Early diagnosis and management of celiac disease in childhood

Meijer, C.R.

5

Prediction models for celiac disease development in children from high-risk families: Data from the PreventCD cohort

Gastroenterology 2022 Aug;163(2):426-436

Caroline R. Meijer*
Renata Auricchio*
Hein Putter
Gemma Castillejo
Paula Crespo
Judit Gyimesi
Corina Hartman
Sanja Kolacek
Sibylle Koletzko
Ilma R. Korponay-Szabo
Eva Martinez Ojinaga
Isabel Polanco
Carmen Ribes-Koninckx
Raanan Shamir
Hania Szajewska
Riccardo Troncone
Vincenzo Villanacci
Katharina Werkstetter
M. Luisa Mearin
*shared first authorship

Supplementary Appendix is available on 1-s2.0-S0016508522004425-mmc1.pdf (els-cdn.com)

ABSTRACT

BACKGROUND AND AIMS: Screening for celiac disease (CD) is recommended in children with affected first-degree relatives (FDR). However, the frequency of screening and at what age remain unknown. Aims: to detect variables influencing the risk of CD-development and develop and validate clinical prediction models to provide individualized screening advice.

METHODS: Analysis of prospective data from the ten years follow-up of the PreventCD-birth cohort involving 944 genetically predisposed children with CD-FDR. Variables significantly influencing the CD-risk were combined to determine a risk score. Landmark analyses were performed at different ages. Prediction models were created by multivariable Cox proportional hazards regression analyses, backward elimination and Harrell's c-index for discrimination. Validation was done using data from the independent NeoCel cohort.

RESULTS: In March 2019, the median follow-up was 8.3 years (22 days-12.0 years); 135/944 children developed CD (mean age 4.3 years (1.1-11.4). CD developed significantly more often in girls (p=0.005) and in HLA-DQ2 homozygous individuals (8-year cumulative incidence 35.4% versus maximum of the other HLA-risk groups 18.2% [p<0.001]). The effect of homozygosity DR3-DQ2/DR7-DQ2 on CD-developing was only present in girls (interaction p=0.04). The prediction models showed good fitting in the validation cohort (Cox regression 0.81(0.54)). To calculate a personalized risk of CD-development and provide screening advice, we designed the Prediction application https://hputter.shinyapps.io/preventcd/.

CONCLUSION: Children with CD-FDR develop CD early in life, and their risk depends on gender, age and HLA-DQ: all factors which are important for a sound screening advice. These children should be screened early in life, including HLA-DQ2/8-typing, and if genetically predisposed to CD, should get further personalized screening advice using our Prediction app.

INTRODUCTION

Celiac disease (CD) is a common autoimmune disorder caused by the ingestion of gluten in genetically susceptible individuals. It is characterized by CD-specific antibodies and HLA-DQ2 and/or HLA-DQ8 haplotypes.[1] CD affects as many as 1-3 % of the general population.[2,3] Among first-degree relatives (FDR) of CD patients, the disease prevalence is much higher, being approximately 10-20% depending on the HLA-DQ and gender.[4-6] This has been prospectively evaluated among others in the PreventCD cohort, consisting of 944 children with at least one FDR with CD and HLA-DQ2 and/or HLA-DQ8. The children were enrolled at birth between 2007 and 2010 in Croatia, Germany, Hungary, Israel, Italy, the Netherlands, Poland and Spain. Initially, a randomized, double-blind, placebo-controlled dietary intervention was performed and the results, published in 2014 in the New England Journal of Medicine, showed that the early introduction of small quantities of gluten and/or breastfeeding did not reduce the risk of CD at three years of age.[5] The data of the follow-up of the PreventCD cohort at the mean age of ten years offers a unique opportunity to study the natural development of CD in children from high-risk families. The aims of this study were, (i) to detect variables that influence the age-dependent risk of CD development in children with affected FDR, and (ii) to build clinically applicable prediction models for CD development among these children to allow for personalized advice for their CD screening.

MATERIALS AND METHODS

PREVENTCD-COHORT

CD diagnosis

Data was frozen on 29th March 2019. All children were assessed regularly from birth onwards for CD development at pre-defined intervals, including seven times during the first three years of age and thereafter annually or at least once between March 2016 and March 2019.[5] We monitored parent-reported health status, weight and height, gluten consumption (up until the age of three years, quantified using standardized questionnaires) and serum IgA against anti-transglutaminase (TGA) (Supplementary Appendix).

(Parents of) children with elevated TGA and/or CD symptoms suggestive of CD, were offered small bowel biopsies to confirm the diagnosis. The date of CD diagnosis was defined as the date of small bowel biopsy or as the date on which TGA were highest. Given that TGA were determined at variable intervals starting from 3 years of age, we considered the age of CD development to be midway between the age at which the last negative TGA was determined and the date of CD diagnosis.

The study was approved by all medical ethics committees of the participating centers. All the authors had access to the study data and had reviewed and approved the final manuscript.

Statistical methods

The statistical analysis plan (SAP) was published online on 29 March 2019 before the analyses were performed using R version 3.6.1 (Supplement 2, pages 83-90 and https://www.preventcd.com/images/stories/Downloads/2019-0402%20Statistical%20Analysis%20Plan_PreventCD_final.pdf). In case a child was lost to follow-up, the child was treated as censored on the date of last visit/TGA determination. For univariate comparison of cumulative incidences of CD between groups, the log-rank test (two-sided) was used.

Prediction models

To develop the models, all the factors that significantly influenced the risk of CD development were combined into a risk score.

Baseline model

Multivariable Cox proportional hazards regression analysis of the baseline was performed in two steps. In the first step, three primary variables already known at the child's birth (gender, HLA-risk group, number of affected FDR, table 1) were entered into the model, irrespective of statistical significance. In accordance with our previous publication, we analyzed the risk for CD in five groups according to HLA-DQ genotype (see Supplementary Appendix).[5] In addition, we also exploratively analyzed the risk for CD in children with DR3-DQ2/DR3-DQ2 separately from those with DR3-DQ2/DR7-DQ2, as the affinity of gluten peptides is higher for DR3-DQ2 than for DR7-DQ2 receptors.[7,8] Because of the low number of children with 3 or more affected FDRs (7), these were considered together in one category. The second step consisted of adding the secondary variables (country of origin, type of affected FDR, maternal diet, delivery mode and early intervention with gluten or placebo, table 1) to the model using backward elimination based on Akaike Information Criterion (AIC), thus guarding against overfitting.[9,10]

Landmark prediction models

Analyses for variables occurring after birth (duration of breastfeeding, duration of exclusive breastfeeding, rotavirus vaccination, infections as reported by parents and gluten intake) were performed at one, two and three years of age (infections until six years of age) (Supplementary Appendix). For each analysis, the information available at the landmark time point was used. Models' backward elimination based on AIC was used. Since quantification of daily gluten intake is usually unknown in the standard medi-

Table 1. Distribution of the baseline variables in the PreventCD cohort (n =944)

VARIABLE	VALUES	N (%)	TOTAL (%)	CD (%)	P-VALUE, UNIVARIATE ANALYSIS
PRIMARY VARIABLES					
1.Gender					0.005
	Male	490 (51.9)	944 (100)	56 (11.4)	
	Female	454 (48.1)		79 (17.4)	
2. HLA risk group*					<0.001
	Group 1	129 (14.2)	911 (96.5)	40 (31.0)	
	Group 2	88 (9.7)		14 (15.9)	
	Group 3	417 (45.8)		58 (13.9)	
	Group 4	66 (7.2)		8 (12.1)	
	Group 5	211 (23.2)		13 (6.2)	
3.Number of affected FDR					0.01
	1	863 (91.4)	944 (100)	115 (13.3)	
	2	74 (7.8)		19 (25.7)	
	3 or more	7 (0.7)		1 (14.3)	
SECONDARY VARIABLES					
4. Country					0.06
	Netherland	133 (14.1)	944 (100)	22 (16.5)	
	Italy	139 (14.7)		20 (14.4)	
	Poland	64 (6.8)		5 (7.8)	
	Spain	249 (26.4)		25 (10.0)	
	Germany	113 (12.0)		13 (11.5)	
	Israel	95 (10.1)		19 (20.0)	
	Croatia	13 (1.4)		0 (0)	
	Hungary	138 (14.6)		31 (22.5)	
5. Type of affected FDR					0.01
	Mother only	407 (43.1)	944 (100)	62 (15.2)	
	Father only	89 (9.4)		10 (11.2)	
	One sib only	367 (38.9)		43 (11.7)	
	Mother+sib(s)	46 (4.9)		15 (32.6)	
	Father+sib(s)	14 (1.5)		3 (21.4)	
	Multiple sibs	19 (2.0)		1 (5.3)	
	Other	2 (0.2)		1 (50.0)	
6. Gluten consumption by the mother during pregnancy					0.04
	No	509 (53.9)	944 (100)	61 (12.0)	
	Yes	435 (46.1)		74 (17.0)	
7. Mode of delivery					0.6
	Vaginally	398 (42.2)	569 (60.3)	57 (14.3)	
	C-section	171 (18.1)		27 (15.8)	
	Unknown	375 (39.7)		51 (13.6)	
8. Early intervention**					0.4
	Placebo	469 (49.7)	944 (100)	63 (13.4)	
	Gluten	475 (50.3)		72 (15.2)	

CD=celiac disease. C. Section= caesarean delivery. FDR= first degree relative. HLA= human leucocyte antigen. N.=number. Sib=sibling.

* data on the HLA risk group were available for 911 of 944 children with HLA typing performed by means of single-nucleotide polymorphisms (SNPs) on the basis of the tag-SNP approach. From 2 children who developed CD no HLA risk group was known; HLA risk groups: 1: DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); 2: DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); 3: DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); 4: DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); 5: DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); 'other': any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

** Early intervention consisted of 100 mg of gluten per day or placebo between 4-6 months of age (Vriezinga 2014).

cal settings in which the prediction models are meant to be used, model building was repeated without quantity of daily gluten intake. For baseline and landmark prediction models, risk scores were calculated by adding the regression coefficients from the multivariable Cox models. The risk scores were divided into low, low-medium, high-medium and high-risk groups and Kaplan-Meier estimates were calculated. Harrell's c-index was calculated to quantify discrimination of the resulting models.

Validation cohort

Validation analysis of the produced models was performed using data of the independent NeoCel cohort, in which all children were assessed regularly from birth for CD development at pre-defined intervals, in a similar way as in the PreventCD cohort (Supplementary Appendix).

The risk score as developed in the PreventCD cohort was calculated for every child in the NeoCel cohort. The children were subsequently allocated to one of the four risk groups. A univariate Cox model with the (continuous) risk score was fitted in the NeoCel cohort. Ideally, this should give a regression coefficient of 1; values significantly smaller than 1 indicate overfitting of the original risk score. Kaplan-Meier estimates were calculated for each of the four risk groups. Harrell's c-index was calculated to quantify discrimination.

RESULTS

PREVENTCD-COHORT

The mean age of the children (n= 944) was 10.3 years (range 8.4-12.0), 52% male, interquartile range (IQR) follow-up from 5.9 to 9.7 years. In total 227 (24%) children stopped participation (Supplementary Appendix). The distribution of the baseline variables of the cohort is presented in table 1.

Diagnosis of celiac disease

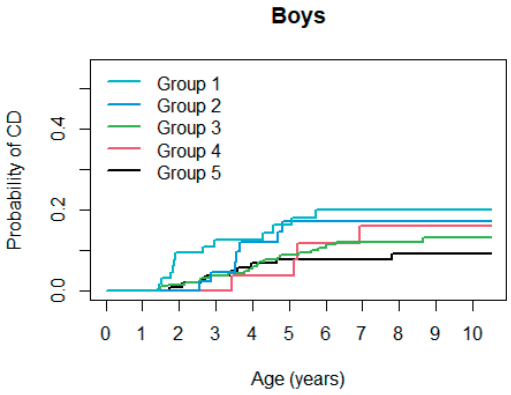
In total, 135 children were diagnosed with CD, including five without small-bowel biopsies according to the non-biopsy ESPGHAN criteria (Figure S1, Supplementary Appendix).[1] In total, 8363 TGA determinations were performed (Figure S2, Supplementary Appendix) with 563 children (59.6%) having at least one determination between March 2016 and March 2019. Mean age at diagnosis was 4.3 years (range 1.1-11.4). The cumulative incidence of CD was 7.5%, 16.6% and 17.5% at three, eight and ten years of age, respectively (Figure S3 Supplementary Appendix).

Variables related to CD development

CD developed significantly more frequently in girls (n=79, 59% vs n=56, 41%) (p=0.005) (Figure S4 Supplementary Appendix). Moreover, the frequency of CD development was significantly higher in children homozygous for HLA-DQ2 (DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2), than children with other HLA-DQ haplotypes, with a cumulative incidence at eight years of 35.4% (n =40) versus maximum 18.2% (HLA risk group 2, n = 14) (P<0.001) (Figure S5 Supplementary Appendix). This difference was even more significant when analysed separately for children with DR3-DQ2/DR3-DQ2 (n=21; 45.0%) compared to those with DR3-DQ2/DR7-DQ2 (n=19; 28.9%) (overall p<0.001) (Figure S6 Supplementary Appendix).

The interaction between gender and HLA risk group was not significant (p=0.10) with hazard ratios for HLA-DQ2 homozygous (DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2) being 13.3 for girls (95% confidence interval [CI], 4.7-38.1; p<0.001)) and 2.4 for boys [95%CI, 1.0-5.7; p = 0.14) (Figures 1a and 1b).

Figure 1a. Cumulative Incidence of celiac disease in the PreventCD cohort (n = 911) at selected ages, according to five HLA-haplotype and male gender (n= 472).

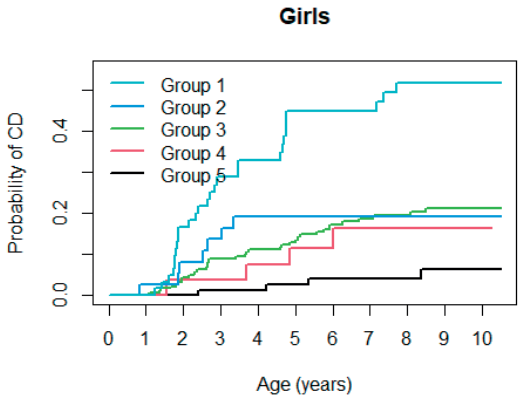


HLA risk	CD/N children at risk at ages					
	0 years	2 years	4 years	6 years	8 years	10 years
Group 1	0/67	6/57	2/48	4/40	0/31	0/10
Group 2	0/48	0/44	5/34	2/29	0/24	0/12
Group 3	0/208	3/190	8/165	8/137	2/107	1/38
Group 4	0/34	0/30	2/50	2/21	1/17	0/7
Group 5	0/115	1/106	6/93	1/82	1/60	0/20

Covariates		Coeff	Se (coef)	Multivariate hazard ratio	95% confidence interval	p-value
HLA risk group (ref: group 5)	Group 1	0.8799	0.4416	2.4108	1.01-5.73	0.14
	Group 2	0.6752	0.5040	1.9644	0.73-5.28	
	Group 3	0.3130	0.3957	1.3676	0.63-2.97	
	Group 4	0.4962	0.6014	1.6424	0.51-5.34	

In addition, in the exploratory analysis separating the HLA-DQ2 homozygosity in HLA DR3-DQ2/DR3-DQ2 from DR3-DQ2/DR7-DQ2, the interaction was significantly different with respect to gender (p=0.04). In girls, the risk to develop CD was significantly increased in both groups of HLA-DQ2 homozygosity, with hazard ratios of 14.8 [95%CI 4.8- 46.0] and 12.5 [95%CI 4.2-37.4] for DR3-DQ2/DR3-DQ2 and for DR3-DQ2/DR7-DQ2, respectively. In boys, the risk to develop CD was also significantly increased in those with DR3-DQ2/DR3-DQ2, but not in those with DR3-DQ2/DR7-DQ2 with hazard ratios of 5.0 (95%CI 2.0-12.6) and 1.0 (95%CI 0.3-3.5) respectively (Figures S7a and S7b Supplementary Appendix).

Figure 1b. Cumulative Incidence of celiac disease (CD) in the PreventCD cohort (n = 911) at selected ages, according to HLA-haplotype divided into five HLA groups and female gender (n = 439).



HLA risk	CD/N children at risk at ages					
	0 years	2 years	4 years	6 years	8 years	10 years
Group 1	0/62	10/49	6/52	9/33	3/20	0/4
Group 2	0/40	3/34	9/61	4/27	0/19	0/8
Group 3	0/209	8/181	21/320	13/155	3/97	2/38
Group 4	0/32	1/27	2/50	1/24	1/10	0/3
Group 5	0/96	0/83	7/166	1/73	0/53	1/12

Covariates		Coeff	Se (coef)	Multivariate hazard ratio	95% confidence interval	p-value
HLA risk group (ref: group 5)	Group 1	2.5903	0.5352	13.3342	4.67-38.07	<0.001
	Group 2	1.5156	0.6269	4.5521	1.33-15.55	
	Group 3	1.4424	0.5271	4.2310	1.51-11.89	
	Group 4	1.1180	0.7077	3.0587	0.76-12.25	

CD= Celiac disease, No=number, CI= confidence interval. Coeff: coefficient. HLA risk group; 1: DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); 2: DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); 3: DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); 4: DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and 5: DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); "other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

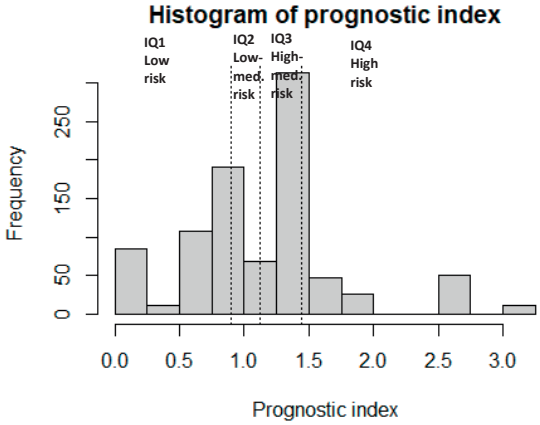
In multivariate analysis, no secondary variable, including early intervention with small quantities of gluten or breastfeeding, showed a significant association with CD development . In the landmark analyses, only a higher amount of average daily gluten intake during the first three years of age was associated with a higher risk to develop CD (p=0.07, p=0.03 and p=0.05 respectively) (Table S1 Supplementary Appendix). The prediction models built with and without the gluten intake per age showed similar results (Table 2).

Table 2. Hazard ratios for the prediction models with and without gluten consumption during the first three years of life in children from celiac families based on data from the PreventCD cohort (n=944)

Age (year)	One		Two		Three	
	With gluten consumption	Without gluten consumption	With gluten consumption	Without gluten consumption	With gluten consumption	Without gluten consumption
Gender (ref male)						
Female	1.65 (1.15-2.36)	1.64 (1.15-2.34)	1.48 (0.99-2.23)	1.47 (0.98-2.21)	1.27 (0.79-2.05)	1.26 (0.78-2.03)
HLA risk group (ref group 5)						
Group 1	6.70 (3.48-12.89)	5.95 (3.10-11.41)	4.68 (2.26-9.71)	3.97 (1.92-8.21)	4.33 (1.80-10.41)	3.60 (1.51-8.61)
Group 2	2.92 (1.31-6.51)	2.76 (1.24-6.15)	2.77 (1.17-6.52)	2.58 (1.10-6.07)	2.73 (0.99-7.54)	2.55 (0.92-7.03)
Group 3	2.51 (1.34-4.68)	2.34 (1.25-4.36)	2.28 (1.18-4.41)	2.07 (1.07-4.00)	2.34 (1.08-5.08)	2.12 (0.98-4.58)
Group 4	2.26 (0.92-5.53)	2.20 (0.90-5.38)	2.22 (0.86-5.73)	2.13 (0.83-5.50)	3.16 (1.14-8.72)	3.00 (1.09-8.30)
Number of FDR (ref 1)						
≥2	1.64 (1.01-2.67)	1.60 (0.99-2.59)	1.75 (1.00-3.05)	1.69 (0.97-2.94)	1.80 (0.94-3.45)	1.72 (0.90-3.31)
Gluten intake*						
Per gram intake	1.28 (1.09-1.50)	-	1.41 (1.15-1.72)	-	1.43 (1.13-1.82)	-

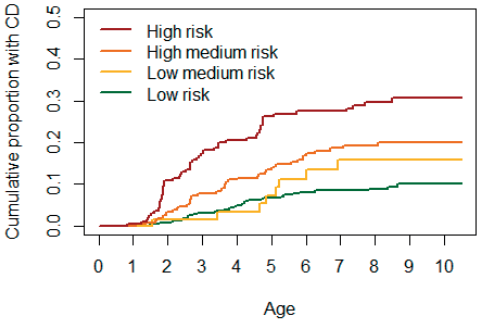
*up to a maximum of 5 grams gluten (see figure S9)

Figure 2a. Histogram of the prognostic index for development of celiac disease; 1. low risk: 0-0.90 points; 2. low-medium risk: 0.91-1.12 points; 3. high-medium risk: 1.13-1.44 points and 4. high risk: >1.45 points.



IQ= interquartile

Figure 2b. Cumulative incidences of celiac disease (CD) at different ages for the four risk groups.



	Risk groups PreventCD cohort			
	High (n)	**High-medium (n)**	**Low-medium (n)**	**Low (n)**
HLA risk group	193	255	68	395
1	129	0	0	0
2	44	44	0	0
3	15	211	61	0
4	5	0	61	0
5	0	0	7	204
CD	52	42	8	31

CD=celiac disease. N=number. HLA=human leucocyte antigen. HLA risk groups: group 1= DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); group 2= DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); group 3 = DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); group 4 = DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and group 5= DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other);"other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

Age (years)	High risk Events/at risk		High-medium risk Events/at risk		Low-medium risk Events/at risk		Low risk Events/at risk	
	Events /at risk	Cum. incidence (95% CI)	Events /at risk	Cum. Incidence (95% CI)	Events /at risk	Cum. incidence (95% CI)	Events /at risk	Cum. incidence (95% CI)
0.5	0/190	0	0/243	0	0/65	0	0/377	0
1	1/188	0.5 (0-1.5)	0/238	0	0/62	0	0/368	0
1.5	4/182	2.7 (0.3-4.9)	2/235	0.8 (0-2.0)	0/62	0	2/360	0.6 (0-1.3)
2	15/162	10.8 (6.2-15.2)	6/224	3.4 (1.1-5.7)	1/59	1.6 (0-4.8)	1/356	0.8 (0-1.8)
2.5	4/156	13.0 (8.0-17.8)	3/219	4.7 (2.0-7.4)	0/59	1.6 (0-4.8)	3/347	1.7 (0.3-3.0)
3	8/145	17.5 (11.8-22.8)	7/205	7.8 (4.2-11.1)	0/55	1.6 (0-4.8)	5/335	3.1 (1.3-4.9)
3.5	4/130	19.9 (13.9-25.6)	1/196	8.2 (4.6-11.7)	1/53	3.5 (0-8.1)	2/318	3.7 (1.7-5.6)
4	1/128	20.6 (14.4-26.3)	7/186	11.5 (7.2-15.6)	0/53	3.5 (0-8.1)	4/311	4.9 (2.6-7.2)
4.5	1/126	21.2 (14.9-27.0)	0/185	11.5 (7.2-15.6)	0/52	3.5 (0-8.1)	4/301	6.2 (3.6-8.7)
5	8/117	26.2 (19.3-32.5)	4/179	13.4 (8.8-17.8)	2/48	7.2 (0.1-13.8)	2/294	6.8 (4.1-9.4)
5.5	1/113	26.8 (19.9-33.1)	3/171	14.9 (10.1-19.5)	2/43	11.2 (2.3-19.4)	2/276	7.4 (4.6-10.2)
6	1/108	27.5 (20.5-33.9)	4/159	16.9 (11.8-21.8)	0/40	11.2 (2.3-19.4)	2/267	8.1 (5.1-11.0)
6.5	0/104	27.5 (20.5-33.9)	2/137	18.0 (12.7-23.0)	1/36	13.5 (3.6-22.3)	1/246	8.5 (5.4-11.4)
7	0/104	27.5 (20.5-33.9)	1/134	18.6 (13.2-23.7)	1/32	15.9 (5.0-25.6)	0/236	8.5 (5.4-11.4)
7.5	2/89	29.0 (21.8-35.5)	1/127	19.3 (13.7-24.5)	0/31	15.9 (5.0-25.6)	0/224	8.5 (5.4-11.4)
8	1/85	29.8 (22.5-36.4)	0/118	19.3 (13.7-24.5)	0/29	15.9 (5.0-25.6)	1/206	8.9 (5.7-12.0)
8.5	1/70	30.8 (23.3-37.5)	1/110	20.0 (14.3-25.3)	0/25	15.9 (5.0-25.6)	1/166	9.4 (6.1-12.7)
9	0/53	30.8 (23.3-37.5)	0/90	20.0 (14.3-25.3	0/20	15.9 (5.0-25.6)	1/130	10.0 (6.5-13.4)
9.5	0/37	30.8 (23.3-37.5)	0/65	20.0 (14.3-25.3	0/16	15.9 (5.0-25.6)	0/92	10.0 (6.5-13.4)
10	0/29	30.8 (23.3-37.5)	0/50	20.0 (14.3-25.3	0/8	15.9 (5.0-25.6)	0/65	10.0 (6.5-13.4)

CD= celiac disease; childr.=children; CI= confidence interval; HLA= Human Leukocyte Antigen; n=number of children

Prediction models

Based on the variables' regression coefficients in this multivariate model, a risk stratification score was constructed for each child (Table 3). Median (1.12) and first and third IQR (IQ1=0.90 and IQ3=1.44) were used as cut-off values for dividing the risk groups into low (0-0.90 points), low-medium (0.91-1.12 points), high-medium (1.13-1.44 points) and high (≥1.45 points) risk score (Figure 2a). The total points score is mapped as a corresponding risk of CD probability (Figure 2b).

Validation of the prediction model in the NeoCel cohort

The distribution of the variables in the NeoCel cohort contributing to the risk scores and probability for CD is presented in Table 4. Figure S8 (Supplementary Appendix) shows the estimated cumulative incidence of CD for each risk group in the NeoCel cohort. Cox regression with the continuous risk score yielded a regression coefficient of nearly 1.0 (0.81 (0.54); p=0.13), indicating good fitting despite the non-significance, with the

Table 3. Multivariate logistic regression model and corresponding risk score of probability of Celiac Disease development in children from celiac families based on data from the PreventCD cohort

	Hazard ratio	95% CI	p-value	Regr. coef./ Points for risk score at birth	Regr. coef./ Points for risk score at 1 year	Regr. coef./ Points for risk score at 2 year	Regr. coef./ Points for risk score at 3 year	Regr. coef./ Points for risk score at 4 year	Regr. coef./ Points for risk score at 5 year	Regr. coef./ Points for risk score at 6 year	Regr. coef./ Points for risk score at 7 year	Regr. coef./ Points for risk score at 8 year
Gender (ref male)												
Female	1.71	1.21-2.42	0.002	**0.54**	**0.49**	**0.38**	**0.23**	**0.38**	**0.47**	**0.85**	**1.42**	**1.09**
HLA risk group (ref group 5)												
Group 1	5.73	3.06-10.74	<0.001	**1.75**	**1.78**	**1.38**	**1.28**	**1.69**	**1.29**	**1.17**	**1.18**	**0**
Group 2	2.76	1.30-5.88	0.008	**1.02**	**1.02**	**0.95**	**0.94**	**0.17**	**0**	**0**	**0**	**0**
Group 3	2.25	1.23-4.10	0.008	**0.81**	**0.85**	**0.73**	**0.75**	**0.94**	**1.08**	**0.75**	**0.03**	**0.39**
Group 4	2.03	0.84-4.90	0.115	**0.71**	**0.79**	**0.76**	**1.10**	**1.25**	**1.61**	**1.40**	**0**	**0**
Number of FDR (ref 1)												
≥2	1.51	0.93-2.44	0.09	**0.41**	**0.47**	**0.52**	**0.54**	**0.62**	**0.57**	**0.67**	**0.71**	**2.1**

CI= confidence interval. FDR=first degree relative. HLA= Human Leukocyte Antigen. ref=reference

Table 4. Distribution of the variables in the Neocel cohort contributing to the risk scores and prediction models for CD (n=162)

Variable	Values	N (%)	Total (%)	CD (%)
1. Gender	Male	79 (48.8)	162 (100)	6 (7.6)
	Female	83 (51.2)		7 (8.4)
2. HLA risk group*	Group 1	3 (2.6)	117 (72.2)	1 (33.3)
	Group 2	13 (11.1)		4 (30.8)
	Group 3	13 (11.1)		0 (0)
	Group 4	54 (46.2)		5 (9.3)
	Group 5	34 (29.1)		2 (5.9)
3. Number of affected FDR	1	137 (84.6)	162 (100)	13 (9.5)
	2 or more	12 (7.4)		0 (0)
4. Risk score groups	High	19 (16.3)	117 (100)	4 (21.1)
	High-medium	12 (10.3)		1 (8.3)
	Low-medium	49 (41.2)		5 (10.2)
	Low	37 (31.6)		2 (5.4)

CD=celiac disease. FDR= first degree relative. HLA= human leucocyte antigen. N=number; * HLA risk group known in 117/162 children (n 1 child who developed CD was not HLA typed). Groups 1: DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); 2: DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); 3: DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); 4:DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); 5: DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); 'other': any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

risk scores based on the data of the PreventCD cohort. The Harrell's c-index of 0.608, somewhat smaller than in the PreventCD cohort, is not surprising, considering the contribution of the factors could be estimated to optimize discrimination in the original PreventCD cohort.

DISCUSSION

Although long term follow-up cohorts of children genetically predisposed for CD have been reported before[11], we here present the longest follow up data from a birth cohort of genetically predisposed children with FDR with CD. Based on this prospective data, we developed prediction models for CD development in children from CD families to facilitate their individualized screening advice for CD.

Our results show first that the risk to develop CD for children with affected FDR during the first ten years of life is significantly higher than previously assumed.[6] Until recently, the lifetime risk of CD for FDR of CD patients was considered to be 5%-10%, yet our data show that at the age of eight years, this is as high as 17%, emphasizing the importance

of a sound advice for early screening.[6,12-17] We also confirm that CD develops in children with affected FDR at a very young age, as the mean age of diagnosis in our cohort was four years of age. This early development has also been shown in screening studies among the general pediatric population, and we can assume that, in general, this can be accepted as part of the natural history of CD.[18-23] We additionally confirm that, as previously reported by us in the same cohort at the age of three years, the risk of CD in these children during their first 10 years of life is strongly related to their gender and HLA-DQ phenotype.[5] In total, at the age ten years, girls have a 7.7% higher cumulative incidence compared to boys (21.5% vs 13.8%). The increased risk for CD in HLA-DQ2 homozygotes as well as the predominance of female gender is well known.[7,8] However, the significant additional effect of the interaction between female gender and certain HLA-DQ2 homozygosity has not been reported before. Contrary to HLA-DR3-DQ2 homozygosity, the increased risk in HLA-DR3-DQ2/DR7-DQ2 homozygosity is only present in females. This different effect of gender appears very early in life, and it persists and increases during the first ten years of age (cumulative incidence 8.0% for boys and 51.3% for girls) (Figure S7a and 7b Supplementary Appendix). The reason for this difference is unknown and intriguing and possible explanations are offered in the Supplementary Appendix.

In contrast to previously reported results by our group, the present results show that the quantity of early gluten intake is associated with a significantly higher risk of CD development, with an increased hazard ratio of 1.07 per gram increase in daily gluten intake.[24] Plausible explanations for the discrepancy are the different statistical methods used to analyse the data, since we now have used landmark analyses to avoid immortal time bias.[24] Since the prediction models with and without adding the amount of gluten intake per age show similar results, we have chosen to use the models without gluten intake, as this is generally unknown in standard clinical setting. Our present findings are in accordance with those from the TEDDY and DAISY studies,[25,26] suggesting that the quantity of gluten ingestion may be a preventive factor for CD. Indeed, the plots of the average daily gluten intake by the children in our study suggest that the risk of CD increases linearly until approximately 5 grams per day, and that more gluten consumption per day does not longer increases the risk of CD (Table 2 and Figure S9, Supplementary Appendix). However, it is important to keep in mind that these data are observational and no causality may be concluded. These observations do not allow us (or others) at this moment to give recommendation to the parents on the prevention of CD in their children. To develop such recommendations the results of RCTs with different quantities of ingested gluten as intervention are needed.

Screening advice

Screening for CD is recommended in children with FDR with this condition, but the frequency of screening and at what age remains unknown.[27,28] Based on our prediction models of CD, an individualized screening advice for children with FDR with CD can be provided (Figure 2b). Children in the high-risk group should be advised to start screening for CD earlier in life and more often than children in other risk groups. This also depends upon the current age of the child, since the risk of CD changes accordingly (Table 3). To calculate the child-tailored risk and give a personalized screening advice, we designed a Prediction application (https://hputter.shinyapps.io/preventcd/) based on both the risk group to which the child belongs and the current age of the child. As basis for our advice, we use the current standard of care of many centres taking care of families with CD, which is comprised of a yearly screening of children with FDR with CD based on the assumption of a 10% cumulative incidence among them. As a result, we advise that every child with a FDR with CD should be screened at presentation, including total IgA and IgA-TGA determination, as well as HLA-DQ2 and DQ8 typing. If the results of the TGA are negative, the risk of developing CD in the next years should be assessed using our Prediction app. If the prediction for CD development is higher than 10% in the next two years, we advise to repeat the screening after six months. If the prediction is between 5-10%, the advice is to repeat the screening after one year and if the prediction is lower than 5%, to repeat the screening after two years. For example, if we assume the case of a 1-year-old girl HLA-DR3-DQ2 homozygous with normal IgA and negative TGA, we will advise her to repeat the screening at 18 months and two years of age (prediction 18.9% in the next two years). For more examples concerning the use of the Prediction app for screening advice, see the Supplementary Appendix.

The strength of our models for CD development and screening advice is that they are based on prospective data from multicentre collaboration with a long follow-up time. All children have been followed in a homogenous manner, with centralized TGA determinations (nine of the ten centres) and assessment of diagnostic biopsies, thereby minimizing the risk of diagnostic bias. The high number of CD-diagnosed cases in our cohort benefits also the design of the prediction model. The multicentre, multinational involvement in the PreventCD cohort, and therefore the plausible influence of different environmental factors in the results and consequently in the produced prediction model, make it applicable in different countries. Lastly, the validation of the prediction model in an external independent high-risk CD cohort with good fitting, supports the implementation to improve medical care and continuously optimize the model. Although individualized screening advice for CD has been reported before,[26] as far as we know, we are the first to provide it including age of initiation and frequency of screen-

ing, in the form of a clinically easy-to-use Prediction app (https://hputter.shinyapps.io/preventcd/).

Possible shortcomings of our study are the variable intervals of TGA determination after the age of three years, implying that the CD development may occur sometime prior to TGA determination. We have taken this into account by averaging the time of CD development between the last negative TGA result and the date of CD diagnosis. Another possible shortcoming is that TGA determination was done in 563/944 children during the last three years of follow up (59.6%).From the 154 children who had no TGA determination during the last three years, we have negative TGA results till a mean age of 5.1 years (3.0 - 8.2 years). However, from the 167 children whose parents withdrawn consent for the study we have negative TGA results till a mean age of 3.2 years (3 months - 9.4 years) and we have included all these data to develop the prediction models and application (see the Supplementary Appendix). Taking all this into consideration, our nearly 60% follow-up rate after 10 years can be considered as quite acceptable.

We have analysed data till the age of 10 years, and our prediction application applies till the age of 8 years. This is inherent to the data available at the time at which the data was frozen for analysis, when all the participants had reached the age of 8 year (range 8.4-12.0). It should be noted that this Prediction app and screening advice have been developed for children from CD families and should therefore not be applied in children from the general population until their use has been broadly validated.

To conclude, children with CD-FDR develop CD early in life, and their risk depends on gender, age and HLA-DQ: all factors which are important for a sound screening advice. These children should be screened early in life, including HLA-DQ2/8 typing, and if genetically predisposed to CD, should get a further personalized screening advice using our Prediction app (https://hputter.shinyapps.io/preventcd/).

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr 2012;54(1):136-60.
2. Vriezinga SL, Schweizer JJ, Koning F, Mearin ML. Celiac disease and gluten-related disorders in childhood. Nat Rev Gastroenterol Hepatol. 2015 Sep;12(9):527-36
3. Lindfors K, Ciacci C, Kurppa K, et al. Celiac Disease. Nat Rev Dis Primers. 2019 Jan 10;5(1):3. doi: 10.1038/s41572-018-0054-z.
4. Lionetti E, Castellaneta S, Pulvirenti A, et al; Italian Working Group of Weaning and Celiac Disease Risk. Prevalence and natural history of potential celiac disease in at-family-risk infants prospectively investigated from birth. J Pediatr. 2012 Nov;161(5):908-14.
5. Vriezinga SL, Auricchio R, Bravi E, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med 2014; 2;371(14):1304-15.
6. Singh P, Arora S, Lal S, Strand TA, Makharia GK. Risk of celiac disease in the first- and second-degree relatives of patients with celiac disease: a systematic review and meta- analysis. Am. J. Gastroenterol. 2015, 110, 1539–1548.
7. Liu E, Lee HS, Aronsson CA, et al; TEDDY Study Group. Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med. 2014 Jul 3;371(1):42-9.
8. Margaritte-Jeannin P, Babron MC, Bourgey M, et al. HLA-DQ relative risks for celiac disease in European populations: a study of the European Genetics Cluster on Celiac Disease. Tissue Antigens. 2004;63:562–567
9. Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control, 19 (6): 716–723, doi:10.1109/TAC.1974.1100705.
10. Hastie T, Tibshirani R, Friedman J. The Elements of Statistical Learning: Data Mining, Inference, and Prediction. Second Edition. Springer, New York, 2009
11. Liu E, Dong F, Barón AE, Taki I, Norris JM, Frohnert BI, et al. High Incidence of Celiac Disease in a Long-term Study of Adolescents With Susceptibility Genotypes. Gastroenterology 2017 May;152(6):1329-1336
12. Oliveira A, Trindade E, Tavares M, Lima M, Dias JA. Celiac disease in first degree relatives of celiac children. Arg Gastroenterol. 2012; 49(3):204-7.
13. Dogan Y, Yildirmaz S, Ozercan IH. Prevalence of celiac disease among first-degree relatives of patients with celiac disease. J Pediatr Gastroenterol Nutr. 2012; 55(2):205-8.
14. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 2003; 163(3):286-92.
15. Almeida PL, Gandolfi L, Modelli IC, Cássia Martins R, Coutinho de Almeida R, Pratesi R. Prevalence of celiac disease among first degree relatives of Brazilian celiac patients. Arg Gastrenterol. 2008; 45(1):69-72.
16. Pittschieler K, Gentili L, Niederhofer H. Onset of celiac disease: a prospective longitudinal study. Acta Pediatr 2003;92(10):1149-1152.
17. Mäki M, Mustalahti K, Kokkonen J, et al. Prevalence of celiac disease among children in Finland. N. Engl. J. Med. 2003 Jun 19;348(25):2517-24. doi: 10.1056/NEJMoa021687.
18. Mustalahti K, Catassi C, Reunanen A, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med. 2010 Dec;42(8):587-95. doi: 10.3109/07853890.2010.505931.

19. Bingley PJ, Williams AJK, Norcross AJ, et al. Undiagnosed celiac disease at age seven: population based prospective birth cohort study. BMJ 2004 Feb 7;328(7435):322-3. doi: 10.1136/bmj.328.7435.322.
20. Katz KD, Rashtak S, Lahr BD, et al. Screening for celiac disease in a North American population: sequential serology and gastrointestinal symptoms. Am J Gastroenterol. 2011 Jul;106(7):1333-9. doi: 10.1038/ajg.2011.21.
21. Hovell CJ, Collett JA, Vautier G, et al. High prevalence of celiac disease in a population-based study from Western Australia: a case for screening? Med J Aust. 2001 Sep 3;175(5):247-50.
22. Jansen M, van Zelm M, Groeneweg M, et al (2018) The identification of celiac disease in asymptomatic children: the Generation R Study. J Gastroenterol. 2018 Mar;53(3):377-386. doi: 10.1007/s00535-017-1354-x53(3):377-386.
23. Catassi C, Kryszak D, Louis-Jacques O, et al. Detection of Celiac disease in primary care: a multicenter case-finding study in North America. Am J Gastroenterol. 2007 Jul;102(7):1454-60. doi: 10.1111/j.1572-0241.2007.01173.x.
24. Crespo-Escobar P, Mearin ML, Hervás D, et al. The role of gluten consumption at an early age in celiac disease development: a further análisis of the prospective PreventCD cohort study. Am J Clin Nutr. 2017 Apr;105(4):890-896.
25. Aronsson CA, Lee HS, Koletzko S, et al. TEDDY Study Group. Effects of Gluten Intake on Risk of Celiac Disease: A Case-Control Study on a Swedish Birth Cohort. Clin Gastroenterol Hepatol. 2016 Mar;14(3):403-409.
26. Mårild K, Dong F, Lund-Blix NA, et al. Gluten Intake and Risk of Celiac Disease: Long-Term Follow-up of an At-Risk Birth Cohort. Am J Gastroenterol. 2019 Aug;114(8):1307-1314.
27. Bai J, Ciacci C. The World Gastroenterology Organisation Global Guidelines recommend testing for CD in asymptomatic children who have first-degree relatives with the disease. J Clin Gastroenterol. Volume 51, number 9. Febr 2017.
28. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Pediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Celiac Disease 2020. J Pediatr Gastroenterol Nutr. 2020 Jan;70(1):141-156.
29. Wessels MMS, de Rooij N, Roovers L, Verhage J, de Vries W, Mearin ML. Towards an individual screening strategy for first-degree relatives of celiac patients. Eur J Pediatr. 2018 Nov;177(11):1585-1592.