TRIAL FULL TITLE	PREVENTCD. Influence of the dietary history in the prevention of coeliac disease: possibilities of induction of tolerance for gluten in genetic predisposed children-THE FOLLOW UP STUDY.
EUDRACT NUMBER	
SAP VERSION	1.0
ISRCTN NUMBER	74582487
SAP VERSION DATE	March 29, 2019
TRIAL STATISTICIAN	Prof. dr. H. Putter
TRIAL CHIEF INVESTIGATOR	Dr. M.L. Mearin
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1. SAP Signatures

I give my approval for the attached SAP entitled "Influence of the dietary history in the prevention of coeliac disease: possibilities of induction of tolerance for gluten in genetic predisposed children-THE FOLLOW UP STUDY" dated March 29, 2019.

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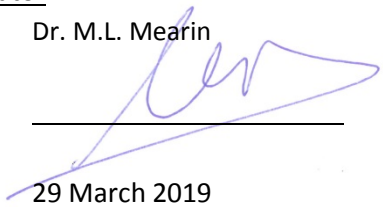
Signature: 

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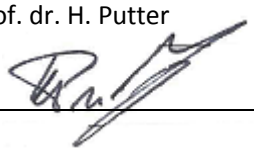
Signature: 

Date: 29 March 2019

2. Abbreviations and Definitions

AE	Adverse Event
AGA	Anti-gliadin antibodies
CD	Celiac disease
EMA	Anti-endomysium antibodies
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
GFD	Gluten free diet
HLA	Human Leukocyte Antigen
IgA	Immunoglobulin A
LUMC	Leiden University Medical Center
PREVENTCD	Multicenter European study funded by the European Commission FP-6-2005-FOOD-4B; Proposal/Contract no 036383:

	Influence of the dietary history in the prevention of coeliac disease: possibilities of induction of tolerance for gluten in genetic predisposed children
SAP	Statistical Analysis Plan
tTGA	Anti-tissue transglutaminase antibodies

3. Introduction

Celiac disease (CD) is a common autoimmune disorder caused by ingestion of gluten in genetically-susceptible individuals. It is characterized by a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 and/or HLA-DQ8 haplotypes and small bowel alterations (Husby, 2012). Gluten is a common name used for proteins (prolamins and glutenins) of cereals such as wheat, barley, and rye. CD is possibly the most common food hypersensitivity in Europe and beyond and affects as many as 1% of the general population (Myleus, 2009).The incidence of CD has increased over the last half-century, resulting in rising interest in identifying risk factors for CD to enable prevention. Early infant feeding practices have been suggested as one of the factors influencing the risk of CD in genetically susceptible individuals. PREVENTCD (www.preventcd.com) is a European multicenter study, which investigates the influence of infant nutrition, and that of genetic, immunologic and environmental factors, on the risk of developing CD. The general objective of PREVENTCD was to significantly reduce the number of people suffering from CD in Europe by developing primary prevention strategies (Hogen Esch, 2010). In this study, 944 children who had at least 1 first-degree relative (FDR) with CD and HLA-DQ2 and/or DQ8 are involved. From age 4 to 6 months, 475 participants received 100 mg of vital gluten daily and 469 received placebo. After 24 weeks, intake of gluten was liberalized in both groups. CD serology was measured periodically. Children with elevated levels of CD antibodies and/or with symptoms suggestive of CD were offered small bowel biopsies to confirm the diagnosis. Meanwhile, two systematic reviews and meta-analyses, which included the above prospective interventional study, concluded that infant feeding practices do not influence the development of CD (Szajewska 2015, Silano 2016). Because of the genetic background of CD, FDRs of celiac patients have a higher risk of developing CD than the general population with a cumulative incidence of 5,2%, 10.8% and 16.4% respectively at the age of 3, 5 and 7 years (Vriezinga, 2014, and unpublished data). The risk of developing CD among FDRs is also influenced by gender and HLA haplotype (Vriezinga 2014). If primary prevention is not possible, secondary prevention is necessary for early detection of the disease to avoid complications.

This statistical analysis plan (SAP) refers to the follow-up of the PREVENTCD cohort and aims to give insight in the natural history and risk for the disease in this unique high risk population of CD.

4. Purpose of the analyses

The primary purpose of the analysis is to detect variables that influence the age-dependent risk of CD in high-risk children with at least 1 FDR with CD and HLA-DQ2 and/or DQ8, using data from the PREVENTCD cohort.

5. Study Objectives and Endpoints

5.1 Study Objectives

The primary objective of this article is:

1. To estimate the cumulative incidence of CD up to the age of 10 years in genetically susceptible children from CD families, in general and according to the early food intervention (gluten vs placebo).

2. To describe the natural history and age at development of CD in a high risk group for CD with respect to
    a. HLA haplotypes
    b. Gender
    c. Country
    d. Early food intervention (gluten vs placebo)
3. To build predictive models for individualizing patient follow-up according to genetic risk in CD-high risk groups taking into account the above named variables.

Taking into account our previous results from the PreventCD cohort published in the NEJM in 2014, the risk of developing CD is expected to differ between:

- Gender,
- Genetic risk for CD in five classes according to HLA-DQ genotype
  Group 1= DR3-DQ2/DR3-DQ2; DR3-DQ2/DR7-DQ2;
  Group 2 = DR7-DQ2/DR5-DQ7;
  Group 3 = DR3-DQ2/DR5-DQ7; DR3-DQ2/DR4-DQ8; DR3-DQ2/other;
  Group 4 = DR7-DQ2/DR7-DQ2; DR7-DQ2/DR4-DQ8; DR4-DQ8/DR4-DQ8;
  Group 5 = DR7-DQ2/other; DR4-DQ8/DR5-DQ7; DR4-DQ8/other
  In addition we will also analyse Group 1 without DR3-DQ2/DR7-DQ2, since the affinity of gluten peptides is higher for DR3-DQ2 than for DR7-DQ2.

In addition we will build predictive models for individual patients taking into account 3 simple HLA-DQ groups:

- Group a: DQ2 homozygous: DR3-DQ2/DR3-DQ2; DR3-DQ2/DR7-DQ2
- Group b: DQ2 heterozygous: DR7-DQ2/DR5-DQ7; DR3-DQ2/DR5-DQ7; DR3-DQ2/DR4-DQ8; DR3-DQ2/other; DR7-DQ2/DR7-DQ2; DR7-DQ2/DR4-DQ8; DR7-DQ2/other
- Group c: DQ8 positive: DR4-DQ8/DR4-DQ8; DR4-DQ8/DR5-DQ7, DR4-DQ8/other

Exploratory subgroup analyses will be performed for the above mentioned variables. Cox regression models with treatment by covariate interaction will be used for the above mentioned variables to test for treatment effect modification by these variables.

5.2 Endpoints

The primary aim of PREVENTCD was to establish whether or not there is a significant difference in the frequency of CD between the children with gluten intervention and the children with placebo.
The primary endpoint of this analysis is the cumulative incidence of CD.

The time scale is age of the subject (child). Time of development of CD for a subject in the study is defined as the age at diagnosis of CD (see below for precise definition). Subjects without CD are censored at the time of last CD antibody determination, defined as either Immunoglobulin A (IgA) anti-tissue transglutaminase antibodies (tTGA) in case of IgA deficiency, tTGA of the IgG class.

CD is diagnosed according to the 1990 and/or 2012 criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (revised criteria 1990, Husby 2012). Small bowel biopsies for the diagnosis of CD are offered to parents of participating children if the following criteria are met:
a) In asymptomatic children biopsies will be performed if they have positive tTGA on two occasions in a 3 month interval (positive tTGA means a result of >7 U/ml)
b) In symptomatic children biopsies are offered independently of the presence of

elevated tTGA.

Biopsies are only performed when medically indicated, that is: only in these children highly suspected for active CD, and not just for purpose of the study. Such children would undergo biopsies also in non-study circumstances. All the biopsies taken during the study are centrally assessed by one pathologist (Professor V. Villanacci, Spedali Civili, Brescia, Italy). The diagnosis of all children is centrally reviewed by a diagnostic committee.
Time of diagnosis of CD is defined as the date of biopsies sampling. According to the ESPGHAN guidelines from 2012, an esophagogastroduodenoscopy with duodenal biopsies can be omitted in children with symptoms of CD and twice tTGA (measured by ELISA) > 10× upper limit of normal (ULN) and positive anti endomysium antibodies (EmA, measured by indirect immunofluorescence). If a child was diagnosed with CD, but endoscopy was not necessary for the diagnosis, as agreed by the diagnostic Committee of PreventCD, the date of the diagnosis is defined as the date at which the CD antibodies were highest. Since from the age of 3 years the specific CD antibodies were determined at least once every 3 years, but at variable intervals, we consider the age at seroconversion to be midway between the age at the last negative antibody determination and the date of the diagnosis of CD.

In case the child is lost to follow-up, the child will be treated as censored at the date of last visit or last biopsy/other measurement.

6. Study Methods

6.1 General Study Design and Plan

PREVENTCD is a multinational, randomized, double-blind, placebo-controlled dietary interventional study involving 944 children who had at least 1 FDR with CD and HLA-DQ2 and/or DQ8. From age 4 to 6 months, 475 participants received 100 mg of vital gluten daily and 469 received placebo. After 24 weeks, intake of gluten was liberalized in both groups. Health status, anthropometric variables, and feeding habits (i.e., breastfeeding and formula feeding) were periodically monitored and gluten consumption was quantified using standardized questionnaires.
Measurements of serum anti-gliadin antibodies (AGA, till 2016) and tTGA were performed centrally at least seven times during the first 3 years of age and thereafter at least once in 3 years. Children with elevated levels of CD antibodies and/or with CD-associated symptoms were offered small bowel biopsies to confirm the diagnosis. The biopsy specimens were histologically assessed at the study sites and were also reviewed by an independent pathologist.
All children with a suspicion of CD were centrally discussed during a diagnostic committee.

The first results of the PREVENTCD-study, published in 2014 in the NEJM, showed no significant difference between the groups receiving the early gluten intervention or placebo in the risk of developing CD at the age of 3 years.
PREVENTCD-2 study is the continuation of the European PREVENTCD study till the age of 12 year to give insight into the effect of the intervention that was carried out in PreventCD-1 later in life and into the natural history of CD.

6.2 Inclusion-Exclusion Criteria and General Study Population

Inclusion criteria:
944 Children who were reported in the analysis for the publication in the NEJM

Exclusion criteria:
We excluded children who were diagnosed with trisomy 21 or Turner syndrome, which are disorders associated with an increased risk of CD. We also excluded children born at <36 weeks, except for healthy

infants born between 34-36 weeks and with a birth weight ≥2 kg. No birth weight restriction was applied to healthy twins born between 34-36 weeks.

6.3 Missing Data

Descriptive statistics will be based on complete cases. For multivariate analysis, multiple imputation with chained equations (including cumulative hazard at time of CD/censoring and CD status indicator in the imputation model (White & Royston 2009) will be used to impute covariates with missing data.

7 General Considerations

7.1 Timing of Analyses

Data for this analysis have been frozen at 29th March, 2019.

7.2 Analysis Populations

For all analysis, we will use all the 944 children who were reported in the analysis for the publication in the NEJM.

7.3 Covariates and Subgroups

The risk of developing CD is expected to differ between:

- Gender,
- Genetic risk for CD in the different groups according to HLA-DQ genotype

Taking into account our previous results from the PREVENTCD cohort published in the NEJM in 2014, the risk of developing CD is not to be expected between:

- Participating country
- Family history (number of affected first degree relatives) at time of inclusion (1, 2, 3 or more)
- Intervention/placebo group

8 Statistical analyses

8.1 Efficacy analyses

For estimating the cumulative incidence of CD, Kaplan–Meier curves will be calculated, with time defined as the patient's age at the diagnosis of celiac disease or at the last assessment or withdrawal from the study (when data were censored). For comparison, a log-rank test (two-sided) is used, stratified according to participating country. The hazard ratio for CD in the gluten group, as compared with the placebo group (with 95% confidence intervals), is provided, on the basis of a Cox proportional hazards regression analysis. All analyses are performed according to the intention-to-treat principle. Different intervention effects were assessed in subgroups by including an interaction term between intervention and subgroup in the Cox proportional-hazards regression analysis.

8.2 Multivariate analysis

All multivariate analyses are based on Cox proportional hazards regression.

8.2.1 Baseline variables

The variables to be considered are:

Primary variables:

- Gender
  - Male
  - Female
- Genetic risk for CD in five groups according to HLA-DQ genotype
  - Group 1= DR3-DQ2/DR3-DQ2; DR3-DQ2/DR7-DQ2;
  - Group 2 = DR7-DQ2/DR5-DQ7;
  - Group 3 = DR3-DQ2/DR5-DQ7; DR3-DQ2/DR4-DQ8; DR3-DQ2/other;
  - Group 4 = DR7-DQ2/DR7-DQ2; DR7-DQ2/DR4-DQ8; DR4-DQ8/DR4-DQ8;
  - Group 5 = DR7-DQ2/other; DR4-DQ8/DR5-DQ7; DR4-DQ8/other
- Intervention
  - Placebo
  - Gluten
- Participating country
  - Croatia,
  - Germany,
  - Hungary,
  - Israel,
  - Italy,
  - the Netherlands,
  - Poland, and
  - Spain.
- Family history (number of affected first degree relatives) at time of inclusion
  - One
  - Two
  - Three or more

Secondary variables:

- Type of first degree relative with CD
  - Mother only
  - One sibling
  - Father only
  - Mother + one or more siblings
  - Multiple siblings, neither parents
  - Father + one or more siblings
  - Mother + father

- Rotavirus vaccination
  - Yes
  - No

- Maternal diet during pregnancy and lactation
  - Gluten-free
  - Gluten-containing (‘normal’)

- Infections during the first 18 months of life

    - No infections
    - Gastro-intestinal infections
    - Respiratory tract infections
    - Gastro- and respiratory tract infections.

- Mother with CD
    - Yes
    - No

- Duration of breastfeeding
    - Never started
    - ≤ 3 months
    - 4-6 months
    - ≥ 6 months

- Duration of exclusive breastfeeding
    - Never exclusive
    - ≤ 3 months
    - 4-6 months
    - ≥ 6 months

- Mode of delivery
    - Vaginally
    - Cesarean section

- Mean daily gluten intake after the dose escalation at 12, 18, 24 and 36 months of age, respectively.

Multivariate Cox proportional hazards regression analysis will be performed in two steps. In the first step the five primary variables will be entered into the model irrespective of statistical significance. The second step will used backward selection (p=0.05) of the secondary variables, with the five primary variables included in the model. A risk score will be defined as the linear predictor provided by the Cox regression model. Harrell's c-index will be calculated to quantify discrimination of the resulting model. The risk score will be divided into four equally sized groups (low risk, medium low risk, medium high risk, high risk); Kaplan-Meier estimates will be calculated for each of the four resulting risk groups.

8.2.2 Landmark analysis

Landmark analyses at 1, 2, and 3 years of age will be performed. For each landmark analysis, children without CD and under follow-up at the landmark age will be included. Besides the baseline variables in Section 8.2.1, also

- Duration of breast-feeding (until the landmark age), as continuous variable
- Daily gluten intake (averaged until the landmark age), as continuous variable
- Occurrence of infection (before the landmark age), as binary variable

will be included. Model building will be as in the multivariate analysis with baseline variables, with the above new variables included in the backward selection. For each landmark analysis, a risk score will be calculated as in Section 8.2.1. Harrell's c-index will be calculated to quantify discrimination of the resulting models. For each landmark analysis, the risk scores will be divided into four equally sized groups

(low risk, medium low risk, medium high risk, high risk); Kaplan-Meier estimates will be calculated for each of the four resulting risk groups.

9 References

1. Husby S et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012;54(1):136-60.
2. Myleus A et al. Celiac disease revealed in 3% of Swedish 12-year-olds born during an Epidemic. J Pediatr Gastroenterol Nutr 2009;49(2):170-6.
3. Hogen Esch CE et al. The PreventCD Study design: towards new strategies for the prevention of coeliac disease. Eur J Gastroenterol Hepatol 2010;22(12):1424-30.
4. Szajewska H et al. Systematic review with meta-analysis: early infant feeding and coeliac disease--update 2015. Aliment Pharmacol Ther 2015;41(11):1038-54.
5. Silano M et al. Infant feeding and risk of developing celiac disease: a systematic review. BMJ Open 2016;25;6(1):e009163.
6. Vriezinga SL et al.Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med 2014; 2;371(14):1304-15.
7. Revised criteria for diagnosis of coeliac disease: report of Working Group of European Society for Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990;65:909-11.
8. White IR, Royston P. Imputing missing covariate values for the Cox model. Stat Med 2009;10; 28(15):1982–98.