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Midpregnancy and cord blood immunologic biomarkers, HLA genotype, and pediatric celiac disease

To the Editor:

Celiac disease (CD) is a multisystem, immune-mediated disorder prevalent in 1% to 2% of many populations worldwide.[1] In CD, genetic and environmental factors interact in triggering a loss of tolerance to gluten. Observations have linked the perinatal environment to CD development in children.[2,3] This study therefore aimed to test whether immunologic biomarkers in midpregnancy or at birth predicted increased risk of childhood CD, while adjusting for genetic and environmental factors.

This case-control study was nested within the Norwegian Mother and Child Cohort (MoBa), a population-based study recruiting pregnant women across Norway during the period 1999-2008.[4] We included 413 MoBa-children diagnosed with CD by the end of 2013; CD was recognized via parental questionnaires and the International Classification of Diseases, 10th Revision code K90.0 in the Norwegian Patient Register. We validated CD in the cohort and found that more than 92% of the diagnoses were confirmed by the parents. We enrolled 568 random controls (see Fig E1 in this article's Online Repository at www.jacionline.org).

Using plasma samples collected around pregnancy week 18, we measured the following 18 cytokines representing T_H1-, T_H2-, T_H17-, and regulatory T-cell–mediated immune responses that have been associated with CD: CCL2, CCL3, CCL4, CXCL10, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-1 receptor antagonist, IL-2 receptor-α, and TNF. Selection of cytokines was also based on their stability in stored samples and potential cross-reactivity. The Online Repository includes an overview of each cytokine. The combined influence of T_H1 and T_H2 immunity was examined by computing summed *Z* scores for selected T_H1 (CCL3, CCL4, CXCL10, IL-12p70, IFN-γ, TNF) and T_H2 (CCL2, IL-4, IL-5, IL-13) cytokines, respectively. In cord blood samples, we measured the concentrations of neopterin and the kynurenine/tryptophan ratio (ie, the kynurenine concentration [nmol/L] divided by the tryptophan concentration [μmol/L]) using a high-throughput liquid chromatography tandem mass-spectrometry.[5] Neopterin and the kynurenine/tryptophan ratio have been used as inflammatory markers reflecting a T_H1 immune activation.[6]

To account for CD-associated genetic susceptibility, we classified the child's HLA genotype as conferring a high risk for CD (DQ2.5/DQ2.5), a moderate risk for CD (DQ2.5/X or a copy of DQ2.2 or DQ8), and a low risk for CD (any other haplotypes). We computed a non-HLA risk score for CD defined as the sum of 44 established risk alleles (see Table E1 in this article's Online Repository at www.jacionline.org).[7]

Logistic regression yielded odds ratios (ORs) for CD according to the log2-transformed concentration of each immunologic biomarker, examined as continuous variables and categorized into 3 groups (see Fig E2 in this article's Online Repository at www.jacionline.org). Subanalyses were restricted to children carrying minimum 1 copy of the DQ2.5 haplotype (CD, n = 306; controls, n = 128) and to children with CD diagnosis by age 5 years (CD, n = 154; controls, n = 568). All analyses were adjusted for maternal CD, pregnancy-related smoking (defined by self-reported data and the concentration of cord blood cotinine, a biomarker of nicotine exposure),[8] parity, calendar year and month of sampling, the child's sex, and HLA and non-HLA genotypes. In addition, analyses on midpregnancy samples were adjusted for maternal type 1 diabetes, and analyses on cord blood were adjusted for degree of visual hemolysis, delivery mode, birth weight, and preterm birth. In a separate model, we also included maternal infection frequency in pregnancy.

Following analyses on a simulated data set, the statistical significance was defined as *P* values of less than .01. This study was approved by the Regional Research Ethics Committee. Details regarding the validation of CD, laboratory methods including methods of genotyping, and covariates definitions are provided in this article's Online Repository at www.jacionline.org.

The median age by December 31, 2013, was 9.5 years among children with CD and 8.5 years among controls (see Table E2 in this article's Online Repository at www.jacionline.org). The median number of non-HLA risk alleles was 44 (range, 31-54) among cases compared with 42 among controls (range, 29-55); the HLA-DQ genotype was strongly associated with CD (see Fig E3 in this article's Online Repository at www.jacionline.org). The concentration of immunologic biomarkers showed only small differences according to previous freeze-thawing of samples (see Fig E4 in this article's Online Repository). ORs for CD per 2-fold increase in cytokine concentration in pregnancy ranged from 0.96 (95% CI, 0.93-1.00) for IL-17A to 1.08 (95% CI, 0.92-1.26) for CXCL10; neither inflammatory markers at birth differed significantly between cases and controls (Fig 1). Multivariate analyses yielded essentially unchanged results (see Fig E5 in this article's Online Repository at www.jacionline.org).

The ORs for childhood CD according to summed *Z* scores of cytokines reflecting midpregnancy T_H1- and T_H2-immunity approximated 1 (T_H1: OR, 0.99; 95% CI, 0.85-1.17; T_H2: OR, 1.01; 95% CI, 0.87-1.16). Analyses restricted to children diagnosed before age 5 years, to children carrying the HLA-DQ2.5 haplotype, or adjustment for maternal infections during pregnancy yielded essentially unchanged results compared with the main analyses (data not shown). Cytokine concentrations were largely similar among mothers with or without CD (see Table E5 in this article's Online Repository at www.jacionline.org).

This large-scale study revealed no significant differences for multiple immunologic biomarkers in pregnancy or at birth. Strengths include detailed, prospectively collected information on covariates, including genetic markers for CD. Although no CD screening was performed, it is unlikely that the association of immune markers in pregnancy with CD should differ largely according to diagnosis obtained by clinical examination versus screening. We acknowledge that the biomarkers in this study may not cover the heterogeneity in immunologic parameters between individuals and our conclusions are restricted to those biomarkers tested.

Research on immunologic biomarkers could offer better understanding of disease processes.[9] However, only 1 study has previously examined the influence of circulatory cytokines in pregnancy on offspring CD.[2] That study reported associations of elevated proinflammatory T_H1 cytokines in early pregnancy with CD in the child.[2] The reason for these contradicting findings compared with the present study is unclear, but could be owing to differing time window of sampling during pregnancy. It is also possible that some of the presented differences are attributable

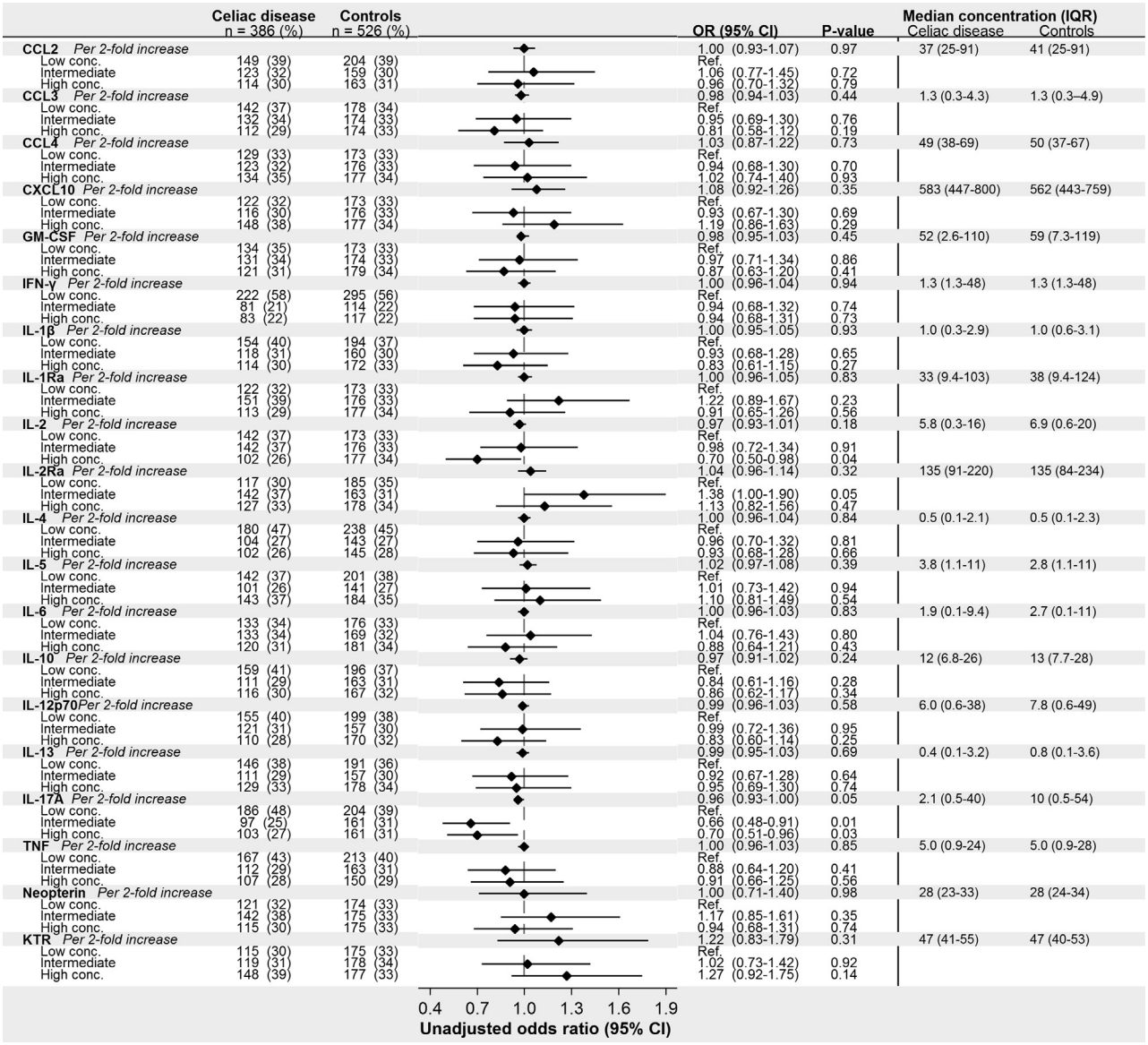

FIG 1. OR for offspring CD according to midpregnancy and cord blood immunologic biomarkers. Concentrations in ng/L, except for neopterin (nmol/L) and kynurenine/tryptophan ratio (KTR; nmol/μmol). Neopterin: 378 cases, 524 controls; KTR: 382 cases, 530 controls. *IL-1Ra*, IL-1 receptor antagonist; *IL-2Rα*, IL-2 receptor-α; *IQR*, interquartile range.

to chance because the previous study was relatively small (29 children with CD).

Our null findings in regard to childhood CD can be interpreted in several ways. First, the measured biomarkers and their mediated immune responses in pregnancy may not be linked to childhood CD. Second, despite our large sample size, factors causing reduced precision of our analysis will increase the risk of a type 2 error, for example, possible errors related to sampling procedures and storage. Third, our results do exclude local immunologic effects of cytokines or transient systemic effects of these acting at specific time windows of pregnancy. Finally, in some comparisons, for example, IL-17A, *P* values ranged between .01 and .05, suggesting that a broader study on T_H17 immunity in pregnancy and offspring CD may be warranted.

In conclusion, this study did not find significant associations between immunologic biomarkers in pregnancy and childhood CD. These findings do not support the hypothesis that the prenatal systemic immune response influences CD development in children.

Karl Mårild, MD, PhD[a]
Maria Vistnes, MD, PhD[a,b]
German Tapia, PhD[a]
Øivind Midttun, PhD[c]
Per M. Ueland, MD, PhD[d]
Marte K. Viken, PhD[e]
Per Magnus, MD, PhD[a]
Jens P. Berg, MD, PhD[f]
Lars C. Stene, PhD[a]
Ketil Størdal, MD, PhD[a,g]

From [a]the Norwegian Institute of Public Health, Oslo, Norway; [b]the Department of Internal Medicine, Diakonhjemmet Hospital, Oslo, Norway; [c]the Bevital AS, Bergen, Norway; [d]the Department of Clinical Science, University of Bergen and the Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway; [e]the Department of Immunology, Rikshospitalet, Oslo University Hospital, Oslo, Norway; [f]the Department of Medical Biochemistry, Oslo University Hospital and the Institute of Clinical Medicine, University of Oslo, Oslo, Norway; and [g]the Department of Pediatrics, Østfold Hospital Trust, Grålum, Norway. E-mail: karlmarild@gmail.com.

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Life-threatening NLRC4-associated hyperinflammation successfully treated with IL-18 inhibition

To the Editor:

Clinical application of several rapidly evolving technologies—next-generation DNA sequencing, biomarker discovery, and targeted cytokine blockade—has been particularly beneficial to understanding an expanding spectrum of genetically defined autoinflammatory diseases.[1] Our understanding of the pathways that cause hemophagocytic disorders, such as macrophage activation syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH), is evolving similarly. MAS and HLH are life-threatening sepsis-like conditions notable for hyperferritinemia, acute cytopenias, and hepatitis. If not promptly recognized and treated, they can progress to consumptive coagulopathy, hemophagocytosis, multiorgan failure, and high mortality. HLH is classically associated with genetic defects in cytotoxicity, whereas MAS is observed as a complication of rheumatic diseases.[1]

We recently implicated gain-of-function mutations in NLRC4, a protein that activates the inflammasome, in a syndrome of recurrent MAS with early-onset enterocolitis (NLRC4-MAS, OMIM#616050).[2,3] Inflammasomes are large innate immune complexes that quickly and exponentially catalyze the activation of pro–IL-1β and pro–IL-18. Although IL-1β blockade is effective in many "inflammasomopathies,"[1,2] the role of IL-1β in MAS is controversial. IL-1 blockade is effective in treating MAS-prone diseases, but was not protective against the development of MAS. The effects of blocking IL-18 are unknown. Patients with NLRC4-MAS have extraordinary and chronic elevation of serum IL-18[2,3]; and although extraordinary IL-18 levels are a feature of MAS more generally, IL-18 is only modestly elevated in other genetic inflammasomopathies.

Classically, myeloid cell–derived IL-18 enhances IL-12–driven IFN-γ production, but IL-18 can also synergize to promote various diverse immunological effects. Notably, IFN-γ is the cytokine most implicated in driving familial forms of HLH,[4] although its role in MAS is more controversial. IL-18 is also highly expressed in intestinal (and other) epithelial cells and has complex roles in intestinal homeostasis and colitis.[5] IL-18 binding protein (IL-18BP) is an endogenous protein that binds tightly to IL-18, preventing signaling but not serologic detection.[6]

Herein, we report a previously healthy white female who developed a parainfluenza upper respiratory tract infection at age 6 weeks. Cough, coryza, and viral RNA cleared, but hectic fevers and erythrodermic rash (Fig 1, *A* and *D*) persisted for several weeks. After 3 weeks of fever, she acutely developed oral intolerance and severe secretory diarrhea that persisted despite cessation of oral intake. Her condition worsened with acute rise in inflammatory markers, relative thrombocytopenia, and rising ferritin, consistent with an MAS-like syndrome. Thorough infection and malignancy workups were unrevealing, and there were no signs of immunodeficiency. Endoscopy showed severe mucosal ulcerations and inflammation extending from stomach through large intestine, with normal staining for intestinal regulatory T (Treg) cells (Fig 1, *B* and *C*; see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Functional assessment of cytotoxicity was normal. Clinical whole-exome sequencing returned a *de novo* heterozygous mutation in *NLRC4* (c.1022T>C, p.Val341Ala) within 2 weeks. This same mutation had previously been associated with a very similar, but dominantly inherited syndrome in a small kindred, including an infant who succumbed soon after birth.[3]

The patient was treated aggressively with corticosteroids (including 11 pulses of 30 mg/kg in 1 month) and IL-1 blockade (10 mg/kg/d anakinra) with minimal response (Fig 1, *D*). The addition of maximal doses of TNF-α-blockade (infliximab, up to 20 mg/kg), cyclosporine, and $\alpha_4\beta_7$-integrin inhibition (vedolizumab) resolved her fever and coagulopathy but did not