Gut sphingolipid metabolites in infants with atopic dermatitis are associated with food allergy

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Food allergy (FA) may be present in the range of 20–80% in atopic dermatitis (AD) [1, 2]. Food sensitization through the skin can cause FA due to damage to the skin barrier, and failure to acquire tolerance to food allergens in the gut can equally cause the development of FA [3]. Gut metabolites can influence the physical gut barrier and intestinal homeostasis [4]. Therefore, it is possible that gut metabolites related to gut immunity play an important role in the development of FA. Sphingolipids are key factors in cell inflammatory response and affect gut epithelial cells and skin barrier integrity and function [5]. Levels of sphingolipid were decrease in FA compared to those of control [6]. In other study, conversely, sphingolipid had significantly higher levels in FA than in controls [7]. Therefore, the sphingolipid levels in FA are still debatable. Gut diacylglycerol (DAG), a product of the metabolic reaction between ceramides and sphingomyelines, was increased in FA [8]. In asthma, one of the important signaling pathways associated with the activation of T lymphocytes involves the generation of a lipid messenger known as DAG [9]. However, there are no reports of FA-associated gut sphingolipid in infants by targeted metabolomics. In our previous study, we showed that when FA is present in various phenotypes of AD in early life, it might be associated with the later development of asthma [10]. The discovery of a biomarker that can distinguish the phenotypes that accompany AD and FA from other AD phenotypes is therefore expedient. Consequently, we aimed to investigate whether FA in AD infants can be classified as gut sphingolipid metabolites using targeted metabolomics.

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The study population consisted of 158 six-month-old infants (46 healthy infants, 30 only AD group, and 82 with combined AD and FA) involved in the Cohort for Childhood Origin of Asthma and Allergic Diseases (COCOA) [11], which was a general population-based birth cohort. The baseline characteristics of the subjects are presented in Table S1. Detailed methods are provided in this article's Online Supplement.

The DAG, ceramide and sphingomyelins were higher in the AD with FA group compared to those of controls and only AD group (Figures S1A-C). Sphingosine was higher in the AD with FA group compared to that of only AD group (Figure S1D). Whereas sphingosine-1-phosphate (S1P) was lower in AD with FA group compared to that of controls (Figure S1E). There were no significant differences in sphinganine among the three groups (Figure S1F). The DAG and sphingomyelins were positively correlated with total IgE and specific IgE to food allergens (Figure S2). The S1P was weak negatively correlated with specific IgE to milk (Figure S2C).

This study showed that gut sphingolipid metabolites can distinguish cases with FA among the AD phenotypes**.** These metabolites were associated with total IgE level and specific IgE levels to food allergens. These results suggest that the difference in the composition of gut sphingolipid metabolites is associated with FA and food sensitization in FA in AD infants. The sphingomyelin, ceramide, and sphingosine can be phosphorylated to S1P. An increase in sphingomyelin, ceramide, sphingosine, and a decrease in S1P in AD infants with FA suggest the problem of the sphingolipid mechanism to synthesize S1P. Milk-derived sphingomyelin promotes an iNKT cell-mediated Th2-type cytokine that boosts sensitization to food allergens [12]. The sphingomyelin and ceramide are significantly increased in patients with inflammatory bowel disease and the colitis animal model [13]. Accumulated ceramide in tight junctions alters the lipid composition, contributing to a disturbed barrier function. Moreover,

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in the colitis model and IL-10 knockout mice, sphingomyelin triggers apoptosis in intestinal epithelial cells and aggravates intestinal inflammation [13]. Therefore, in AD, changes in gut sphingolipid lead to the development of FA due to gut barrier damage and inflammation.

In our study, the gut sphingolipids in only AD tended to decrease more than that of controls (Figure S1). In a previous study, it was found that a sphingolipid module comprising several metabolites involved in de novo sphingolipid synthesis metabolism was significantly more elevated in subjects with food sensitization compared to those with FA [8]. The major difference was that the changes in the sphingolipid module of feces were performed with untargeted metabolomic profiling without considering AD [8], whereas, in this study, single sphingolipid metabolite analysis through targeted metabolomics was conducted according to the AD phenotype.

In this study, among many of the sphingolipids, 15 sphingolipids were chosen because they contain major fatty acid compositions and commercially available. As time has passed, more sphingolipids have become commercially available. However, regrettably, these newly available sphingolipids were not included in the scope of this study. Not all gut sphingolipids including ceramides, DAG, and sphingomyelin could be measured in this study, but nevertheless, the most of measured gut sphingolipid show differences between only AD and AD with FA groups. Further studies are needed to study various other sphingolipids, such as ceramide-1-phosphate (C1P), lactosylceramide (LacCer), hexosylceramide (HexCer) and dihydroceramides (DHCer).

To date, previous studies on sphingolipid metabolites in FA and AD have been limited to serum and skin samples by untargeted metabolite profiling. To our knowledge, this is the first study exploring gut sphingolipid metabolite profiles using targeted metabolomics in AD according

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to FA.

Restricted diets in children with FA might eventually affect their metabolic profiling. Therefore, the alteration of gut sphingolipids may be the result of dietary restrictions to suppress FA symptoms. Therefore, it is unclear in this study whether this result is the cause of FA or a secondary phenomenon caused by a diet restriction due to FA. However, since milk and eggs are sphingolipid-rich foods, sphingolipids tended to increase in subjects with milk or egg allergy probably due to a mechanism other than the effect of food restriction. Further research related to dietary restrictions is needed in this regard. In conclusion, our results indicate that gut sphingolipid metabolites may play a role in the development of FA in infants with AD.

Conflict of interest

The authors have no conflicts of interest relevant to this study to declare.

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