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Original Article

Interactions Between *IL-17* Variants and *Streptococcus* in the Gut Contribute to the Development of Atopic Dermatitis in Infancy

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ABSTRACT

Purpose: Interleukin (IL)-17 variants and perturbations in the gut microbiota may influence the development of atopic dermatitis (AD). However, unifying principles for variants of host and microbe interaction remains unclear. We sought to investigate whether *IL-17* variants and gut microbiota affect the development of AD in infancy.
Methods: Composition of the gut microbiota was analyzed in fecal samples from 99 normal healthy and 61 AD infants at 6 months of age. The associations between total immunoglobulin E (IgE), the scoring atopic dermatitis (SCORAD), short-chain fatty acids, transcriptome and functional profile of the gut measured in these subjects and *Streptococcus* were analyzed. IL-6 and IL-8 in the human intestinal epithelial cell line (HIEC-6) were measured after stimulation of IL-17 and *Streptococcus mitis*.
Results: In this study, *Streptococcus* was enriched in infants with AD and was higher in those with the GA + AA of *IL-17* (rs2275913) variant. *Streptococcus* was positively correlated with IgE and SCORAD in infants with AD and GA + AA of *IL-17*. Butyrate and valerate were negatively correlated with *Streptococcus* and were decreased in infants with AD and GA + AA. Bacterial

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Disclosure

There are no financial or other issues that might lead to conflict of interest.

genes for oxidative phosphorylation induced by reduced colonization of *Clostridium* were decreased compared with normal and GG. In transcriptome analysis, lactate dehydrogenase A-like 6B was higher in infants with AD compared with healthy infants. IL-6 and IL-8 were increased in IL-17 and/or *S. mitis*-stimulated HIEC-6 cells.

Conclusions: These findings suggest that increased *Streptococcus* and A allele of *IL-17* (rs2275913) may contribute to the pathogenesis of AD via modulation of the immune system in infancy.

Keywords: Atopic dermatitis; gastrointestinal microbiome; *Streptococcus*; *IL-17*

INTRODUCTION

Atopic dermatitis (AD) is one of the most common inflammatory diseases in children. Several studies have shown that the prevalence of AD has increased significantly over the last 3 decades, particularly in developed countries.[1] Although reasons for this increase are not fully understood, the etiology of AD is believed to be multifactorial with an interaction between genetics, immune, and environmental factors, such as birth mode, use of antibiotics, intake of westernized food, and low helminth burden, which is in line with the 'hygiene hypothesis.'[2,3] These environmental factors contribute to an alteration in the composition of the gut microbiota and a subsequent change in the immune system.[4,5] Many recent studies have indicated that the gut microbiota is involved in the pathogenesis of allergic diseases, including AD, in infants.[6-8] These data imply that the gut microbiota play a critical role in the development of AD.

Host genetic variants can affect the variation of the gut microbiota between individuals. For example, the gut microbiota of monozygotic twins has a significantly higher degree of similarity than that of dizygotic twins.[9,10] In addition, *Bacteroides fragilis* delivers immunomodulatory molecules to immune cells through the outer membrane vesicles (OMVs). Immune cells from human subjects with a major risk variant in *ATG16L1* inflammatory bowel disease (IBD)-associated genes are defective in regulatory T cells responses to OMVs.[11] Taken together, these studies indicate that host genetics influences the composition of the human gut microbiota and can do so in ways that impact on host disease development. However, no studies have investigated the interaction between the host genetic variants and the gut microbiota of individuals in relation to AD.

Th17 cells define a subset of T-helper cells that mainly produce interleukin (IL)-17A, IL-17F, and IL-22, which play a critical role in inflammation.[12] In gut mucosal immunity, IL-17-expressing cells were increased in the gut lamina propria of patients with Crohn's disease[13] and the expression of IL-17 was increased in the mucosa and serum of subjects with IBD.[14] In addition, several bacteria are involved in the differentiation of Th17 cells in the lamina propria of mice[15,16] and IL-17 is associated with the colonization of *Streptococcus pyogenes* in the human genital tract, but not in the gut.[17] From a genetic perspective, A allele of *IL-17* variant (rs2275913) in the promoter affects the expression of IL-17[18] and is associated with the development and severity of AD.[19,20] These previous studies demonstrate that the *IL-17* variant (rs2275913) and gut microbiota are likely to affect the development of AD, either individually or in combination. However, this process remains unclear in patients with AD. To address this, the current study sought to investigate whether the gut microbiota is controlled by the *IL-17* variant rs2275913 and whether an interaction between the 2 factors affects the development of AD in infants.

MATERIALS AND METHODS

Subjects

Patients were enrolled from the Cohort for Childhood Origin of Asthma and Allergic Diseases birth cohort and the Asthma and Allergy Clinic of the Asan Medical Center of South Korea. Infants who received antibiotics prior to fecal collection were excluded. AD was diagnosed at 6 and 12 months of age by a pediatric allergist and total serum immunoglobulin E (IgE) levels were measured using the UniCAP fluoroenzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden) at 12 months of age. The scoring atopic dermatitis (SCORAD) index[21] was assessed by a pediatric allergist at 6 and 12 months of age. This study was approved by the Institutional Review Boards (IRBs) of the Asan Medical Center (IRB No. 2008-0616 and 2015-1031), Samsung Medical Center (IRB No. 2009-02-021), Severance Hospital (IRB No. 4-2008-0588), the CHA Medical Center (IRB No. 2010-010), and the Seoul National University Hospital (IRB No. H-1401-086-550). Written, informed consent was obtained from the parents of each infant.

Fecal sample collection, 16S ribosomal RNA (rRNA) gene pyrosequencing, and MiSeq sequencing

Fecal samples were collected from infants at 6 months of age and were immediately frozen at −80°C before being processed for DNA extraction. Briefly, DNA was extracted using the Power Microbiome RNA/DNA Isolation kit (Mo Bio/Qiagen, Carlsbad, CA, USA) according to the manufacturer's instructions. For pyrosequencing (106 infants) and MiSeq sequencing (54 infants), the extracted DNA was amplified using barcoded primers targeting the V1-V3 region of the 16S rRNA gene. The details are described in a previous study.[6,22] To resolve the difference between 2 sequencing methods, commonly detected bacteria were included in the microbiome analysis.

Genotyping of the *IL-17* (rs2275913) variant

Genomic DNA was isolated from cord blood using the Gentra Puregene® Blood kit (Qiagen, Germantown, MD, USA). The *IL-17* variant (rs2275913) was genotyped using a TaqMan assay (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. The assay ID number for the SNP was C_15879983_10. The final volume from polymerase chain reaction (PCR) was 5 μL, containing 10 ng genomic DNA, 2.5 μL TaqMan Universal PCR Master Mix, and 0.13 μL of 40 × assay mix. All PCRs were performed in 384 well plates using a 384 Well Veriti thermal cycler (Applied Biosystems). The endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative controls were included to ensure genotyping accuracy.

Measurement of short-chain fatty acids (SCFAs)

The levels of SCFAs (acetate, propionate, butyrate, and valerate) were measured from fecal samples of infants at 6 months of age. Briefly, 100 mg fecal samples were diluted at a ratio of 1:4 (w/v) in deionized water. The samples were then vigorously vortexed and supernatants were collected after centrifugation. Supernatants were extracted by shaking with internal standard solution, 20 μL 0.6 N HCl, and 2 mL diethyl ether. After centrifugation, the upper layer was collected and was completely dried under vacuum. The samples were reconstituted with 100 μL acetonitrile. SCFAs were analyzed by gas chromatography-mass spectrometry (7890A/5975A; Agilent, Waldbronn, Germany) and a HP-5MS 30 m × 250 μm × 0.25-μm column (Agilent 19091S-433). The details are described in a previous publication.[23]

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Whole-metagenome sequencing and analysis

A total of 88 samples with a sufficient amount of feces were selected from each group (19 normal with GG genotype, 34 normal with GA + AA genotypes, 11 AD with GG genotype, and 24 AD with GA + AA genotypes) and used for whole-metagenome sequencing. The details are described in a previous publication.[6]

Colonocyte isolation from fecal samples and transcriptome

Transcriptome data were generated for 20 subjects (10 normal and 10 AD). For the isolation of exfoliated colonocytes, 0.5 g of the fecal samples were thawed, vortexed with 10 mL phosphate-buffered saline (PBS), and filtered through a 40-μm cell strainer (SPL, Seoul, Korea). The resulting filtrate was gently overlaid on Histopaque-1077 (Sigma Aldrich, St. Louis, MO, USA) and centrifuged at 400 × *g* for 30 minutes at room temperature. After centrifugation, the layer containing cells was washed twice with 10 mL PBS. Total RNA was extracted using the RNeasy Mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Gene expression analysis was performed using Affymetrix GeneChip Human Gene 2.0 ST Arrays (Thermo Fisher Scientific, Inc., Waltham, MA, USA). All of the procedures, including array hybridization and scanning, were performed independently according to the standard protocol.

Human intestinal epithelial cell-6 (HIEC-6) cell stimulation by IL-17 and *Streptococcus mitis*

The HIEC-6 cell line (CRL3266) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and was maintained at 37°C in a humidified incubator at 5% CO_2 in opti-MEM (Gibco, Grand Island, NY, USA) culture medium supplemented with 20 mM HEPES buffer, 10 mM GlutaMAX, 10 ng/mL epidermal growth factor, 4% fetal bovine serum, and 1% penicillin-streptomycin. *S. mitis* strain (ATCC® 49456™) was obtained from the ATCC and grown overnight in brain heart infusion broth supplemented with 0.4% yeast extract and 1% b-cyclodextrin at 37°C in a humidified bacterial incubator at 10% CO_2. The *S. mitis* was pelleted, washed in sterile PBS, and resuspended in sterile opti-MEM media without antibiotics at a concentration of 5×10^7 bacteria/mL. Recombinant human IL-17 (R&D Systems, Lille, France) was resuspended in sterile PBS. HIEC-6 cells (5×10^5) were seeded in a 60 mm cell culture dish. The next day, the cells were washed with PBS and cultured in media without antibiotics. HIECs were cultured in opti-MEM (Gibco) culture medium either alone or in the presence of IL-17 only for 24 hours, *S. mitis* (multiplicity of infection 1:100) only for 6 hours, or a combination of both. In the case of the combination, after 18 hours of IL-17 stimulation, *S. mitis* was added and incubated for 6 hours; the cells were harvested at the same time.

Measurement of IL-6 and IL-8

The amount of the pro-inflammatory cytokines IL-6 and IL-8 produced by HIEC-6 in response to IL-17 alone, *S. mitis* alone or a combination of both was measured using real-time PCR. Total RNA was extracted using the RNeasy Mini kit (Qiagen) and complementary DNA was synthesized using the WizScript™ cDNA Synthesis Kit (Wizbio, Seongnam, Korea) according to the manufacturer's instructions. Real-time PCR was carried out using predesigned TaqMan primers and probes for IL-6 (assay No, Hs00174131_m1), IL-8 (assay No, Hs00174103_m1), and glyceraldehyde 3-phosphate dehydrogenase (assay No, Hs02786624_g1) as an endogenous control. Analysis was carried out using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems) in accordance with the manufacturer's instructions.

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Statistical analysis

Continuous variables were compared among the groups by the Kruskal-Wallis test, and categorical variables were compared using Fisher's exact test. Continuous variables after adjustment for diagnosis of AD, delivery mode, and feeding type were compared using the general linear model. Linear discriminant analysis effect size (LEfSe) was used to elucidate differences in bacterial taxa among the groups. The difference of *Streptococcus* according to IL-17 variant and AD was compared by analysis of variance (ANOVA), followed by Tukey's test. Correlations between 2 continuous variables were analyzed by Spearman correlation coefficient. In whole-metagenome sequencing analysis, the number of bacteria contributing to the metabolic pathway used genus instead of species. Differentially expressed genes (DEGs) were filtered through statistical estimation of fold change (FC) ≥ 1.5 and $P < 0.05$ based on Student's *t*-test. Functional enrichment analysis was performed to identify significant biological pathways, including gene ontology terms. Results of real-time PCR are reported as the mean ± standard error of triplicate samples. ANOVA followed by Tukey's test was applied to *in vitro* studies. A *P* value < 0.05 was considered to indicate a significant difference.

RESULTS

Characteristics of the study subjects

A total of 160 infants (99 normal healthy controls, 61 patients with AD) were included. There were no significant differences in sex, gestational age, birth weight, delivery mode, or feeding type between healthy infants and infants with AD (**Table 1**). The total IgE levels were higher in the AD group compared with the normal healthy group ($P = 0.030$, **Table 1**).

Gut microbiota composition

The gut microbiota were first analyzed in the normal healthy and AD groups. Diversity indices (observed OTUs, Chao1, and Shannon diversity index) showed no significant differences between the 2 groups (**Supplementary Fig. S1**). However, a difference was seen between the groups in gut microbial taxa using LEfSe. Excluding low abundance (<1% mean relative abundance in each group and distributed to <30% of the subjects), the results indicated that *Streptococcus* and *Lactobacillus* were markedly increased in the AD group, while *Clostridium*, *Clostridium g4* and *Clostridium g6* were enriched in the normal healthy group (**Fig. 1A**). The relationship between the *IL-17* variant (rs2275913) and development of AD at 6 months of age was analyzed by a dominant model. The *IL-17* variant (rs2275913) was not associated with a significant change in the development of AD, SCORAD score at 6 month of age or total IgE levels at 12 months of age (**Supplementary Fig. S2**). The association between the *IL-17* variant

Table 1. Demographic characteristics of the study subjects

Characteristics	Normal healthy	AD	*P* value
No.	99	61	
Male/female	53/46	40/21	0.142*
Gestational age (wk)	39.40 ± 0.11	39.15 ± 0.20	0.723†
Birth weight (g)	3,265.92 ± 43.52	3,159.38 ± 60.51	0.295†
Delivery mode (% cesarean)	30.30	24.59	0.471*
Feeding type (% breast milk)	32.32	37.70	0.608*
Log IgE (IU/mL)	1.22 ± 0.06	1.51 ± 0.10	0.030†
Log SCORAD	ND	22.90 ± 2.26	NA
SCFAs (measurement %)	54.55	26.23	0.001*

Data are shown as the mean ± standard error.
AD, atopic dermatitis; IgE, immunoglobulin E; ND, not done; NA, not available; SCORAD, scoring atopic dermatitis; SCFA, short chain fatty acids.
*Fisher's exact test; †Mann-Whitney test.

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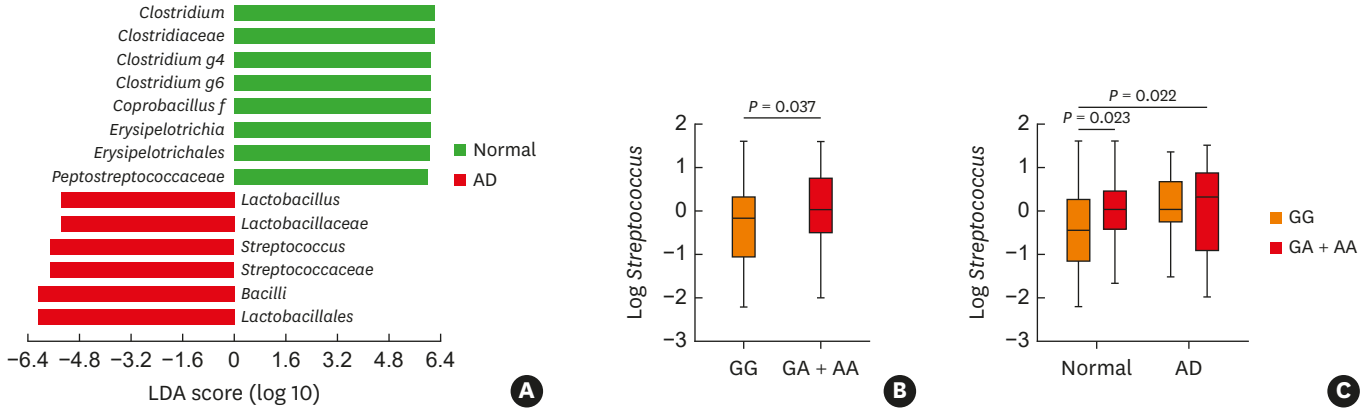

Fig. 1. Altered bacterial microbiota composition in patients with AD. (A) Differences in the gut bacterial communities between the normal healthy and AD groups, shown using LDA effect size analysis. (B) The relative abundance of *Streptococcus* according to the *IL-17* variant rs2275913. *P* value was adjusted by AD, delivery mode, and feeding type. (C) The relative abundance of *Streptococcus* according to the *IL-17* variant rs2275913 and AD at 6 months of age. Data are presented as mean ± standard error (B, C).
AD, atopic dermatitis; LDA, linear discriminant analysis.

(rs2275913) and AD-related gut microbial taxa (*Streptococcus*, *Lactobacillus*, *Clostridium*, *Clostridium g4*, and *Clostridium g6*) was then determined. *Streptococcus* was significantly increased in the GA + AA genotype of *IL-17* (rs2275913) variant after adjusting for diagnosis of AD, delivery mode, and feeding type (**Fig. 1B**). *Streptococcus* was enriched in infants with AD who had the GA + AA genotype versus normal healthy infants who had the GG genotype of *IL-17* (**Fig. 1C**). However, *Clostridium*, *Clostridium g4*, *Clostridium g6*, and *Lactobacillus* did not differ according to the genotype of *IL-17* variant (rs2275913; **Supplementary Fig. S3**). Analysis of persistence of AD according to *Streptococcus* mean value and the *IL-17* variant rs2275913 showed that AD patients with high (above mean) *Streptococcus* at 6 months of age and the GA + AA genotype of *IL-17* had more sustained persistence of AD until 12 months of age (**Supplementary Table S1**). In addition, *Streptococcus* abundance was not associated with the level of total IgE, regardless of the *IL-17* variant in normal healthy infants (**Fig. 2A**), but it showed a positive correlation with the level of total IgE and SCORAD in infants with AD who had the GA + AA genotype of the *IL-17* variant, but not in those with the GG genotype (**Fig. 2B and C**).

Complex association of SCFA-*IL-17* variant (rs2275913)-*Streptococcus* and AD

Butyrate and valerate, but not acetate and propionate, showed a negative correlation with the relative abundance of *Streptococcus* (**Fig. 3A-D**). The 4 groups according to the *IL-17* variant and AD showed no significant differences in terms of acetate and propionate levels (**Fig. 3E and F**). However, the abundance of butyrate and valerate was lowest in infants with AD who had the GA + AA genotype of *IL-17* variant (**Fig. 3G and H**). In addition, butyrate and valerate were negatively correlated with SCORAD at 12 months of age (**Supplementary Fig. S4**).

Different functional profiles according to *IL-17* variant (rs2275913) and AD

The relative abundance of genes associated with oxidative phosphorylation (OXPHOS), glycosaminoglycan degradation, PI3K-Akt signaling pathway, focal adhesion, antigen processing and presentation, and nucleotide-binding oligomerization domain-containing protein (NOD)-like receptor signaling pathway was significantly different among the 4 groups (**Fig. 4A** and **Supplementary Table S2**). *Clostridium* contributed to differences in the expression of genes associated with OXPHOS (**Fig. 4B**).

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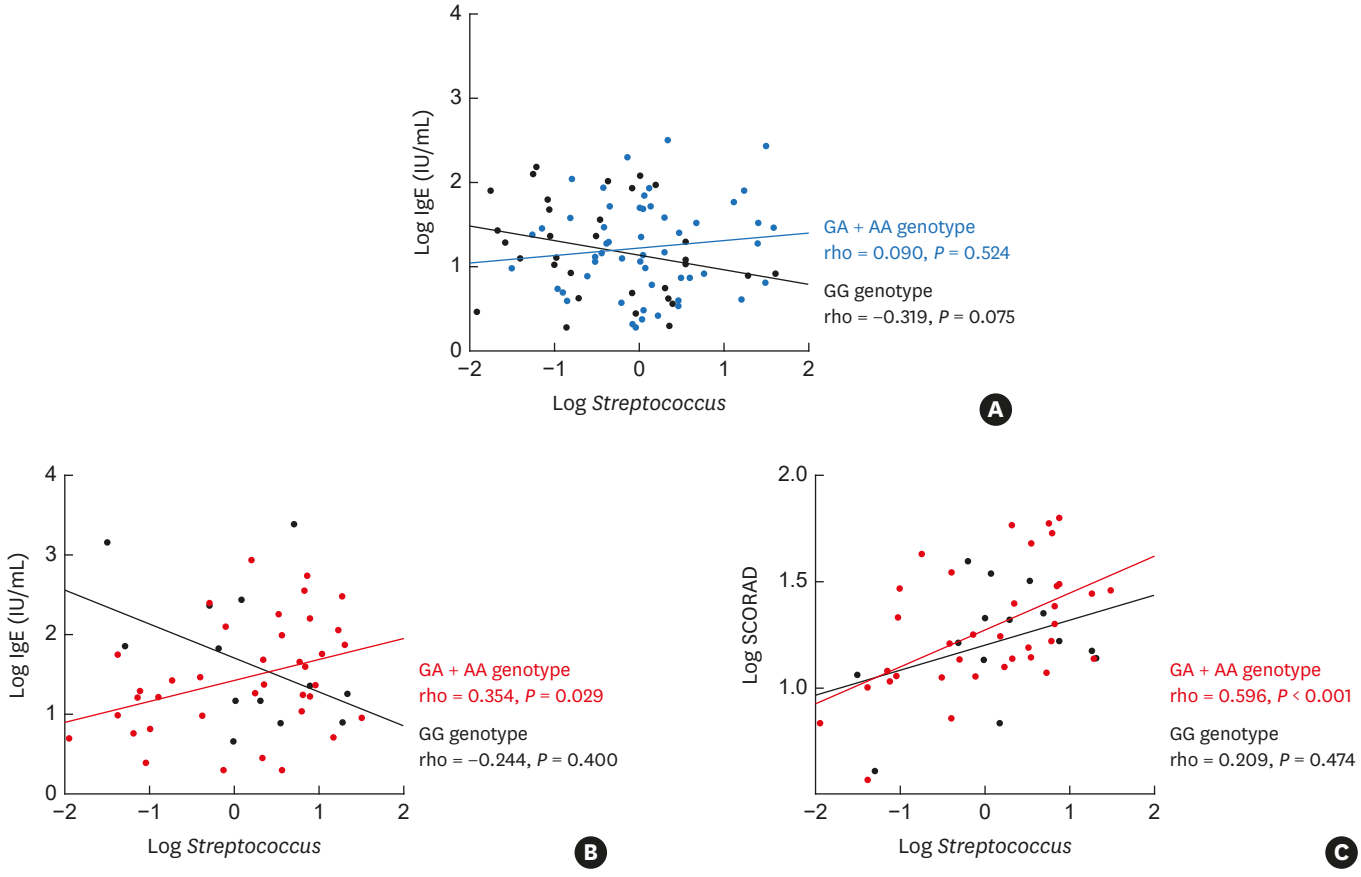

Fig. 2. Correlation between relative abundance of *Streptococcus* and total IgE levels according to the genotype of the *IL-17* variant rs2275913 in (A) normal healthy and (B) AD infants. (C) Correlation between *Streptococcus* and SCORAD score in AD infants at 6 months of age in AD infants.
IgE, immunoglobulin E; SCORAD, scoring atopic dermatitis; AD, atopic dermatitis.

Gene expression profiling of colonocytes between normal the healthy and AD groups

The colonocytes exhibited the most robust changes in gene expression with 651 DEGs between normal heathy and AD groups (**Supplementary Fig. S5A**). The 245 DEGs were up-regulated and 406 DEGs were down-regulated in infants with AD compared with normal subjects. The DEG mainly enriched to GO terms related to cellular process, single-organism process, and metabolic process (**Supplementary Fig. S5B**). Among the genes involved in metabolic process and cellular metabolic process (**Supplementary Table S3**), expression of lactate dehydrogenase A-like 6B (LDHAL6B) was higher in AD patients than in normal subjects (FC = 1.55 and $P = 0.003$). This gene is involved in the pathway that synthesizes lactate from pyruvate.

Inflammation after stimulation of HIEC-6 cells by IL-17 and *S. mitis*

To explore whether IL-17 and *Streptococcus* could affect intestinal inflammation, the expression of IL-6 and IL-8 was measured in HIEC-6 cells after stimulation by IL-17 and/or *S. mitis*. IL-17 and *S. mitis* induced the production of IL-6 and IL-8, respectively (**Fig. 5**). Simultaneous stimulation of these 2 factors tended to induce IL-6 and IL-8 to a greater extent than IL-17 or *S. mitis* alone (**Fig. 5**).

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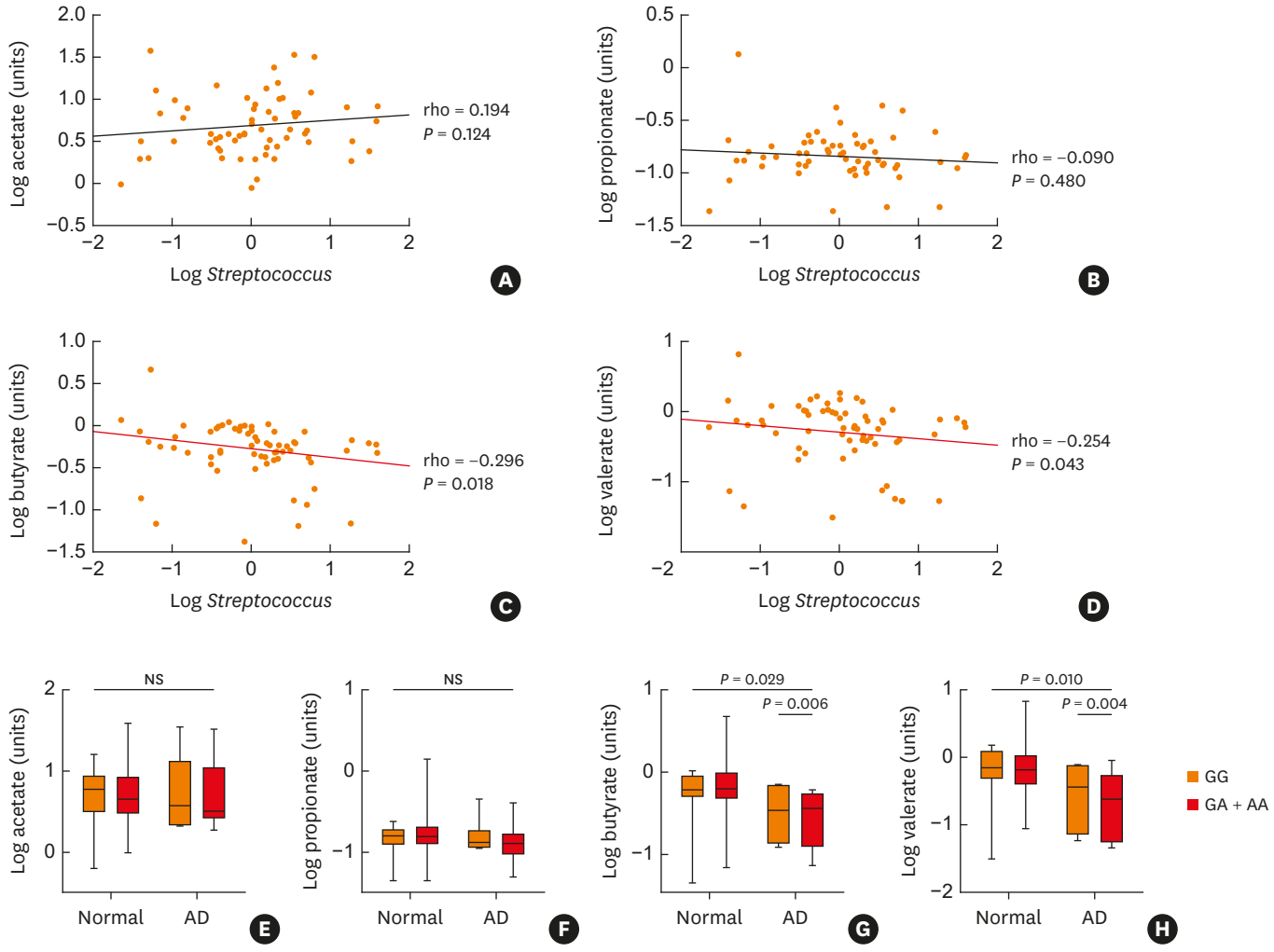

Fig. 3. Correlation between relative abundance of *Streptococcus* and (A) Acetate, (B) propionate, (C) butyrate, and (D) valerate. Comparison of (E) acetate, (F) propionate, (G) butyrate, and (H) valerate according to AD and the *IL-17* variant rs2275913. Data are presented as mean ± standard error.
AD, atopic dermatitis; NS, not significant.

DISCUSSION

This study has shown differences in gut microbiota, total IgE, SCORAD, SCFAs, functional genes of microbiota, and colonocyte expression according to the *IL-17* gene variant rs2275913 and AD in infants, and increased inflammatory cytokine production following IL-17 and *S. mitis* stimulation *in vitro*. An abundance of *Clostridium* or *Streptococcus* was higher in the healthy and AD groups, respectively, and a greater abundance of *Streptococcus* was seen in infants with the GA or AA genotype compared with GG of *IL-17* gene variant (rs2275913). Total IgE and SCORAD at 6 months of age were positively correlated with *Streptococcus* abundance in infants with AD who had the GA or AA *IL-17* variant rs2275913, but not in GG. Butyrate and valerate were lowest in infants with AD who had the GA or AA genotype, and were negatively correlated with *Streptococcus* and SCORAD at 12 months of age. In addition, differences in functional metabolic genes involved in OXPHOS and the contribution of *Clostridium* were found among groups according to the *IL-17* variant and AD. A high LDHAL6B expression was demonstrated in colonocytes of AD infants when compared with normal subjects. Moreover,

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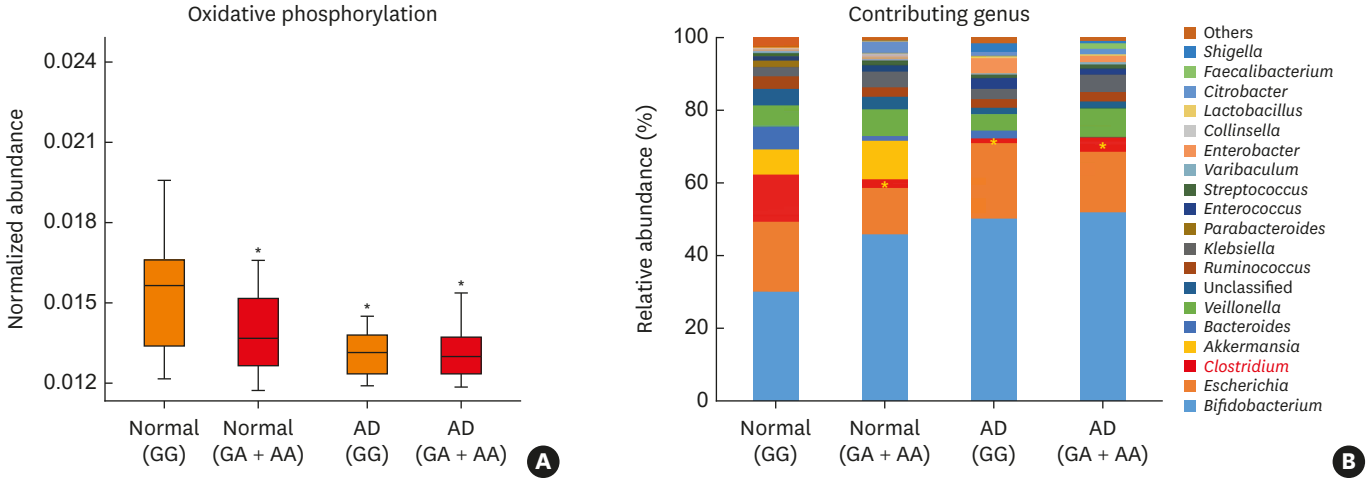

Fig. 4. (A) Comparison of functional genes related to oxidative phosphorylation and (B) their contributing genus according to AD status and the *IL-17* variant rs2275913 using whole-metagenome analysis of the gut microbiota in infants.
AD, atopic dermatitis.
*Indicate significant difference vs. the normal (GG) group.

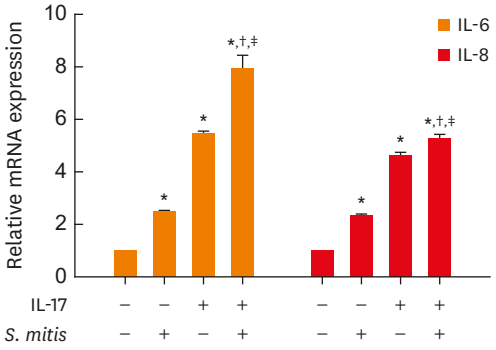

Fig. 5. Expression of IL-6 and IL-8 in normal human intestinal epithelial cell-6 cell line after stimulation by IL-7 and infection with *S. mitis*. Data are presented as mean ± standard error.
mRNA, messenger RNA; IL, interleukin.
*P < 0.05 vs. control group; †P < 0.05 vs. *S. mitis* (+) group; ‡P < 0.05 vs. IL-17 (+) group.

IL-17 and *S. mitis* induced the production of IL-6 and IL-8 in HIEC-6 cells. Collectively, these data demonstrate that the *IL-17* variant rs2275913 might be an important host genetic factor determining the development of AD in infants via an interaction with the gut microbiota and epithelium, and through increased inflammation and reduced SCFAs. These findings suggest that the interaction between host genetics and gut microbiota is important in the development of AD.

This study is the first to describe an association between the host genetic variant rs2275913 of *IL-17* and the microbiota (*Streptococcus*) in the development of AD in infants using multi-omics. Host genetics, immunity, and microbiota are intricately linked. Understanding host-microbe interactions is important to gain a better insight into human health. Early in the microbiome study, environmental factors, such as diet and medication, were known to affect the microbiota[24] and host genetics has recently been shown to be an important factor determining gut microbiota.[10,25] Many studies have used knockout mice to demonstrate a relationship between host-specific genes and microbiota. Mice lacking the *Card9* gene are more susceptible to colitis by alteration of the microbiota,[26] and *NOD2*-deficient mice

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have a reduced ability to prevent colonization of pathogens.[27] Recently, an association between single gene variants and microbial taxa was demonstrated in a targeted approach of candidate variants, where clear molecular mechanisms have been established. For example, a variant in the *NOD2* gene that has a well-known relationship with IBD is associated with *Enterobacteriaceae* in patients with IBD.[28] The *LCT* gene, responsible for lactose tolerance, and the *FUT2* gene, responsible for the transfer of the terminal fucose residues to the mucosal glycans, have been associated with the abundance of *Bifidobacterium.*[25,29] However, there is no study of the interaction between variants and microbiota in allergic diseases. Therefore, the current study presents compelling evidence, although future studies will be required to confirm the findings.

The rs2275913 variant in the *IL-17* gene promoter is significantly associated with allergic disease.[19,20,30] Two alleles of rs2275913 differentially have luciferase activity and bind the transcription factor NFAT, leading to differences in IL-17 secretion.[18] The IL-17 level from PHA-stimulated peripheral blood mononuclear cells (PBMCs) of healthy individuals with the A allele of rs2275913 was significantly higher than the G allele.[18] The plasma level of IL-17 was higher in healthy subjects with the AA genotype than with the GG genotype.[31] In addition, IL-17 contributes to the regulation of *S. pyogenes* (group A *Streptococcus*) colonization and promotes local cellular infiltration in the female genital tract.[17] It can be assumed from these previous studies that IL-17 increased by the A allele of rs2275913 may cause the development of AD with alterations in the gut microbiota in infants. To assess the influence of *IL-17* variant only on the gut microbiota, we analyzed the composition of gut microbiota after adjusting for AD status, feeding type, and delivery mode, which can affect the gut microbiota. We found that *Streptococcus* was higher in infants with the GA or AA genotype than with the GG genotype. A previous study has reported that *S. mitis*, *Streptococcus sanguí*, and *Streptococcus gordonii* induced IL-6 production in healthy smooth muscle cells.[32] In the current study, investigation of whether *S. mitis* induces inflammatory cytokines in HIEC showed increased mRNA levels of IL-6 and IL-8 in HIEC-6 cells after stimulation by *S. mitis*. IL-17 is also known to increase IL-6 and IL-8 in HIEC in a dose-dependent manner[33] and contribute to chronic inflammation.[34] Therefore, HIEC-6 cells were cultured with or without IL-17 alone or in combination with *S. mitis*. IL-17 had a greater effect than *S. mitis* and a 2.5-FC induced greater mRNA expression of IL-6 and IL-8 when combined with *S. mitis*. However, further research is required to understand how IL-17 and *Streptococcus* influence each other.

In general, the gut of infants after birth is an aerobic environment and is initially dominated by various facultative bacteria, such as representatives of *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus*.[35] Once available oxygen is consumed, anaerobes such as *Bifidobacterium*, *Bacteroides*, and *Clostridium* gradually proliferate and eventually establish an anaerobic environment in the first year of life.[36] Microbial colonization of the infant during this critical time is important to maintain homeostasis of the immune system. SCFAs, which are the major metabolic products of anaerobic bacterial fermentation, have been suggested to be the link between microbiota and host immunity, including inflammation.[37] One of the anaerobic and butyrate-producing colon bacteria, *Clostridium* (*Clostridiaceae* family), was enriched and butyrate was higher in the normal healthy group compared with AD. In whole-metagenome analysis, functional genes involved in OXPHOS were highest in normal subjects who had the GG genotype and *Clostridium* influenced OXPHOS. Colonocytes utilize bacterially produced butyrate as their primary energy source.[38] Butyrate is known to stimulate mitochondrial OXPHOS[39] and Treg cells use OXPHOS to synthesize ATP.[40] Butyrate also regulates the immune response and Treg/Th17 balance through expansion of Treg cell populations in the

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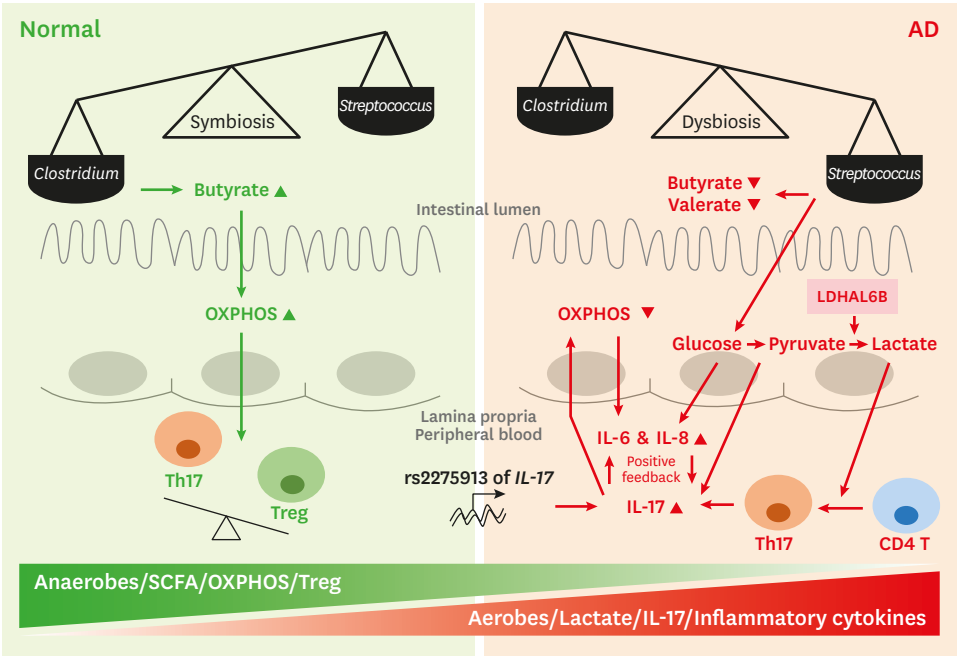

Fig. 6. Schematic of putative interactions between host gene (*IL-17*) and microbiota-derived SCFA and metabolism on AD development.
OXPHOS, oxidative phosphorylation; SCFA, short chain fatty acid; AD, atopic dermatitis; IL, interleukin.

colon and peripheral blood.[41,42] It is assumed that these steps are a mechanism mediated by symbiosis and low levels of IL-17 in normal infants (**Fig. 6.** left panel). However, *Streptococcus* was markedly increased in the AD group in the current study, and butyrate and valerate were lowest in infants with AD who had GA or AA genotypes of the *IL-17* variant. Butyrate and valerate were negatively correlated with *Streptococcus* and SCORAD score at 12 months of age. Although it is not clear whether *Streptococcus* directly affected the reduction in SCFAs in the current study, the data suggest that SCFA production was reduced by relatively decreased anaerobic bacteria due to increased *Streptococcus*. In addition, we found that *S. mitis* and IL-17 induced IL-6 and IL-8 inflammatory cytokines in HIEC cells. SCFAs, particularly butyrate, have been shown to decrease the production of IL-6 and IL-17 in PBMCs after stimulation with lipopolysaccharide.[43] IL-6 and IL-17 are closely connected and each forms a positive feedback loop.[44] IL-17 decreases the expression of the genes for OXPHOS[45] and mitochondrial dysfunction-like reduction of OXPHOS induces pro-inflammatory cytokines in the intestine.[46] Moreover, IL-17 modifies lactate dehydrogenase activity and results in increased lactate production in the colon.[47] In CD4 T cells, lactate also induces a switch towards the Th17 subset.[48] Taken together, these data suggest that increased expression of IL-17 by A allele of the *IL-17* variant rs2275913 and dysbiosis (which can be defined as an increase in *Streptococcus* with an alteration in energy metabolism in gut epithelium) induced intestinal and systemic inflammation, leading to the development of AD in early life (**Fig. 6.** right panel).

Our study has several limitations. First, the number of subjects assessed for SCFA, SCORAD at 12 months of age and colonocyte expression was relatively small compared with the number assessed for gut microbiota. However, the difference in SCFA among the four groups according to *IL-17* variant and AD, and the correlation between SCFA and SCORAD, at 12 months of age was quite clear. Further investigation involving a larger number of subjects will be required to confirm these findings. Secondly, we analyzed only one variant of *IL-17*

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to understand *IL-17* gene-microbe interactions. There are many variants in the *IL-17* gene and these may also affect the gut microbiota. However, rs2275913 of *IL-17* is located in the promoter and affects IL-17 expression level.[18] Therefore, this variant can be considered to be representative among *IL-17* variants. For a more thorough understanding of the association between the *IL-17* gene and gut microbiota, *IL-17* fine mapping could be conducted. Thirdly, we used *S. mitis* to identify the induction of inflammation *in vitro* despite the absence of species level outcomes. There are many species in *Streptococcus* at genus level, among which *S. thermophilus* is a probiotic that is generally used in the production of yogurt.[49] However, it is unlikely that the increased *Streptococcus* seen in the AD group is *S. thermophilus* because almost all probiotics sold in Korea contain *Lactobacillus* and *Bifidobacterium*. We selected *S. mitis* for the *in vitro* study because previous study has reported that previous study have shown that it is present in not only the oral but also the intestines,[50] moreover *S. mitis* induces IL-6 production.[32] However, further studies are required to clarify the precise species and to understand the role of the gut microbiota in the development of AD.

In conclusions, this study identified a significant association between *Streptococcus* and AD, and the interactions between *Streptococcus* and the *IL-17* variants of the host. Moreover, we described how the interactions between genetic factors (*IL-17*) and the environment (gut microbiota) cooperate to result in AD in infants. Our results suggest the possibility that alteration of the gut *Streptococcus*, which may be mediated in part by *IL-17* host genetic variants, can contribute to the development of AD in infants via intestinal and systemic inflammation and a reduction in SCFA. This scheme may represent a new perspective for the etiology of allergic diseases associated with gene-host gut microbiome interactions.

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SUPPLEMENTARY MATERIALS

Supplementary Table S1

Persistence of AD from 6 to 12 months according to the abundance of *Streptococcus* at 6 months of age and the *IL-17* variant rs2275913

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Supplementary Table S2

Different functional pathways according to the *IL-17* variant rs2275913 and AD status at 6 months of age

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Supplementary Table S3

List of genes involved in metabolic process and cellular metabolic process

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Supplementary Fig. S1

Alpha diversity indexes (OTUs, Chao1, and Shannon) between the normal healthy and AD groups. Data are presented as mean ± standard error.

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Supplementary Fig. S2

(A) Association between the *IL-17* variant rs2275913 and the development of AD at 6 months of age. (B) SCORAD in AD infants at 6 months of age. (C) IgE levels at 12 months of age in normal healthy and AD infants according to *IL-17* genotypes. Data are presented as aOR ± 95% confidence interval (A) or mean ± standard error (B, C).

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Supplementary Fig. S3

The relative abundance of *Clostridium* according to the *IL-17* variant rs2275913. *P* value was adjusted by atopic dermatitis, delivery mode, and feeding type.

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Supplementary Fig. S4

Correlation between relative abundance of (A) acetate, (B) propionate, (C) butyrate, and (D) valerate and SCORAD score at 12 months of age.

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Supplementary Fig. S5

Transcriptome analysis of colonocytes in infants. (A) Unsupervised hierarchical clustering of differentially expressed gene changes between the normal and AD groups. (B) Gene ontology terms related to biological process and significant terms of DAVID functional annotation chart.

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