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Investigating the early metabolic fingerprint of celiac disease – a prospective approach

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ABSTRACT

Objectives and study: In the development of Celiac Disease (CD) both genetic and environmental factors play a crucial role. The Human Leukocyte Antigen (*HLA*)-DQ2 and *HLA*-DQ8 loci are strongly related to the disease and are necessary but not sufficient for the development of CD. Therefore, increasing interest lies in examining the mechanisms of CD onset from the early beginning. Differences in serum and urine metabolic profiles between healthy individuals and CD patients have been reported previously. We aimed to investigate if the metabolic pathways were already altered in young, 4 month old infants, preceding the CD diagnosis.
Methods: Serum samples were available for 230 four month old infants of the PreventCD project, a multicenter, randomized, double-blind, dietary intervention study. All children were positive for *HLA*-DQ2 and/or *HLA*-DQ8 and had at least one first-degree relative diagnosed with CD. Amino acids were quantified after derivatization with liquid chromatography – tandem mass spectrometry (MS/MS) and polar lipid concentrations (acylcarnitines, lysophosphatidylcholines, phosphatidylcholines, and sphingomyelins) were determined with direct infusion MS/MS.
We investigated the association of the metabolic profile with (1) the development of CD up to the age of 8 years (yes/no), (2) with HLA-risk groups, (3) with the age at CD diagnosis, using linear mixed models and cox proportional hazards models. Gender, intervention group, and age at blood withdrawal were included as potential confounder.
Results: By the end of 2014, thirty-three out of the 230 children (14%) were diagnosed with CD according to the ESPGHAN criteria. Median age at diagnosis was 3.4 years (IQR, 2.4–5.2). Testing each metabolite for a difference in the mean between healthy and CD children, we (1) could not identify a discriminant analyte or a pattern pointing towards an altered metabolism (Bonferroni corrected $P > 0.05$ for all). Metabolite concentrations (2) did not differ across the HLA-risk groups. When investigating the age at diagnosis using (3) survival models, we found no evidence for an association between the metabolic profile and the risk of a later CD diagnosis.
Conclusion: The metabolic profile at 4 months of age was not predictive for the development of CD up to the age of 8 years. Our results suggest that metabolic pathways reflected in serum are affected only later in life and that the HLA-genotype does not influence the serum metabolic profile in young infants before introduction of solid food.

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1. Introduction

Celiac disease (CD) is a systemic immune-mediated disorder which is triggered by gluten and other prolamins in wheat, barley, rye, and other cereals in genetically susceptible individuals. CD has systemic effects but is mainly characterized by enteropathy with villous atrophy that may result in diarrhea, growth faltering and malnutrition, and other symptoms such as constipation or fatigue [1]. The only known effective treatment is a lifelong, strict gluten-free diet (GFD) [2]. Environmental and genetic factors play an important role in the pathogenesis of CD. Apart from the main environmental trigger gluten, a protein complex formed by gliadins and glutenins [3], other environmental factors such as breastfeeding, time of gluten introduction, or the mode of delivery have been proposed to be associated with the risk for CD. While the influence of some of these environmental factors has been clarified or is still a question of debate [4,5], the relevance of the risk alleles of the Human Leukocyte Antigen (HLA)-DQ2 and HLA-DQ8 for the development of CD is well established: Over 90% of CD patients carry the alleles encoding HLA-DQ2 molecule; most of the remaining CD patients carry the HLA-DQ8 heterodimers. However, although around 25% of the European general population is positive for the HLA-DQ2 heterodimer (and ~50% if HLA-DQ8 is also included), only a small fraction (~1%) will develop CD [6–8]. This indicates that the HLA-DQ2 and/or HLA-DQ8 risk alleles are necessary but not sufficient for the development of the disease [3]. Thus, other genetic and/or environmental factors besides gluten must be involved in the pathogenesis of CD and a closer investigation of the mechanisms involved in the activation and development of the disorder is needed.

Metabolomics, the study of small-molecule metabolite profiles, facilitates the characterization of several pathological conditions such as obesity or cardiovascular diseases [9,10]. The metabolites are intermediates and end products of cellular regulatory processes, and their levels can be regarded to be the result of the interaction of genome, epigenome, transcriptome, proteome, and the environment [11,12]. Investigating the metabolic profile of CD patients is thus a logical consequence. So far, only few studies investigated the differences in metabolic profiles between CD patients and healthy controls [13], and even fewer studies focused on the serum metabolic profile. The results of those studies, however, are quite promising revealing alterations in energy metabolism [14] and suggesting that metabolic alterations may precede the development of small intestinal villous atrophy [15].

Besides this cross-sectional view, it is also of major interest to investigate whether the metabolic profiles of children who will progress to CD later in life differ already at early age. The identification of metabolic markers would represent a significant advance and targets for early interventions and preventive strategies. For instance, studies on type 1 diabetes which shares common alleles with CD, have identified metabolic phenotypes that characterize the early pathogenesis of the disease [16,17]. Using data from the prospective cohort of the PreventCD study, we tested if the metabolic profile of 4 month old children at genetic risk for CD was associated with (1) the development of CD up to school age, (2) the HLA-risk groups, or (3) the age at CD diagnosis.

2. Material and methods

2.1. Study design

The PreventCD project is a prospective, randomized, double-blind, placebo-controlled, dietary-intervention study in children with high risk for CD [5,18]. The first child was included on May 26th, 2007, and the follow-up for this analysis closed on August 26th, 2015. Recruitment of the infants was done consecutively through CD organizations in Croatia, Germany, Hungary, Israel, Italy, the Netherlands, Poland, and Spain. Infants between 0 and 3 months of age were recruited if (i) they had at least one first-degree family member with CD confirmed by small-bowel biopsy, and (ii) were HLA-DQ2 or HLA-DQ8 positive, or otherwise carrying the allele DQB1*02 [18]. Premature infants or infants with syndromes associated with an increased risk of CD, such as trisomy 21 or Turner's syndrome, were excluded. The infants were randomized to the intervention groups and either received gluten (200 mg of vital wheat gluten mixed with 1.8 g of lactose) or placebo (2g of lactose) between 4 and 6 months of age. Randomization took place after blood withdrawal.

The study was approved by the medical ethics committee at each participating center and complied with Good Clinical Practice guidelines. The authors vouch for the veracity and completeness of the data and analyses reported and for the adherence of the study to the protocol.

2.2. Assessment of CD

Children with elevated levels of antibodies indicating CD and/or with clinical suspicion of CD are offered to undergo a small-bowel biopsy to diagnose the disorder. The diagnosis of CD was based on the histologic findings of the small-bowel biopsies, according to the criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) [19]. The age of the patient at the day of biopsy is considered to be the age at the diagnosis of CD.

2.3. Genotyping

Genotyping for HLA-DQ-alleles was performed by single nucleotide polymorphisms (SNPs) based on the tag-SNP approach (Department of Genetics University Medical Center Groningen, the Netherlands). The HLA-risk groups were defined as follows: (1) DR3-DQ2/DR3-DQ2 (DQ2.5/DQ2.5); 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); DR3- DQ2/other (DQ2.5/other); (4) DR7-DQ2/DR7-DQ2 (DQ2.2/DQ2.2); DR7-DQ2/DR4-DQ8 (DQ2.2/DQ8); DR4- DQ8/DR4-DQ8; (DQ8/DQ8)(5) DR7-DQ2/other (DQ2.2/other); DR4-DQ8/DR5-DQ7 (DQ8/DQ7); DR4-DQ8/other (DQ8/other), where other refers to any HLA-DQ haplotype except for DR3-DQ2, DR7-DQ2, DR4-DQ8 or DR5-DQ7 [5,20]:

2.4. Sample collection & quantification of metabolites

Measurements of serum antigliadin antibodies, TG2A, and metabolomics were performed centrally. Blood samples were collected and centrifuged and the frozen serum samples (−20 °C) were sent for determination of CD antibodies to Thermo Fisher Scientific, Freiburg, Germany. The rest of the serum was stored at −20 °C and sent to the central sera bank of the project at the department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, where it was stored also at −20 °C. Serum samples were available for 230 children who previously have been selected for a study on breastmilk composition during the first months of life and serum composition at 4 month (paper under submission). We took advantage of this collection for the purpose of this study. The sera of these 4 month old infants collected before the start of the dietary intervention were transferred on dry ice to LMU Munich and stored at −80 °C until metabolomic analysis.

Analysis of amino acids (AA) was performed as described previously [21]. Briefly, 10 μl of serum was prepared by derivatization

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