Oral Abstract Session 3

Atopic Dermatitis and Food Allergy: Following the Evidence

May 11, Saturday 2019
Kangmo Ahn (Sungkyunkwan Univ.),
Gwang-Cheon Jang (NHIC ILSAN Hosp.)
**Phenotypes of Wheeze and Atopic Dermatitis in Japanese Children: A Birth Cohort Study**

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**Purpose:** The aim of this study was to identify phenotypes of wheeze and atopic dermatitis (AD) in Japanese children and to assess the associated factors for these phenotypes.

**Methods:** Relevant data were extracted from a birth cohort study, the Tokyo Children’s Health, Illness and Development study. The study recruited 1701 pregnant women between 2003 and 2005. In total, 1550 babies were enrolled and followed up until the age of 13 years. Wheeze and AD was assessed at approximately 1-year intervals based on the ISAAC questionnaire. We analyzed the trajectory of children aged 1–9 years by group–based trajectory analysis. A multinomial logit model was built to evaluate the relationships between phenotypes and risk factors.

**Results:** Five typical trajectories of wheeze were identified. 43.7% never/infrequent wheeze, 32.2% transient early wheeze, 6.2% school–age-onset wheeze, 8.6% early–childhood-onset remitting wheeze, and 9.2% persistent wheeze. Infant tobacco exposure increased the odds of membership in the transient early wheeze trajectory compared to the never/infrequent wheeze trajectory. As for AD, we identified four phenotypes, 17.6% early-onset AD, 10.1% persistent AD, 9.5% late-onset AD, and 62.7% never/infrequent AD in the children.

**Conclusion:** Using the group–based trajectory modeling approach, we identified five and four phenotypes of trajectories for childhood wheeze and AD in Japanese population.

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**Therapeutic Advantage of Bacterially Delivered Toll-Like Receptor 8 miRNA**

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**Background:** Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases in children. Skin inflammation is caused by complex interactions between genetic disposition and aberrant innate/adaptive immune responses.

**Objective:** Toll–like receptors (TLRs) are key molecules in innate/adaptive immune response by recognizing various molecular motifs associated with pathogens. Among them, TLR8 is implicated in eczematous skin reactions. We investigated the therapeutic effects of TLR8 suppression using miRNA on the modulation of inflammatory response.

**Methods:** We used Salmonella as a vector to deliver TLR8 miRNA. The recombinant strain of Salmonella typhimurium (ST) expressing TLR8 miRNA (ST-miRTLR8) was prepared for knockdown of TLR8. After oral administration of ST-miRTLR8 into mice, we observed cytokine levels, skin pathology and scratching behaviors in an AD–like mouse model.

**Results:** TLR8 down–regulation decreased macrophage–derived chemokine concentrations in activated human mast cells. Th2 cytokine levels were significantly decreased after oral administration of ST-miRTLR8. Serum IgE and interleukin–4 production were suppressed whereas IFN–γ was induced after treatment. Scratching behaviors and skin inflammation were also improved. In addition, attenuated S. typhimurium safely accumulated in mouse macrophages and showed adjuvant effects.

**Conclusions:** This study shows the recombinant miRNA which express the TLR8 gene has therapeutic effects by suppressing Th2 inflammation. TLR gene modulation using miRNA via Salmonella vectors would have a double protective effect in the treatment of AD.

**Key Words:** Atopic dermatitis, TLR8, microRNA
Integrated Multi-omics Approach of Human Gut Microbiome and Metabolome in Allergen Sensitised Eczema and Healthy Infants

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Purpose: Short chain fatty acids (SCFA) are metabolites produced by gut microbes following fermentation of dietary fibre and have immunomodulatory/anti-inflammatory properties. We have previously shown that infants with allergen sensitized eczema had lower levels of stool butyric acid in the first year of life compared to controls. This study aimed to investigate the functional gut microbiome signature of healthy and eczema infants by leveraging a multi-omics approach.

Methods: From the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort, a sub-cohort of 64 subjects categorized by clinical outcome at 18 months of age: (1) non-allergen sensitised eczema (NAE) (n=15), (2) allergen sensitized eczema (AE) (n=14) and (3) non-eczema controls (n=35), were selected. Fecal samples (n=164) at week 3, months 3, 6 and 12 were analyzed using microbial metagenomics and metatranscriptomics sequencing and metabolomics (SCFA by LC/MS/MS and global by GC/TOFMS). Spearman correlation and longitudinal multivariate analysis were used to deconvolute the putative interactions between the different omics datasets while adjusting for possible confounders.

Results: Based on longitudinal analysis, our previous observation of decreased butyric acid levels in AE compared to controls (adj p<0.05) corresponded with low production of other metabolites in the butanoate metabolism pathway and high accumulation of those belonging to the glycolysis pathway. Metagenomics and metatranscriptomics data substantiated the butanoate and glycolysis metabolism pathways were correspondingly perturbed in AE compared to controls (adj p<0.05). Microbiome-metabolome correlation analysis showed a depletion of butyric acid-producing bacterial families (Bacteroidaceae, Erysipelotrichaceae, Eubacteriaceae, Lachnospiraceae and Ruminococcaceae) in AE compared to controls (adj p<0.05).

Conclusions: The development of AE in early life is associated with a functional perturbation of the gut microbiome which is characterized by a delayed production of butyric. This may shed light in the potential underlying mechanism between host–microbiome and their metabolites in the context of allergy development.

Chitinase 3-Like 1 Drives Allergic Skin Inflammation Via Th2 Immunity and M2 Macrophage Activation

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by defective skin barrier and Th2 immune responses. Chitinase 3-like 1 (CHI3L1), also known as breast regression protein 39 (BRP-39) in mice and human homologue YKL-40, plays important roles in Th2 inflammation and allergen sensitization. CHI3L1 has been implicated in a variety of diseases including asthma characterized by inflammation, apoptosis, and tissue remodeling, but its role in AD remains elusive.

Objective: The aim of this study was to investigate the role of CHI3L1 in the development and progression of AD.

Methods: We investigated YKL-40 level in the serum of AD patients by ELISA. Using a murine model of AD induced by ovalbumin (OVA), we investigated Th2 immune responses, M2 macrophage activation, and skin barrier gene expression using wild-type (WT) and YKL-40 null mutant (BRP-39−/−) mice.

Results: YKL-40 level was significantly increased in serum of AD patients. Consistently, both mRNA and protein expression levels of BRP-39 were higher in OVA-sensitized WT mice than in control mice. OVA-sensitized BRP-39+/− mice showed decreased epidermal thickness, lower total serum IgE, Th2 cytokine levels, and CD4+ effector T cell populations than OVA-sensitized WT mice. Induction of BRP-39 was dominant in dermal macrophages and BRP-39 promoted M2 macrophage activation. In addition, BRP-39 deficiency attenuated dysregulation of skin barrier and tight junction genes.

Conclusion: These findings demonstrate that CHI3L1 mediates the development of AD induced by OVA, affecting Th2 inflammation, M2 macrophage activation and skin barrier function.

Key Words: Atopic dermatitis, Chitinase 3-like 1, Type 2 immunity
Differential IgE Binding and Crosslinking to Shrimp Allergens: Implication for Shrimp Allergy Diagnosis

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Purpose: Shrimp is a frequent cause of food allergy, but little is known about the relationship between IgE reactivity to shrimp allergens and clinical symptoms. This study aimed to evaluate the IgE binding and crosslinking patterns to shrimp allergens.

Methods: Chinese subjects with a history of shrimp allergy and a positive serum specific IgE (sIgE) level (≥0.35 kUA/L) to shrimp extract were recruited. Recombinant shrimp allergens including tropomyosin (TM), arginine kinase (AK), myosin light chain (MLC), sarcoplasmic calcium-binding protein (SCP), troponin C (TnC), triosephosphate isomerase (TIM) and fatty-acid-binding protein (FABP) were synthesized using standard molecular methods. sIgE binding and crosslinking to the individual allergens were measured by ELISA and the IgE crosslinking–induced luciferase expression (EXiLE) test using the RS–ATL8 cell line, respectively.

Results: Thirty-one shrimp allergic subjects (71% male; median age=15 [2-43]) were recruited. IgE binding assessed by ELISA revealed TnC as the leading sensitising allergen (22/31, 71%), followed by TM, FABP, AK, SCP, TIM and MLC, by which only 3 subjects were sIgE positive (i.e. OD 450 nm ≥0.2) to MLC. Sera of these subjects were then tested for IgE crosslinking using the EXiLE test. Positive luciferase signal (i.e. fold change ≥2 representing positive IgE crosslinking) was only detected in 14 subjects (45.2%) when TnC was used as stimulant, although the maximum fold change induced by TnC was the highest comparing to other allergens. However, SCP represented the leading cross–linking allergen (19/31, 61.3%), closely followed by TIM (18/31, 58.1%) and MLC (17/31, 54.8%).

Conclusions: The differences in IgE binding and crosslinking attributed to both the differential physiochemical nature of shrimp allergens in immobilised and liquid phases, as well as the functionality of IgE imply the importance in selecting appropriate test for diagnosing shrimp allergy.

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Air Flow Reduces Skin Temperature and Epidermal Filaggrin Breakdown Products in Children with Atopic Dermatitis

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Purpose: Environmental control is important for the management of atopic dermatitis (AD). We aimed to evaluate the influence of air flow from the air conditioner on skin barrier function and filaggrin breakdown products (FBPs) in children with AD.

Methods: In a crossover study, 28 patients with AD (aged 6 to 16 years) were randomly allocated to one of air conditioner modes (conventional and wind-free) in the first phase, and vice versa in the second phase. Each phase lasted 2 hours and was separated by 1-hour wash-out period. Skin temperature, transepidermal water loss (TEWL), and skin pH were measured on the right forearm at pre- and post-exposure time points. We collected stratum corneum specimens from the volar forearms before and after exposure to both air conditioner modes, and measured contents of filaggrin (FLG) and FBPs using Immuno-Dot Blot and hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry, respectively.

Results: Skin temperature was significantly decreased after exposure to either wind-free or conventional mode (P<0.001), but the percent change was higher in the conventional mode compared to that in the wind-free mode (P<0.001). TEWL decreased in the wind-free mode (P=0.009) and skin pH increased in the conventional mode (P<0.001). However, there were no changes in TEWL and skin pH after the exposure to the conventional mode and wind free mode, respectively. The levels of pyrrolidone carboxylic acid (PCA) and cis-UCA were significantly reduced after the exposure to the conventional mode (all P<0.033), but no changes of FBPs were found in the wind-free mode. In addition, there were no differences in the levels of epidermal filaggrin between pre- and post-exposure to either conventional or wind–free air conditioner mode.

Conclusions: Our results indicate that skin barrier function in children with AD might be altered by the exposure to air flow from the air conditioner.

Key Words: Atopic dermatitis, Skin barrier function, Air flow, Skin pH, Filaggrin breakdown products
Epidemiology of Food Allergies in Young Adults in Japan

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Purpose: The epidemiology of food allergies (FA) in adulthood has not been well investigated in Japan. We aimed to clarify the epidemiology of FA in young adults.

Methods: As a primary survey, a questionnaire was administered to 602 students enrolled at Showa University in 2018. Students who had current food avoidance or were diagnosed with FA by a physician were selected (secondary survey). Further, allergy specialists conducted detailed investigations and skin prick tests, and classified the students into the FA group, FA-suspected group, and non-FA group (third survey). Students classified into the FA-suspected group ingested suspect food at hospital or home, and were confirmed as having an FA if an allergic reaction was observed (fourth survey).

Results: Of 602 subjects, 593 (98.5%; mean age, 18.3±0.7 years; male, 35.4%) were analyzed. Fifty-seven (9.6%) subjects currently avoided some foods, and 30 (5.1%) had FA diagnosed by a doctor. Of the subjects entered into the secondary survey, 36 who agreed to the interview were analyzed. According to the third survey, 10 subjects were diagnosed with FA and 20 subjects were suspected of having an FA. The prevalence of suspected FA or FA diagnosed by an allergy specialist was 5.1%, and the prevalence of FA based on food intake was 1.9%.

Conclusions: We reported three types (questionnaire-based, inquiry-sensitization based, and food-intake based) of epidemiological data on FA prevalence in young adults. A relatively high number of adults avoid foods without accurate diagnosis after adolescence.

Interaction between IL-17 Variant and Streptococcus in Gut Contribute to the Development and Severity of Atopic Dermatitis in Infancy

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Background: Atopic dermatitis (AD) is associated with variants in many genes including IL–17 and recent studies show that perturbations in gut microbiota may influence a pathogenesis of AD. However, unifying principles for variants of host and microbe interaction remains unclear.

Objective: We sought to investigate whether IL–17 variant and gut microbiota affect the development of AD in infancy.

Methods: As a primary survey, a questionnaire was administered to 602 students enrolled at Showa University in 2018. Students who had current food avoidance or were diagnosed with FA by a physician were selected (secondary survey). Further, allergy specialists conducted detailed investigations and skin prick tests, and classified the students into the FA group, FA-suspected group, and non-FA group (third survey). Students classified into the FA-suspected group ingested suspect food at hospital or home, and were confirmed as having an FA if an allergic reaction was observed (fourth survey).

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Conclusions: We reported three types (questionnaire-based, inquiry-sensitization based, and food-intake based) of epidemiological data on FA prevalence in young adults. A relatively high number of adults avoid foods without accurate diagnosis after adolescence.
Wheat is the Major Cause of Food Allergy Presented to a Major Tertiary Allergy Referral Center in Bangkok

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Purpose: Prevalence of food allergy is increasing worldwide, including Thailand. Multiple factors including socio-economic, geographic, genetic and dietary factors could be major factors causing differences in types of foods allergy in different parts of the World. Wheat allergy has become increasingly observed in Thailand over the past two decades. We sort to examine details of food allergy cases presented to our centers over the past 4 years to determine frequencies of various types of foods causing allergy among Thai patients.

Methods: This is a retrospective data of cases presented to the Samitivej Allergy Institute (SAI), a referral allergy center in Bangkok Thailand with complaints of food allergy between 2014 to 2018. In this preliminary report, cases with convincing history of food allergies with positive skin prick test and/or specific IgE were examined to determine frequencies of different types of food allergies.

Results: 645 children and adults presented to SAI who met criteria for food allergies were identified during the study period. The prevalence of food allergic cases was constantly increasing over the past 4 years. Wheat was the most frequently food causing food allergy (29%, 188/645) followed by egg (25.3%, 163/645), shellfish (16.3%, 105/645), cow’s milk (11.2%, 72/645), peanut (7.9%, 51/645), fish (4.2%, 27/645), treenut (2.8%, 18/645) and soy (0.9%, 6/645). One fifth (92 patients) were identified as multiple food allergy. Surprisingly, cow milk is not as common as expected. Allergy to peanut and treenuts, although not as common as other food, is being identified. Allergy to unusual foods such as coconut as well to banana are identified.

Conclusions: There are increasing numbers of patients with food allergy presented to SAI over the past 4 years. Wheat allergy is the most common observed among this group of patients followed by egg, cow’s milk and shellfish. Further detail study on epidemiology of various group of food allergy, particularly of wheat allergy is warranted and is on-going at our Institute.

Imbalance of Gut Streptococcus, Clostridium, and Akkermansia Determines the Natural Course of Infantile Atopic Dermatitis

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Background: Atopic dermatitis (AD) often manifests in early childhood with a variable disease course among individuals. The role of gut microbiota on the natural course of AD are not yet fully understood.

Objective: We investigated whether the composition and function of gut microbiota at 6 months of age could affect the natural course of AD in early childhood.

Methods: We compared the composition and function of gut microbiota at 6 months of age among children without AD up to 24 months, in whom AD had disappeared after 12 months, and with persistent AD up to 24 months. Fecal samples from 132 infants were analyzed using pyrosequencing, including 84 healthy control, 22 transient AD and 26 persistent AD subjects. The functional profile of the gut microbiome was analyzed by whole–metagenome sequencing.

Results: The composition of the microbiota differed between the AD groups. Low levels of Streptococcus and high amounts of Akkermansia were evident in transient AD cases and low Clostridium, Akkermansia and high Streptococcus were found in children with persistent AD. The relative abundance of Streptococcus positively correlated with the SCORing of AD (SCORAD) values (rho= 0.484, P=.002) whereas that of Clostridium negatively correlated with SCORAD (rho= −0.556, P=.013). The persistent AD group showed decreased gut microbial functional genes related to oxidative phosphorylation compared with healthy controls (P=.001).

Conclusion: Different compositions and functions of the early gut microbiome are related to the natural course of AD in young children.

Key Words: Atopic dermatitis, Natural course, Gut microbiome
Confirmation of Wheat-Dependent Exercise Induced Anaphylaxis (WDEIA) by Exercise-Food Challenge Test is Crucial to Prevent Further Episodes of Anaphylaxis

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Purpose: WDEIA is a severe, potentially life threatening type of food allergy. However, the reactions are not always reproducible, thus this lead to multiple episodes of the reaction before the diagnosis has been confirmed. This study aim to establish the exercise-food challenge protocol, in order to confirm the diagnosis and to investigate the effect on the number of the reactions before and after the confirmation test.

Methods: Retrospective case review of patients between the aged of 1–25 years with a convincing history of WDEIA was conducted. Exercise-food challenge test protocol developed recently among our institute were performed among these patients, in addition to the SPT to wheat extracts, and sIgE to wheat, and omega-5-gliadin. The protocol consists of 4-day schedule conducted during hospital admission. The result of the challenge test, and episodes of the reaction were obtained.

Result: Ten patients were enrolled to this study with the median age of 15.8 years (range 10.5–21.4). The median age of onset was 12 years (range 5–12), while the median time to diagnosis was 20 months (range 0–45). Of these, history of chronic urticaria before the diagnosis of WDEIA were reported in 5 patients (50%). The exercise-food challenge test were performed in 9 patients, and 6 (66.7%) of them gave the positive result. The median number of exacerbations per year before the diagnosis was 2 (range 1–10), compared with 1 (range 0–3) after the diagnosis. Of the 6 participants with positive challenge test, 2 (33.3%) had ≥1 exacerbation per year after the challenge result, whereas all of the 4 participants without the positive test had ≥1 exacerbation in the same period.

Conclusion: Time to diagnosis was delayed among patients with WDEIA and multiple episodes of the reaction were reported, even after the diagnosis. Positive exercise-food challenge test result seems to reduce further episodes of the reaction.