residual WASP function may be associated with milder clinical phenotypes, and a functional assay that could demonstrate lowered but not defective function in this population may allow disease risk stratification as well as diagnosis via one assay.

In summary, we demonstrated the efficacy of a whole-blood flow cytometry–based staining protocol as a tool to quickly diagnose patients with WAS with up to 89% sensitivity and 100% specificity. However, WAS diagnosis cannot be excluded in clinically suspicious cases when WASP is present. Inclusion of clinical markers and other possible functional assays in a combined algorithm could improve the overall accuracy for diagnosing WAS.

Samuel C. C. Chiang, PhD[a]
Sue M. Vergamini, BS[a]
Ammar Husami, BS[b]
Lisa Neumeier, MT[a]
Kathryn Quinn, MS[a]
Teresa Ellerhorst, BS[a]
Linda Sheppard, BS[a]
Carrie Gifford, MBA[a]
David Buchbinder, MD[c]
Avni Joshi, MD[d]
Marianne Ifversen, MD, PhD[e]
Gary I. Kleiner, MD, PhD[f]
James B. Bussel, MD[g]
Shanmuganathan Chandrakasan, MD[h]
Robert D. Pesek, MD[i]
Tamara C. Pozos, MD, PhD[j]
Melissa J. Rose, DO[k]
Amy M. Scurlock, MD[i]
Kejian Zhang, MBA, MD[b,l]
Yenan T. Bryceson, PhD[m,n]
Jack Bleesing, MD, PhD[a]
Rebecca A. Marsh, MD[a]

From the Divisions of [a]Bone Marrow Transplantation and Immune Deficiency and [b]Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; [c]CHOC Children's Hospital, Orange, Calif; [d]the Division of Pediatric Allergy and Immunology, Mayo Clinic Childrens Center, Rochester, Minn; [e]the Department of Pediatric and Adolescent Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [f]the Department of Pediatrics, University of Miami Miller School of Medicine, Miami, Fla; [g]Weill Cornell Medical College, New York, NY; [h]the Division of Bone Marrow Transplant, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, Ga; [i]Arkansas Children's Hospital and the University of Arkansas for Medical Sciences, Little Rock, Ark; [j]Pediatric Infectious Diseases and Immunology, Children's Minnesota, Minneapolis and St Paul, Minn; [k]Nationwide Children's Hospital and the Ohio State University College of Medicine, Columbus, Ohio; [l]Coyote Bioscience, Campbell, Calif; [m]the Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden; and [n]Broegelmann Research Laboratory, Department of Clinical Sciences, University of Bergen, Bergen, Norway. E-mail: sam.chiang@cchmc.org.

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Intestinal microbial-derived sphingolipids are inversely associated with childhood food allergy

To the Editor:

Food allergy is a life-threatening disease that is common and increasing in prevalence, yet the factors leading to its development are poorly understood.[1] Microbial composition has been associated with risk of food allergy,[2] and integrative analysis of the human intestinal microbiome and metabolome could provide insights into mechanisms of microbial-associated pathogenic changes.[3] Here, we performed a prospective, untargeted, integrative analysis of the intestinal bacterial microbiome and metabolome during infancy, testing associations with the development of clinical food allergy and sensitization to foods at age 3 years. Our goal was to identify microbial-associated metabolites that were associated with food allergy or sensitization.

For detailed methods, see this article's Methods section in the Online Repository at www.jacionline.org. Subjects were offspring of participants in the Vitamin D Antenatal Asthma Reduction Trial (NCT00920621),[4] a multicenter randomized controlled trial of vitamin D supplementation in pregnancy to prevent asthma in offspring. The study protocol was approved by the institutional review boards at each center and all participants provided written informed consent. Food allergy and sensitization at age 3 years were based on parental questionnaire responses and serum specific IgE testing. Stool samples were collected between age 3 and 6 months from 333 subjects. Microbiome composition analysis by bacterial 16S rRNA sequencing and metabolomic analysis with ultraperformance LC/MS-MS were performed on stool samples from 12 children with food allergy (see Table E1 in this article's Online Repository at www.jacionline.org), 32 with food sensitization, and 37 controls.

Subjects were well matched on baseline characteristics, with a few exceptions including age, solid food introduction at stool sample collection, and asthma/recurrent wheeze at age 3 years (see Table E2 in this article's Online Repository at www.jacionline.org). Of several potential determinants of the intestinal microenvironment analyzed, only mode of delivery differed by phenotype, with a higher percentage of subjects born by Cesarean section

SPHINGOLIPID MODULE	BILE ACID MODULE	DIACYLGLYCEROL MODULE	
3-ketosphinganine	Ursocholate	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	1-palmitoyl-2-linoleoyl-digalactosylglycerol (16:0/18:2)*
Sphinganine	Ursodeoxycholate	oleoyl-linolenoyl-glycerol (18:1/18:3) [2]*	**palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]***
3-hydroxypalmitate	Glycoursodeoxycholate	linoleoyl-linoleoyl-glycerol (18:2/18:2) [2]*	palmitoyl-linoleoyl-glycerol (16:0/18:2) [1]*
13-methylmyristate	Tauroursodeoxycholate	linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]*	**oleoyl-linoleoyl-glycerol (18:1/18:2) [2]**
N-palmitoylserine	Isoursodeoxycholate	**linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]***	oleoyl-linoleoyl-glycerol (18:1/18:2) [1]
Trimethylamine N-oxide	Dopamine	linoleoyl-linolenoyl-glycerol (18:2/18:3) [1]*	**2-aminophenol**
Hypoxanthine	**Tryptamine**	oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	Xylose
	D-urobilin	oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	
	I-urobilinogen	palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	

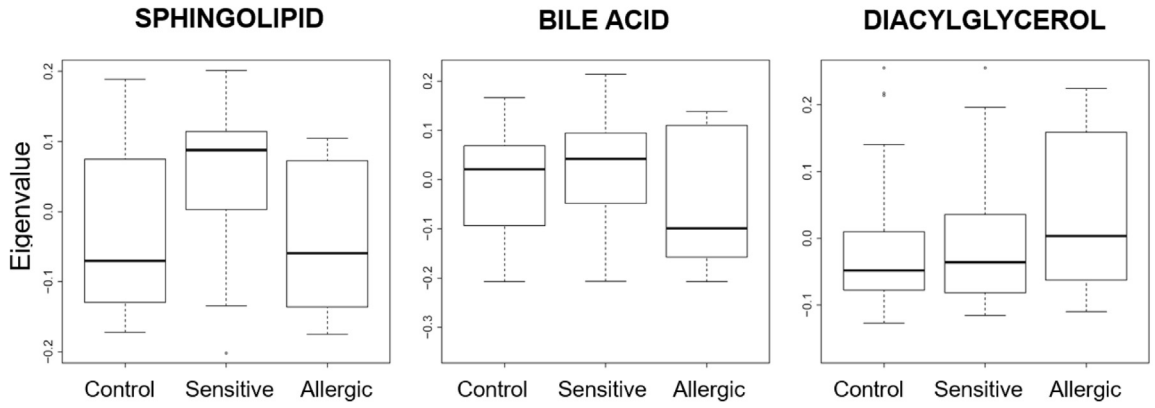

FIG 1. Metabolite members of metabolite modules associated with food allergy or sensitization and box plots of module eigenvalues. Box plots summarize module eigenvalues for subjects with food allergy (n = 12), food sensitization (n = 32), and controls (n = 37). *Compounds with annotations that have not been officially confirmed on the basis of a standard. Bolded compounds were associated with phenotype in analyses of individual metabolites.

among those with food allergy and a lower percentage among those with food sensitization. Accordingly, we adjusted for age in all analyses, performed sensitivity analyses of key results adjusting for other potential confounders, and tested for associations between mode of delivery and phenotype-associated microbiome and metabolome perturbations.

Logistic regression analyses revealed several individual metabolites that differed in relative abundance by food allergy/sensitization phenotype (see Table E3 in this article's Online Repository at www.jacionline.org). Weighted gene coexpression network analysis identified 29 modules of highly correlated and likely functionally related metabolites. Eigenvalues of 3 modules were associated ($P < .05$) with food allergy or sensitization (Fig 1; see Table E4 in this article's Online Repository at www.jacionline.org). We focused on a module that included several metabolites associated with *de novo* sphingolipid synthesis (sphinganine, 3-ketosphinganine, 3-hydroxypalmitate, *N*-palmitoylserine, 13-methylmyristate) that had significantly higher eigenvalues in subjects with food sensitization than in those with food allergy ($P = .02$) or controls ($P = .02$), and nonsignificantly higher eigenvalues in controls than in those with food allergy ($P = .15$). This pattern suggests that this module might be associated with protection from clinical food allergy, with the most pronounced protective effect among food-sensitized individuals.

16S rRNA sequencing revealed 6 operational taxonomic units (OTUs), all of the genus *Bacteroides*, that were positively associated with the sphingolipid metabolite module and positively associated with food sensitization compared with food allergy (see Tables E4 and E5 in this article's Online Repository at www.jacionline.org). Sphingolipids are produced by a minority of bacteria, including *Bacteroides* species.[5] Mediation analysis showed that 95% of the association between having nonzero relative abundance of at least 1 of the 6 *Bacteroides* species OTUs and food sensitization was mediated by the sphingolipid module, with the proportion mediated ranging from 53% to 84% for individual sphingolipid metabolites (*P* value for indirect effect < .05 for all) (see Table E6 in this article's Online Repository at www.jacionline.org). We investigated the possibility that Cesarean section could increase food allergy