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**Background:** Genome-wide association studies (GWAS) of asthma and asthma-related traits, including our previous TENOR study, have consistently identified *ORMDL3-GSDMB, IL33, IL1RL1-IL1R1, RAD50-IL13, TSLP-WDR36*, and *HLA-DR-DQ* regions.  

**Methods:** In this study, GWAS of asthma was performed in non-Hispanic white population from STAMPEED study (813 cases and 1564 controls). Our GWAS results were compared with the published GWAS of asthma and autoimmune diseases (AD).  

**Results:** Multiple SNPs in *TNFAIP3* interacting protein 1 (*TNIP1*) on chromosome 5q32-q33.1 were associated with asthma in STAMPEED: rs1422673 (*P* = 3.44 × 10⁻⁷) and rs10036748 (*P* = 1.41 × 10⁻⁶). rs422673 was weakly associated with asthma in the published GABRIEL study (*P* = 0.018 for meta-analysis) but not in the TENOR study (*P* = 0.18 but same trend). *TNIP1* may interact with *TNFAIP3* and inhibit TNFα-induced NFκB inflammation pathway. Joint analyses were performed on 6 SNPs in GSDMB (rs2872507), *IL33* (rs3939286), *IL1RL1* (rs1341828), *IL13* (rs20541), *TSLP* (rs1837253), and *HLA-DR* (rs2395185) in STAMPEED and TENOR populations, but only limited variance can be explained (percentage of deviance = 1.5−1.9%); the area under the receiver operating characteristic curve (AUC) = 0.58−0.59. Minor allele T of rs20541 in *IL13* is the risk allele for asthma but the protective allele for psoriasis. Minor allele A of rs2872507 in GSDMB is the protective allele for asthma but the risk allele for rheumatoid arthritis, Crohn’s disease and ulcerative colitis. T allele of rs10036748 in *TNIP1* is the minor protective allele for asthma, but the minor or major risk allele for systemic lupus erythematosus in non-Hispanic white or Chinese population, respectively.  

**Conclusions:** Our study provides genetic evidence that asthma and AD have opposite immunopathogenesis directions.  

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**118**  

**EGCG Downregulates Mucin Gene Expression Through the Mapk Signaling Pathway in Asthma**  

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**Background:** Mucus plays an important role in protecting human airway from external environments. Highly glycosylated mucin proteins are the major components of mucus, responsible for its viscoelastic properties. Excessive mucus is a major manifestation of inflammatory respiratory diseases. Epigallocatechin-3-gallate (EGCG) is major component of green tea extract and known to provide numerous functions, such as anti-oxidant effect, anti-tumor effect, anti-diabetic effect and anti-inflammatory effect. But precise mechanisms are still unclear.  

**Methods:** Using NCI-H292 human airway epithelial cells, we measured phorbol 12-myristate 13-acetate (PMA)-induced MUC5B mRNA expression with the treatment of indicated doses of EGCG. We also measured PMA-induced MUC5B protein secretion with the treatment of indicated doses of EGCG using ELISA technique in NCI-H292 cells. To test the brief signaling pathways, we performed activation study of mitogen-activated protein kinase (MAPK) pathways, which is well-known to signaling the PMA-induced mucin gene over-expression, using Western blot technique in NCI-H292 cells. And then we performed in vivo study using ovalbumin-induced asthmatic mice model and control mice group. In ovalbumin-sensitized asthmatic mice model, EGCG was treated with indicated dose. And then ovalbumin was challenged and we sacrificed the mice. Tissue samples from the mice were stained with PAS (periodic acid-Schiff) for mucin distribution in bronchioles of each group. Immunocytochemical stain was performed using MUC5B specific antibody. MUC5B mRNA and protein levels was measured using extracted lung tissues.  

**Results:** PMA-induced MUC5B mRNA and protein level was significantly decreased after treatment of EGCG at all doses in NCI-H292 cells. PMA-induced phosphorylation of p38 MAPK was significantly decreased after treatment of EGCG at all doses in NCI-H292 cells. Results from in vivo studies showed that decreased bronchial mucin distribution in the group of pretreated with EGCG using ELISA technique in NCI-H292 cells. To test the brief signaling pathways, we performed activation study of mitogen-activated protein kinase (MAPK) pathways, which is well-known to signaling the PMA-induced mucin gene over-expression, using Western blot technique in NCI-H292 cells. And then we performed in vivo study using ovalbumin-induced asthmatic mice model and control mice group. In ovalbumin-sensitized asthmatic mice model, EGCG was treated with indicated dose. And then ovalbumin was challenged and we sacrificed the mice. Tissue samples from the mice were stained with PAS (periodic acid-Schiff) for mucin distribution in bronchioles of each group. Immunocytochemical stain was performed using MUC5B specific antibody. MUC5B mRNA and protein levels was measured using extracted lung tissues.  

**Conclusions:** PMA-induced MUC5B mRNA and protein over-expression in both NCI-H292 cells and extracted tissues from asthmatic mice were significantly decreased with the treatment of EGCG. We demonstrated that...
EGCG downregulates mucin gene expression through the MAPK signaling pathway in asthma.

119 Enhancer of Zeste Homolog 2: A Pivotal Role in Pulmonary Artery Smooth Muscle Cell Proliferation

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Background: Pulmonary arterial hypertension (PAH) is a progressive and a devastating disease characterized by excessive proliferation of pulmonary artery smooth muscle cells (PASMCs). The pathogenesis of PAH is not fully understood and treatment options are limited. Studies suggest that PAH and cancer share apoptosis resistant state featuring excessive cell proliferation. Proliferation of cancer cells is mediated by increased expression of Enhancer of Zeste Homolog 2 (EZH2), a mammalian histone methyltransferase that contributes to the epigenetic silencing of target genes. However, the role of EZH2 in PAH has not been studied. In this study, we hypothesized that EZH2 could play a role in PASMC’s proliferation.

Methods: In the present study the effects of EZH2 overexpression on human PASMC’s proliferation were tested. PASMCs were transfected with wild type EZH2 cDNA or GFP using the Lonza 4D nucleofector system. After transfection, cells were incubated for 48 hours at 37°C. PASMC’s proliferation and cell cycle analysis were performed by flow cytometry; PASMC’s apoptosis was determined using annexin V staining, and cell migration was tested by the wound healing assay. Expression levels of EZH2 were confirmed by real time PCR.

Results: The overexpression of EZH2 in PASMC’s enhances proliferation, migration, and decreases the rate of apoptosis when compared to GFP transfected cells. There was a 3.5-fold increase in proliferation and a 1.5-fold increase in the percentage of cells in the G2/M phase in the EZH2 transfected cells while there was a significant decrease in the rate of apoptosis in the PASMCs.

Conclusions: These findings suggest that EZH2 plays a role in the migration and proliferation of PASMC’s. It also suggest that EZH2 could play a role in PAH development and serve as a potential target for new therapies for PAH.

120 The Features of Airway Remodeling Are More Severe in Female Mice

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Background: Epidemiological studies have already shown that females are dominant in terms of the sex ratio of adult asthma prevalence and severe asthma. It has also been reported that female mice are more susceptible to the development of allergic airway inflammation and airway hyperresponsiveness (AHR) than males. However, there have been few reports of studies on sex difference in the pathogenesis of severe asthma, especially airway remodeling in an animal model. In this study, we investigated sex difference in formation of airway remodeling using a long-term antigen challenged asthma model.

Methods: Following ovalbumin (OVA)/alum intraperitoneal injection, male or female mice (BALB/c) were challenged with aerosolized 1% OVA on 3 days/week for 5 weeks, and we investigated the sex difference in AHR, airway inflammation, as well as airway remodeling.

Results: In OVA-sensitized and -challenged (OVA/OVA) female mice, AHR, the number of eosinophils and lymphocytes, as well as Th2 cytokines and growth factors in BAL fluid were increased compared with OVA/OVA male mice. On the other hand, there is no significant difference in the level of eotaxin in BAL fluid. The histological features of airway remodeling, including goblet cell hyperplasia, subepithelial fibrosis and myofibroblast hypertrophy, were also increased in OVA/OVA female mice. Moreover, serum total and OVA-specific IgE were significantly elevated in OVA/OVA female mice.

Conclusions: These results indicate that female mice are dominant in terms of forming airway remodeling as compared with male mice. The involvement of sex difference for sensitization and growth factor release in lung tissue based on inflammatory cell infiltration is indicated for the mechanism of sex difference of airway remodeling.

121 Ovalbumin-induced Bronchial Asthma is Compromised in Apoptosis Signal-Regulating Kinase-Deficient Mice

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Background: Apoptosis signal-regulating kinase 1 (ASK1), a member of mitogen-activated protein kinase kinase kinases (MAP3Ks) protein family, plays a crucial role in the induction of apoptosis and inflammation in some cell types. Allergic asthma is a chronic inflammatory airway disease characterized by airway hyperresponsiveness (AHR), inflammatory cell infiltration, and airway remodeling. In the present study, we examined whether ASK1 is involved in the induction of bronchial asthma using a mouse model of airway inflammation.

Methods: ASK1-deficient (ASK1−/−) and wild-type (WT) control mice were sensitized with ovalbumin (OVA) in saline intraperitoneally on consecutive 7 days. Eighteen days later, mice received intranasal administration of OVA aerosol and were assayed for AHR, cytokine production, cell proliferation, antibody (Ab) production, and lung tissue histopathology at 24 hours after the last serial OVA administration. Levels of Ab and cytokines were determined by enzyme-linked immunosorbent assay (ELISA).

Results: Control WT mice showed inflammatory infiltrates in airways in response to OVA to a greater extent than ASK1−/− mice. The number of cells, especially eosinophils accumulating in airways, was reduced in ASK1−/− mice relative to control mice. OVA-induced AHR is also compromised in ASK1−/− mice. Anti-OVA IgE Ab production in ASK1−/− mice was substantially reduced, although levels of other isotypes were comparable to those in control mice. Levels of some Th2 cytokines (IL-5 and IL-13) and pro-inflammatory cytokine TNF-a in BAL fluid from ASK1−/− mice were substantially diminished relative to control, although a comparable level of a typical Th2 cytokine IL-4 and anti-inflammatory cytokine IL-10 was produced. Although the BAL fluid TNF-a levels from ASK1−/− mice were severely diminished, lymph node cells from ASK1−/− mice produced comparable levels of TNF-a to WT in vitro. Intranasal administration of recombinant TNF-a caused a comparable increase in AHR between ASK1−/− and WT mice, whereas the TNF-a-induced accumulation of inflammatory cells was severely reduced in ASK1−/− mice.

Conclusions: ASK1 appears to be involved in the induction of OVA-induced bronchial asthma, probably through cytokine production such as TNF-a and IL-13. Moreover, TNF-a sensitivity in response to OVA is also regulated by ASK1.

122 Role of the CC-Chemokine Receptor CCR9 in the Regulation of Inflammatory Process During Allergic Airway Inflammation

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Background: The CC-chemokine receptor CCR9 plays an important role in the recruitment of CCR9+ T cells, which are important for the development of the allergic reaction, during the inflammatory process. In this study, we investigated the role of CCR9+ T cells in the regulation of the inflammatory process during allergic airway inflammation.

Methods: Following ovalbumin (OVA)/alum intraperitoneal injection, male or female mice (BALB/c) were challenged with aerosolized 1% OVA on 3 days/week for 5 weeks, and we investigated the sex difference in AHR, airway inflammation, as well as airway remodeling.