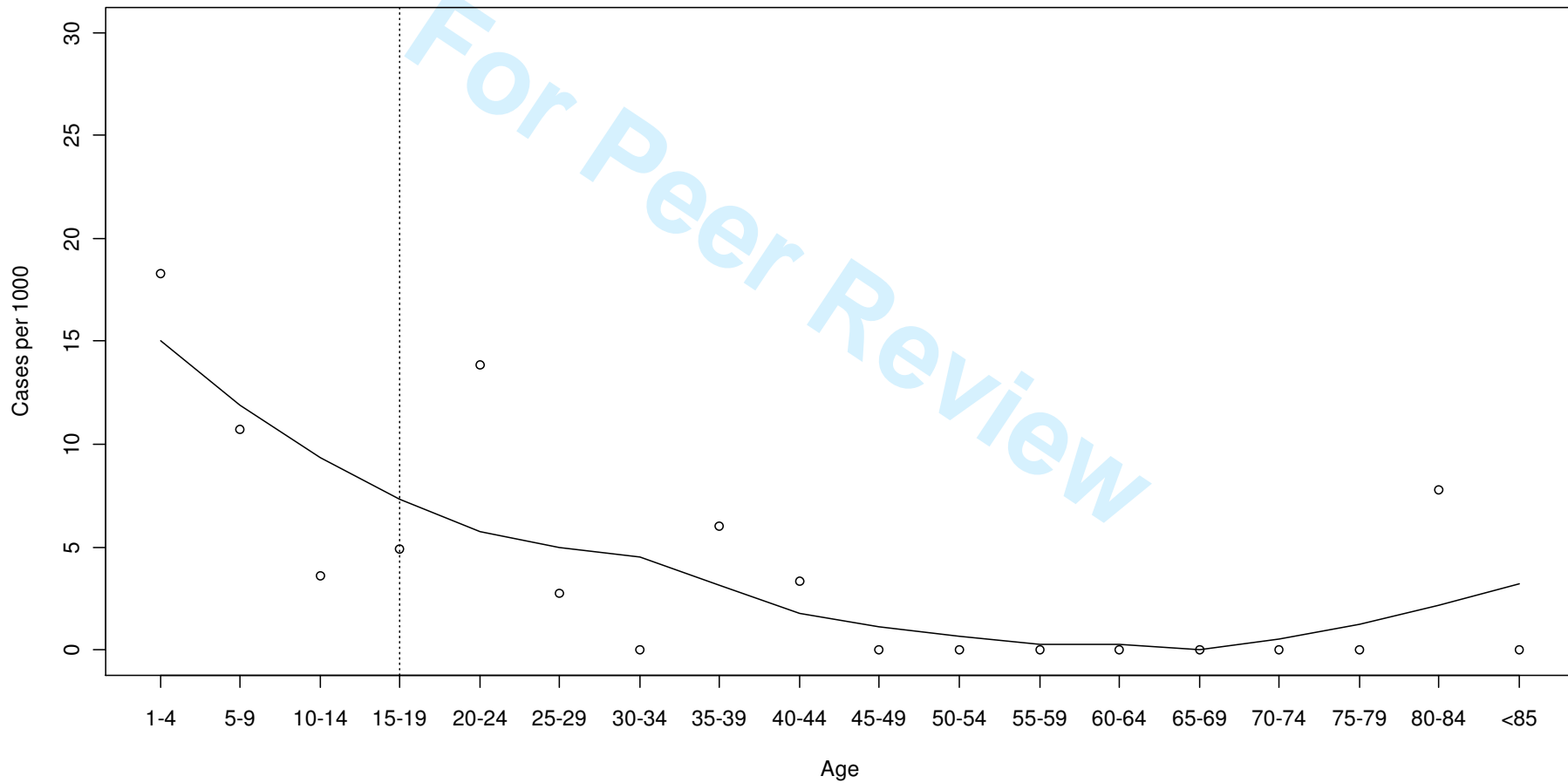
Percent change of prevalence by age -4.78% (95% CI: -7.36; -2.14)

Supplemental Table 2. 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

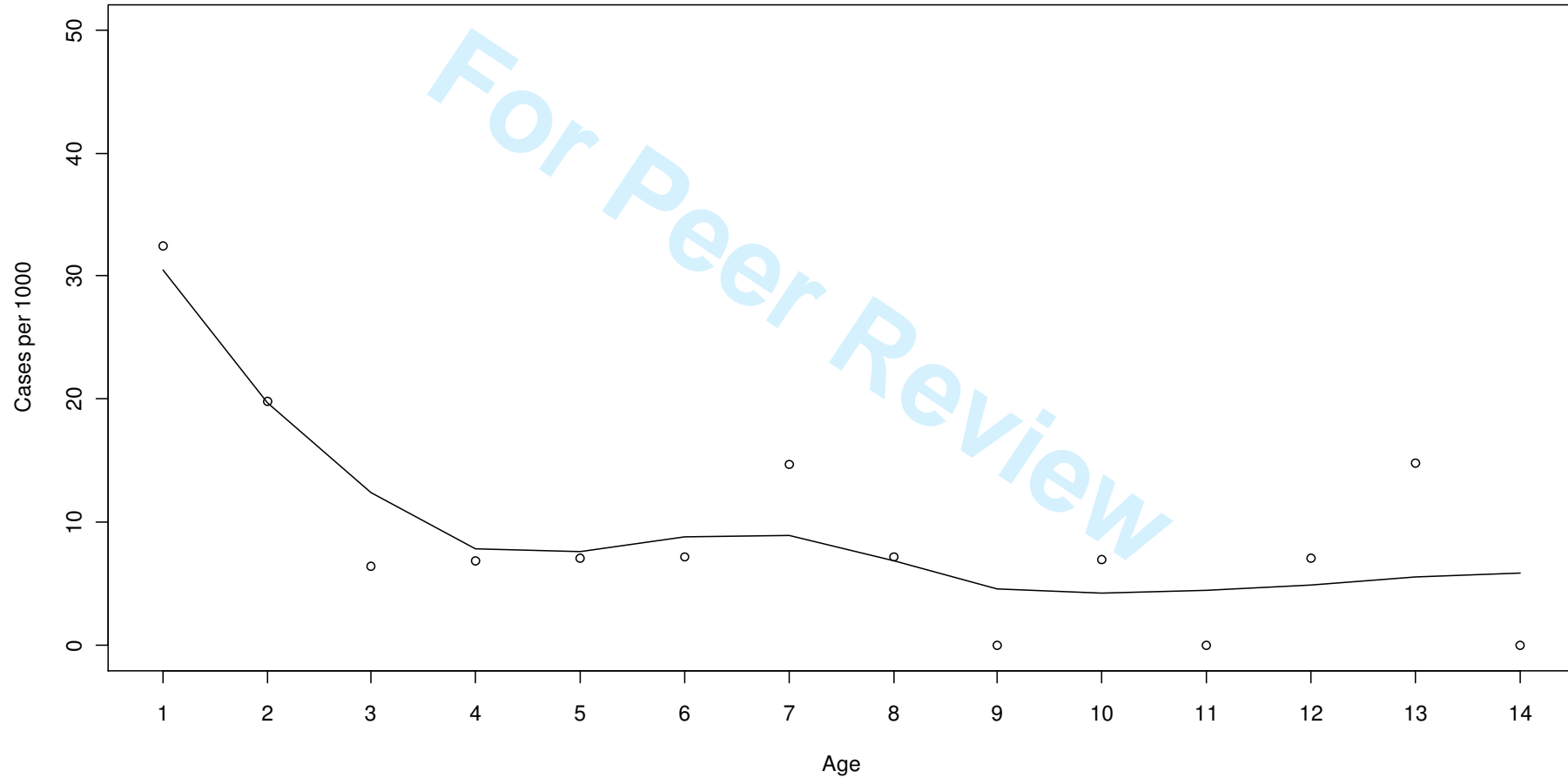
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Supplemental Figure 2. Decreased CD prevalence related to older age excluding previously diagnosed CD cases (cases already known to have celiac disease at the time of recruitment). This Figure corresponds to data available in Supplemental Table 3.



For Peer Review

Supplemental Figure 3. 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 Supplemental Table 4.



Supplemental Table 3. 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%).

Supplemental Table 4. 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%).

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 $Y_i \sim \text{Binomial}(N_i, p_i)$, 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)

```
1
2
3
4
5
6
7 age.cat<-function(x)
8 {
9   if (x<=4) y<-2.5
10  if ((x>4)&&(x<=9)) y<-7.5
11  if ((x>9)&&(x<=14)) y<-12.5
12  if ((x>14)&&(x<=19)) y<-17.5
13  if ((x>19)&&(x<=24)) y<-22.5
14  if ((x>24)&&(x<=29)) y<-27.5
15  if ((x>29)&&(x<=34)) y<-32.5
16  if ((x>34)&&(x<=39)) y<-37.5
17  if ((x>39)&&(x<=44)) y<-42.5
18  if ((x>44)&&(x<=49)) y<-47.5
19  if ((x>49)&&(x<=54)) y<-52.5
20  if ((x>54)&&(x<=59)) y<-57.5
21  if ((x>59)&&(x<=64)) y<-62.5
22  if ((x>64)&&(x<=69)) y<-67.5
23  if ((x>69)&&(x<=74)) y<-72.5
24  if ((x>74)&&(x<=79)) y<-77.5
25  if ((x>79)&&(x<=84)) y<-82.5
26  if (x>84) y<-87.5
27  y
28 }
29
30 age.6<-function(x)
31 {
32   if ((x>=0)&&(x<=14)) y<-1
33   if ((x>14)&&(x<=29)) y<-2
34   if ((x>29)&&(x<=44)) y<-3
35   if ((x>44)&&(x<=59)) y<-4
36   if ((x>59)&&(x<=64)) y<-5
37   if ((x>64)) y<-6
38   y
39 }
40
41 ##### Read Data
42 ## Directory: Working directory where files should be
43 ## fitxer: file with individual data
44 # Note that the process transforms individual data into groups
45 according to age-groups defined
46 ## in functions age.cat and age.6
47
48 library(epitools)
49 directori<-"C:/2010 - Celiacs Cohort/Definitiva/"
50 fitxer<-"CeliacsR.txt"
51 BD<-as.data.frame(read.table(paste(directori,fitxer,sep=""),header=T))
52 summary(BD)
53 BD$ED<-as.numeric(lapply(as.numeric(BD$Edat),age.cat))
54 BD$ED6<-as.numeric(lapply(as.numeric(BD$Edat),age.6))
55 # Note: in the original dataset Edat is Age variable
56
57 ##### Analysis 6 Age groups: 0-14, 15-29,30-44,45-59,60-74,75+
58
59 out.matrix.6<-as.data.frame(matrix(0,6,7))
60 names(out.matrix.6)<-
  c("Age","N","NCel","Prev","Prev1000","LIPrev","LSPrev")
  out.matrix.6$Age<-seq(7,85,15)
```

```

1
2
3 out.matrix.6$N<-as.numeric(table(BD$ED6))
4 out.matrix.6$NCel<-as.numeric((table(BD$ED6,BD$celiac))[,2])
5 out.matrix.6$Prev<-out.matrix.6$NCel/out.matrix.6$N
6 out.matrix.6$Prev1000<-(out.matrix.6$NCel/out.matrix.6$N)*1000
7 for (i in 1:6)
8 {
9 prev.tmp<-binom.exact(out.matrix.6$NCel[i],out.matrix.6$N[i])
10 out.matrix.6$Prev[i]<-prev.tmp$proportion
11 out.matrix.6$Prev1000[i]<-prev.tmp$proportion*1000
12 out.matrix.6$LIPrev[i]<-prev.tmp$lower*1000
13 out.matrix.6$LSPrev[i]<-prev.tmp$upper*1000
14 }
15
16 plot(out.matrix.6$Age,out.matrix.6$Prev1000,ylim=c(0,30),ylab="Cases
17 per 1000",xlab="Age",xaxt="n")
18 model.loess<-loess(out.matrix.6$Prev1000~out.matrix.6$Age)
19 model.pred<-abs(predict(model.loess))
20 lines(out.matrix.6$Age,model.pred)
21 age.grp<-c("0-14","15-29","30-44","45-59","60-64",>64")
22 axis(side=1,at=out.matrix.6$Age,labels=age.grp)
23
24
25 ### Log-Binomial model
26 ### In this model Age refers to Age
27
28 model.bin.b<-glm(cbind(NCel,N-
29 NCel)~Age,family=binomial(link="log"),data=out.matrix.6)
30 model.bin<-summary(glm(cbind(NCel,N-
31 NCel)~Age,family=binomial(link="log"),data=out.matrix.6))
32 lmodel.bin.li<-model.bin$coef[2,1]-1.96*model.bin$coef[2,2]
33 lmodel.bin.ls<-model.bin$coef[2,1]+1.96*model.bin$coef[2,2]
34 lmodel.bin.med<-model.bin$coef[2,1]
35 model.bin.li<-(exp(lmodel.bin.li)-1)*100
36 model.bin.ls<-(exp(lmodel.bin.ls)-1)*100
37 model.bin.mean<-(exp(lmodel.bin.med)-1)*100
38
39 ##### Note that model.bin.li is the lower limit of the percentage
40 change of prevalence confidence interval,
41 ##### model.bin.ls is the upper limit and model.bin.med is the mean
42 value
43
44 print(paste("Percent Change of PRevalence:",round(model.bin.mean,2),"
45 95% CI(",round(model.bin.li,2),";",round(model.bin.ls,2),")")
46
47 #####
48 ### Trend test of prevalence: Another look at the significance to the
49 percent change of prevalence
50 prop.trend.test(out.matrix.6$NCel,out.matrix.6$N)
51
52 ##### Analysis 5-year Age groups
53
54 out.matrix<-as.data.frame(matrix(0,18,7))
55 names(out.matrix)<-
56 c("Age","N","NCel","Prev","Prev1000","LIPrev","LSPrev")
57 out.matrix$Age<-seq(2.5,87.5,5)
58 out.matrix$N<-as.numeric(table(BD$ED))
59 out.matrix$NCel<-as.numeric((table(BD$ED,BD$celiac))[,2])
60 out.matrix$Prev<-out.matrix$NCel/out.matrix$N
out.matrix$Prev1000<-(out.matrix$NCel/out.matrix$N)*1000
for (i in 1:18)

```

```
1
2
3 {
4 prev.tmp<-binom.exact(out.matrix$NCel[i],out.matrix$N[i])
5 out.matrix$Prev[i]<-prev.tmp$proportion
6 out.matrix$Prev1000[i]<-prev.tmp$proportion*1000
7 out.matrix$LIPrev[i]<-prev.tmp$lower*1000
8 out.matrix$LSPrev[i]<-prev.tmp$upper*1000
9 }
10
11 #Global prevalence and its 95% Confidence interval: 21 CD among 4230
12 individuals
13
14 binom.exact(21,4230)
15
16 plot(out.matrix$Age,out.matrix$Prev1000,ylim=c(0,30),ylab="Cases per
17 1000",xlab="Age",xaxt="n")
18 model.loess<-loess(out.matrix$Prev1000~out.matrix$Age)
19 model.pred<-abs(predict(model.loess))
20 lines(out.matrix$Age,model.pred)
21 age.grp<-c("0-4","5-9","10-14","15-19","20-24","25-29","30-34","35-
22 39","40-44","45-49","50-54","55-59","60-64","65-69","70-74","75-
23 79","80-84","85+")
24 axis(side=1,at=out.matrix$Age,labels=age.grp)
25 abline(v=17.5,lty=3)
26
27 ### Log-Binomial model
28 ### In this model Age refers to Age
29
30 model.bin.b<-glm(cbind(NCel,N-
31 NCel)~Age,family=binomial(link="log"),data=out.matrix)
32 model.bin<-summary(glm(cbind(NCel,N-
33 NCel)~Age,family=binomial(link="log"),data=out.matrix))
34 lmodel.bin.li<-model.bin$coef[2,1]-1.96*model.bin$coef[2,2]
35 lmodel.bin.ls<-model.bin$coef[2,1]+1.96*model.bin$coef[2,2]
36 lmodel.bin.med<-model.bin$coef[2,1]
37 model.bin.li<-(exp(lmodel.bin.li)-1)*100
38 model.bin.ls<-(exp(lmodel.bin.ls)-1)*100
39 model.bin.mean<-(exp(lmodel.bin.med)-1)*100
40
41
42 ##### Note that model.bin.li is the lower limit of the percentage
43 change of prevalence confidence interval,
44 ##### model.bin.ls is the upper limit and model.bin.med is the mean
45 value
46
47 print(paste("Percent Change of PRevalence:",round(model.bin.mean,2),"
48 95% CI(",round(model.bin.li,2),";",round(model.bin.ls,2),")"))
49
50 #####
51 ### Trend test of prevalence: Another look at the significance to the
52 percent change of prevalence
53 prop.trend.test(out.matrix$NCel,out.matrix$N)
54
55
56 ##### PAEDIATRIC #####
57
58 library(epitools)
59 directori<-"C:/2010 - Celiacs Cohort/Definitiva/"
60 fitxer<-"PediatricR.txt"
```

```

1
2
3   BD.Nen<-
4   as.data.frame(read.table(paste(directory,fitxer,sep=""),header=T))
5   BD.Nen$ED3<-as.numeric(lapply(as.numeric(BD.Nen$Age),age.3.Nen))
6   summary(BD.Nen)
7   out.matrix.Nen<-as.data.frame(matrix(0,14,7))
8   names(out.matrix.Nen)<-
9   c("Age","N","NCel","Prev","Prev1000","LIPrev","LSPrev")
10  out.matrix.Nen$Age<-1:14
11  out.matrix.Nen$N<-as.numeric(table(BD.Nen$Edat))
12  out.matrix.Nen$NCel<-
13  as.numeric((table(BD.Nen$Edat,BD.Nen$celiac))[,2])
14  out.matrix.Nen$Prev<-out.matrix.Nen$NCel/out.matrix.Nen$N
15  out.matrix.Nen$Prev1000<-(out.matrix.Nen$NCel/out.matrix.Nen$N)*1000
16  for (i in 1:14)
17  {
18  prev.tmp<-binom.exact(out.matrix.Nen$NCel[i],out.matrix.Nen$N[i])
19  out.matrix.Nen$Prev[i]<-prev.tmp$proportion
20  out.matrix.Nen$Prev1000[i]<-prev.tmp$proportion*1000
21  out.matrix.Nen$LIPrev[i]<-prev.tmp$lower*1000
22  out.matrix.Nen$LSPrev[i]<-prev.tmp$upper*1000
23  }
24
25  ### GLOBAL Prevalence
26  binom.exact(25,2010)
27
28  plot(out.matrix.Nen$Age,out.matrix.Nen$Prev1000,ylim=c(0,50),ylab="Cases
29  per 1000",xlab="Age",xaxt="n")
30  model.loess<-loess(out.matrix.Nen$Prev1000~out.matrix.Nen$Age)
31  model.pred<-abs(predict(model.loess))
32  lines(out.matrix.Nen$Age,model.pred)
33  ed.grp<-c(1:14)
34  axis(side=1,at=out.matrix.Nen$Age,labels=ed.grp)
35
36  ### Log-Binomial model
37  ### In this model Age refers to Age
38
39  model.bin.Nen<-summary(glm(cbind(NCel, N-
40  NCel)~Age,family=binomial(link="log"),data=out.matrix.Nen))
41  lmodel.bin.Nen.li<-model.bin.Nen$coef[2,1]-
42  1.96*model.bin.Nen$coef[2,2]
43  lmodel.bin.Nen.ls<-
44  model.bin.Nen$coef[2,1]+1.96*model.bin.Nen$coef[2,2]
45  lmodel.bin.Nen.med<-model.bin.Nen$coef[2,1]
46  model.bin.Nen.li<-(exp(lmodel.bin.Nen.li)-1)*100
47  model.bin.Nen.ls<-(exp(lmodel.bin.Nen.ls)-1)*100
48  model.bin.mean.Nen<-(exp(lmodel.bin.Nen.med)-1)*100
49
50  ##### Note that model.bin.li is the lower limit of the percentage
51  change of prevalence confidence interval,
52  ##### model.bin.ls is the upper limit and model.bin.med is the mean
53  value
54
55  print(paste("Percent Change of
56  Prevalence:",round(model.bin.mean.Nen,2)," 95%
57  CI(",round(model.bin.Nen.li,2),";",round(model.bin.Nen.ls,2),")")
58
59  #####
60  ### Trend test of prevalence: Another look at the significance to the
    percent change of prevalence
    prop.trend.test(out.matrix.Nen$NCel,out.matrix.Nen$N)

```

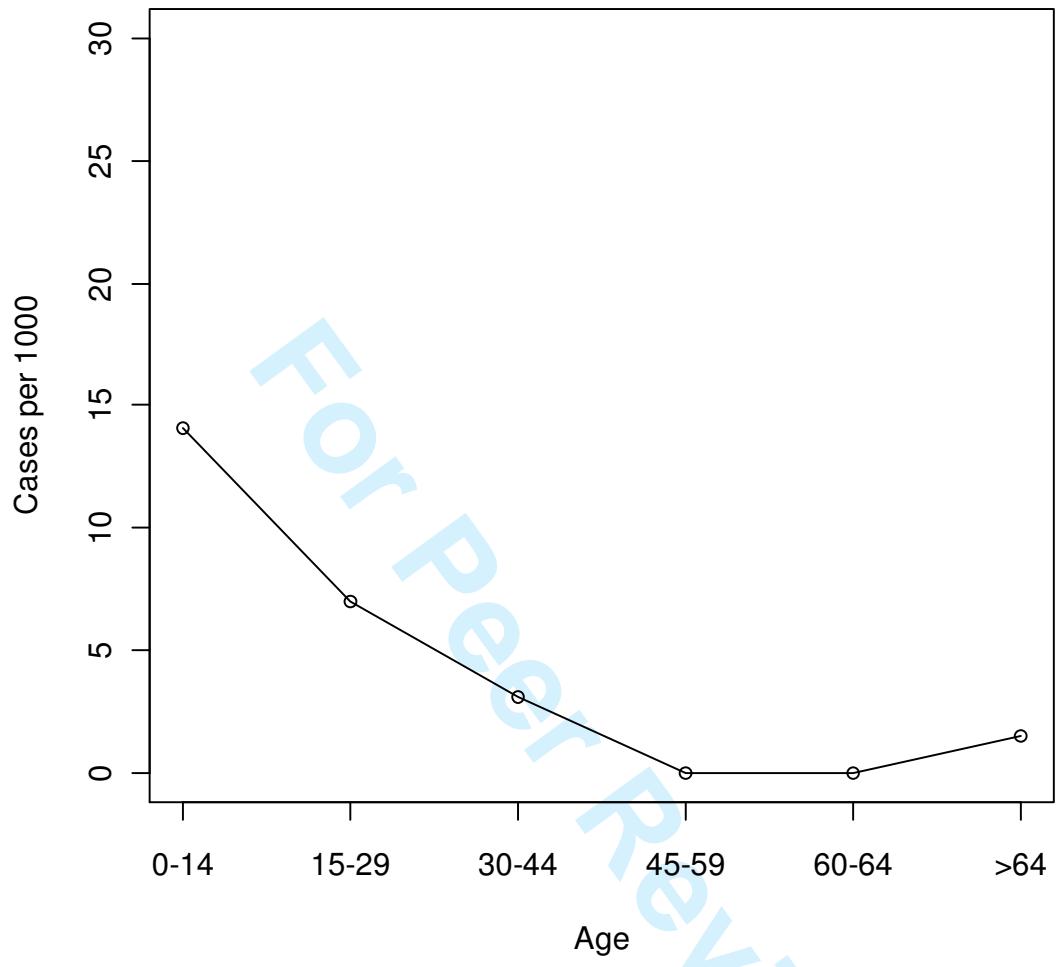
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Chi-Square test: $p=0.001243$; CI= Confidence interval

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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)