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**Regulatory Function of MicroRNA-149 on
Thromboxane A2 receptor Expression in
Asthmatics**

by

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Major in Molecular Medicine

Department of Biomedical Sciences

The Graduate School, Aju University

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**A Dissertation Submitted to The Graduate School of
Ajou University in Partial Fulfillment of the
Requirements for the Degree of Master in Biomedical
Sciences**

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-ABSTRACT-

Regulatory Function of *MicroRNA-149* on *Thromboxane A2* *Receptor* Gene Expression in Asthmatics

Background and objectives: The regulation of inflammatory responses in human diseases is mediated by the coordinated control of inflammatory gene expression at the transcriptional level as well as at the post-transcriptional level. One mechanism of post-transcriptional regulation is through control by microRNAs, which bind to the 3' untranslated region (3' UTR) of target genes and regulate mRNA stability or translation. The aim of this genetic association study was to identify *miR-149* polymorphisms and evaluate their relationship with asthma with a view to further understanding the pathogenesis of asthma.

Subjects and methods: One hundred and eighty-four patients with asthma and 168 healthy normal controls (NC) were enrolled in this study. Single nucleotide polymorphisms (SNPs) in *miR-149* (rs2292832) were genotyped using a TaqMan® allelic discrimination assay. Plasma *miR-149* expression was evaluated using quantitative RT-PCR with a TaqMan® MicroRNA Reverse Transcription Kit and TaqMan® MicroRNA Assays. The expression of the *thromboxane A2 receptor* (TBXA2R) gene in peripheral blood mononuclear cells (PBMCs) was measured by quantitative RT-PCR. Human Embryonic Kidney 293T (HEK293T) cells were transfected with an EGFP-tagged *TBXA2R* 3'UTR plasmid construct and a *miR-149*

mimic or a *miR-149* inhibitor using lipofectamine. EGFP expression was evaluated by measuring fluorescence intensity.

Results: Asthma patients had a significantly lower frequency of the *miR-149* C allele compared to that of the NC group ($P = 0.014$). The expression levels of plasma *miR-149* in asthma patients were significantly lower than that of the NC group ($P = 0.002$). Plasma *miR-149* expression levels showed a negative correlation with both total IgE ($r = -0.320$; $P = 0.027$) and peripheral eosinophil count ($r = -0.311$, $P = 0.030$). The expression of the *TBXA2R* gene in PBMCs was significantly higher in asthma patients compared to that in the NC group ($P = 0.024$). Transfection with the *miR-149* mimic significantly suppressed *TBXA2R* expression ($P = 0.04$), whereas transfection with the *miR-149* inhibitor significantly increased *TBXA2R* expression ($P = 0.02$).

Conclusion: These findings suggest that the *miR-149* C-allele (rs2292832) is associated with the phenotype of asthma and that *miR-149* negatively regulates *TBXA2R* expression. Therefore, *miR-149* may contribute to the pathogenesis of asthma.

Keyword: Asthma, *miR-149*, *TBXA2R*

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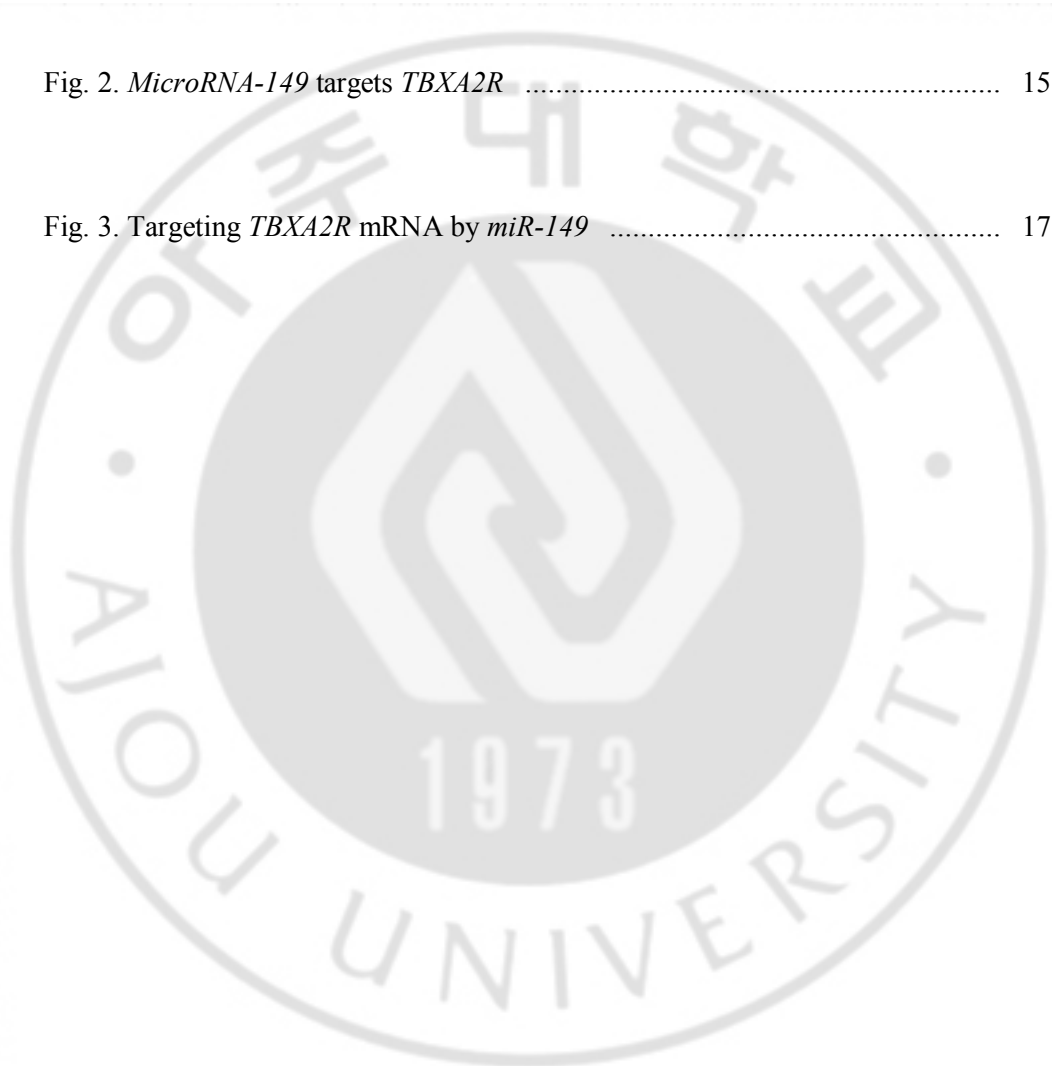
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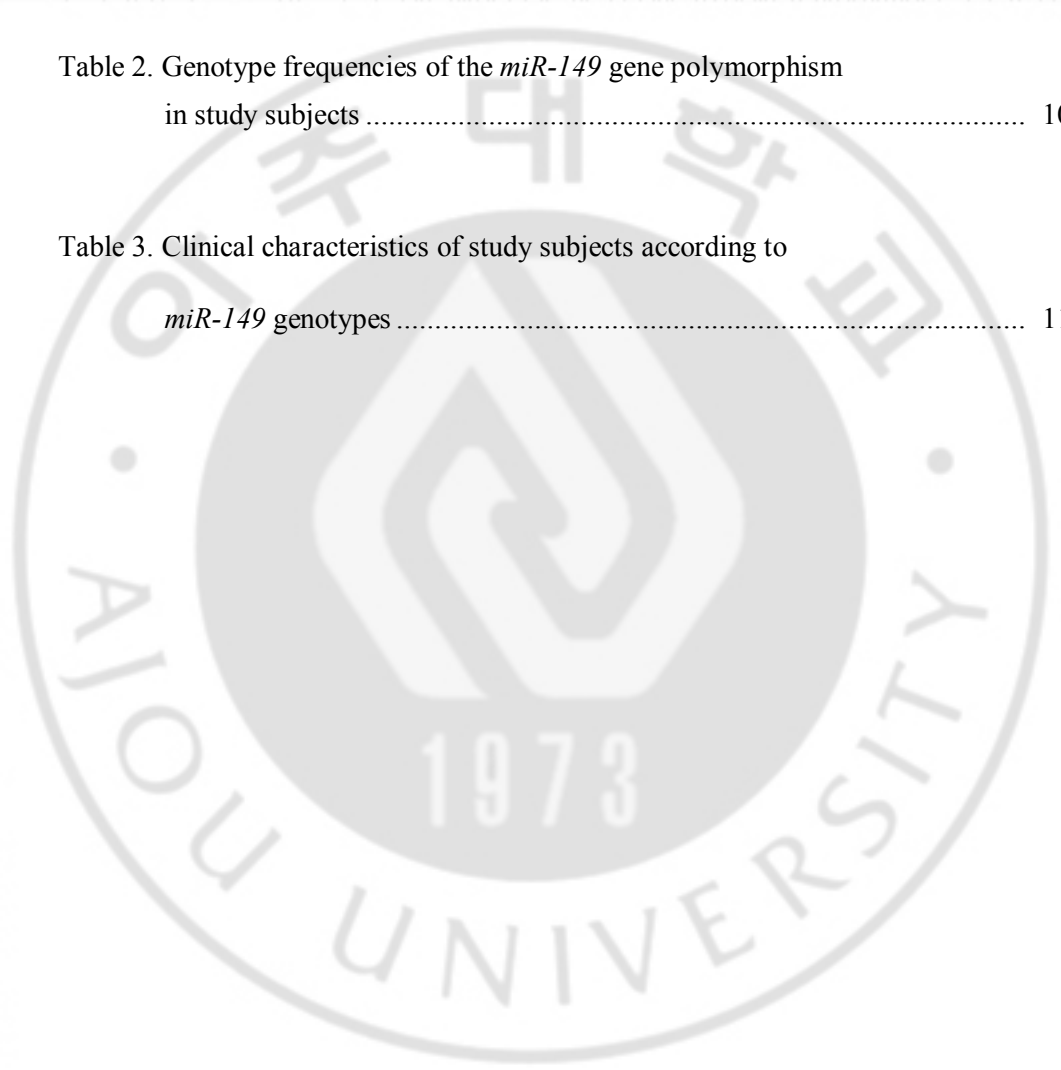


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I . INTRODUCTION

Asthma is a respiratory disorder characterized by reversible airflow obstruction, and airway inflammation and bronchoconstriction (Lim et al., 2014; Shim et al., 2015; Durham et al., 2016). Worldwide, asthma is a significant medical burden and its prevalence is increasing every year. In Korea, the current prevalence of asthma has been reported to be in the range 3.6 – 5.8% and further increases with age (Song et al., 2014). Asthma is now considered a heterogeneous disease with various, complex pathogenic mechanisms (Hinks et al., 2016; Silkoff et al., 2016). Therefore, it is necessary to identify biomarkers that will aid in further defining the differing asthma phenotypes.

MicroRNAs are 22~25 nt noncoding RNAs which regulate gene expression by targeting mRNAs for translational repression. They play critical roles in a number of diverse biological processes, such as infection, immune response and inflammation (Lindsay, 2008; Wei et al., 2014). Of relevance here, several studies have shown that microRNAs are involved in the pathogenesis of asthma. For example, in asthma patients, *microRNA-19a* (*miR-19a*) is upregulated in airway T cells, and promotes T helper 2 cell (Th2) cytokine production in the airway. (Simpson et al., 2014). A separate study also showed that, in severe asthmatics, *miR-19a* is upregulated and it enhances bronchial epithelial cell proliferation through binding to *TGF-beta receptor 2* mRNA (Haj-Salem et al., 2015). Another study reported that reduced *miR-146a* expression improved lung function by enhancing production of prostaglandin E2 increasing cyclooxygenase-2 levels and thereby increasing production of prostaglandin (Wang et

al., 2015). Additionally, in lung macrophages, inhibition of *miR-9* resulted in reduced phosphorylation of the glucocorticoid receptor (GR) thereby restoring dexamethasone-induced nuclear translocation of GR and suppressing steroid resistance (Li et al., 2015). *MicroRNA-138* represses airway smooth muscle cell (ASMC) proliferation by directly to the 3'UTR of *3-phosphoinositide-dependent protein kinase-1* mRNA (Liu et al., 2015). Taken together, these finding provide evidence that microRNAs can modulate the expression of several target genes important in regulating the pathogenesis of asthma.

Genetic polymorphisms within a microRNA may also play a role in the progression of asthma (Jiménez-Morales et al., 2012; Chang et al., 2015). For example, recent studies have shown the association of genetic polymorphisms in *miR-146a* (Jiménez-Morales et al., 2012), and the *miR-152* family (Tan et al., 2007) with respiratory diseases. Additionally, genetic polymorphisms in *miR-196a2* (Li et al., 2011) and *miR-499* (Li et al., 2011) were found to be associated with chronic obstructive pulmonary disease (COPD). The CC/GC genotype of *miR-146a* (rs2910164) was also associated with increased lung function in COPD patients (Wang et al., 2015). In a Chinese population, those carrying the T allele in *miR-149* may be protected from the risk of developing asthma (Su et al., 2011).

Thus, the present study was designed to investigate the genetic association of *miR-149* polymorphisms in asthmatics, and to study the potential regulatory mechanisms by which *miR-149* may function in the pathogenesis of asthma.

II. MATERIALS AND METHODS

Study subjects

We enrolled 186 patients with asthma, 168 healthy normal controls (NC) from Ajou University Hospital (Suwon, Korea). Asthma was diagnosed at the first evaluation based on a history of respiratory symptoms as well as the results of airway. Asthma patients were stratified according to sputum inflammatory cell profiles. Atopy was defined as one or more positive reactions on skin prick tests with common aeroallergens (Bencard, Bradford, UK). Serum total IgE was measured using ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden). All subjects underwent an interview, chest radiography and lung function measurements as well as a methacholine inhalation challenge. All consents were obtained from all the subjects. The institutional review board of Ajou University Hospital (Suwon, Korea) approved the study.

Genotyping of miR-149 single nucleotide polymorphism

Genomic DNA was extracted from whole blood using the Puregene DNA purification kit (Gentra, Minneapolis, MN, USA) according to manufacturer's protocol. *MicroRNA-149* gene was amplified by PCR using the forward primer 5'-GAGGCTCCCAGGCCTTC-3' and the reverse primer 5'-AGAGGTGAGGCCCGAAAC-3'. Single nucleotide polymorphism (SNP) in *miR-149*

was genotyped by TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA).

Quantitative measurement of miR-149 expression in plasma

MicroRNAs were isolated from 200µl of plasma using miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. cDNA was synthesized from 10ng of microRNA using TaqMan® MicroRNA Reverse Transcription Kit and TaqMan MicroRNA Assays (Applied Biosystems) according to the manufacturer's instruction. The expression level of *miR-149* was normalized to *miR-16* expression level. Amplified products were detected using TaqMan Universal Master Mix II, no UNG Assays (Applied Biosystems) and calculated using the $2^{-\Delta\Delta CT}$.

Quantitative measurement of TBXA2R expression in PBMC

Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) using TRIzol reagent (ThermoFisher Scientific) according to the manufacturer's instructions. After denaturation of RNA and annealing with oligo dT 18mers (72°C, 5 min), cDNA was synthesized from 1µg of RNA using 200U of MMLV-RNase (Promega, Madison, WI, USA) under optimized reaction conditions (RT buffer: 50mM pH 8.3 Tris-HCl, 75mM KCl, 3mM MgCl₂, 10mM DTT, 2mM dNTPs, 0.5U/ml Rnase inhibitors

(Promega) at 42°C for 1 hour and extended at 70°C for 10min. To confirm the synthesis of cDNA or normalization of cDNA with PCR, RT-PCR for GAPDH was performed with primers 5'- GAAGGTGAAGGTCGGAGT -3' as forward primer and 5'- GAAATCCCATCACCATCTTC -3' as reverse primer using 0.03U of Taq polymerase (Solgent, Daejeon, Korea) under the following buffer and PCR condition.

Plasmid construction for 3'UTR of TBXA2R

PCR was used to amplify a fragment of 288bp from 3' untranslated region (3'UTR) of TBXA2R using the primers as followed: Forward 5'- ACAAGTAAAGCGGCCGCGAAGTGGACAGAGCGCCC -3'; Reverse 5'- TAGATGCATGCTCGAGGACCCTCTTCCAATGTCTG -3'. PCR product was purified using an agarose gel purification kit (GeneAll Biotechnology, Seoul, Korea). The products were digested with Not I and Xho I restriction enzymes (Takara, Shuzo, Japan), followed by ligation into an EGFP reporter-pcDNA3 plasmid, which was produced as previously described (Kim et al., 2009), using In-Fusion cloning kit (Clontech).

Transfection of TBXA2R 3'UTR plasmid DNA and miR-149 mimic or miR-149 inhibitor into HEK293T cell

Human Embryonic Kidney 293T (HEK293T) cells were cultured in Dulbecco's Modified Eagle Mediums (DMEM) (Gibco, Grand Island, NY, USA) with 10% heat-

inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (50µg/ml). Cells were grown at 37 degree in humidified atmosphere with 5% CO₂. HEK293T cells (5 x 10⁵/ml) were seeds into each well of 12-well plates, grown in DMEM serum free.

HEK293T cells (5 x 10⁵) were cultured in twelve wells overnight before transfection. The next day, cells were transfected with EGFP-tagged TBXA2R 3'UTR plasmid DNA (1µg), Renilla plasmid DNA (10ng), 5nM *miR-149* mimics and controls or 50nM *miR-149* inhibitor and controls (Qiagen) by using lipofectamine (Invitrogen, Carlsbad, CA, USA). After 6 hours, each well of 12-well plate was filled with 1 mL of DMEM with 10% FBS and incubated for 48 hours at 37°C in 5% CO₂ humidified atmosphere. After 48 hours, cell supernatants were collected. Cells were lysed with 100µl passive lysis buffer (Promega) per well. 10µl of lysate was assayed for luciferase activity using a dual-luciferase reporter assay (Promega) and using a Clarity Luminescence microplate reader (BioTek, Winooski, VT, USA) following the manufacturer's protocol. EGFP expression was evaluated by measuring fluorescence intensities in the Synergy HT multi-detection microplate reader (BioTek Instruments, Winooski, VT, USA). The EGFP expression levels were normalized to the luciferase activity. Transfections were performed in triplicate.

Statistical analysis

Genotype frequencies were analyzed between the study subjects. Logistic regression analysis with co-dominant, dominant and recessive models was applied to control for age and sex as co-variables. Differences in clinical characteristics were analyzed using the chi-square tests for categorical variables and independent *t*-tests for continuous variables. The level of *miR-149* expression and *TBXA2R* expression were compared by Mann-Whitney *U*-test. Dual luciferase assay was measured using independent *t*-test. *P* values < 0.05 were judged significant. All statistical analysis was performed using SPSS, version 22.0 (SPSS Inc., Chicago, IL, USA).

III. RESULTS

Clinical characteristics of the study subjects

Table 1 shows the clinical demographics of the study subjects. Asthma patients were significantly older than the NC group ($P < 0.001$). There were also significantly more females and a higher incidence of atopy in asthma patients compared to that in the NC group ($P = 0.031$ and $P < 0.001$, respectively). In keeping with the higher incidence of atopy, serum total IgE levels were also significantly higher in asthma patients compared to that in the NC group ($P < 0.001$). Finally, the levels of serum IgE to *D. preronyssinus* (D1) and *D. farinae* (D2) were significantly higher in asthma patients compared to that in the NC group ($P = 0.011$ and $P < 0.008$, respectively).

Table 1. Clinical characteristics of the study subjects

	Asthma (n=186)	NC (n=168)	<i>P</i> value
Age (year)†	41.34±15.08	30.11±10.47	< 0.001
Sex (Female/total)‡	101/186(54.3%)	72/168(42.9%)	0.031
Atopy (presence/total)‡	101/165(61.2%)	46/123(37.4%)	< 0.001
Total IgE (IU/L)†	505.13±1043.45	129.73±229.56	< 0.001
PC ₂₀ (mg/mL)†	9.67±15.51	NA	NA
Baseline FEV1 (% Pred)†	89.13±21.33	NA	NA
IgE to D1 (KU/L)†	9.69±20.14	4.64±16.1	0.011
IgE to D2 (KU/L)†	12.83±25.58	6.99±23.4	0.008
Sputum eosinophil count (%)†	23.97±32.87	NA	NA
Sputum neutrophil count (%)†	60.56±33.29	NA	NA
Total eosinophil count (%)†	498.25±1357.14	NA	NA

NC, normal control; n, number of subjects; NA, not applicable; PC₂₀: the provocative concentration of methacholine required to cause a 20% fall in FEV1, FEV1: forced expiratory volume in 1 second, D1: *Dermatophagoides pteronyssinus*, D2: *Dermatophagoides farinae*. † Data presented as mean ± SD. *P* values were obtained using Student's T test. ‡ Data presented as prevalence (%). *P* values were obtained using Pearson's test. Bold numbers implicate statistically significant *P* values.

Genetic association of miR-149 gene polymorphism with asthma

The genotype frequency of the *miR-149* gene is significantly different between asthma patients and the NC group (Table 2). Asthma patients showed a significantly lower frequency of the rs2292832 genotype (CC/CT) in *miR-149* compared to that in the NC group ($P = 0.014$). The clinical characteristics broken down according to the *miR-149* genetic polymorphisms are shown in Table 3. The numbers of females and the incidence of atopy were higher in those carrying the *miR-149* TT genotype compared to that in the non-carriers ($P = 0.029$ and $P < 0.001$, respectively). In addition, there were no significant associations between the *miR-149* genotype and other clinical parameters including age, total IgE, PC₂₀, FEV1, IgE to D1, IgE to D2 and total eosinophil count.

Table 2. Genotype frequencies of the *miR-149* gene polymorphism in study subjects

	Asthma (n=186)	NC (n=168)	<i>P</i> value*
TT	149(80.1%)	115(68.5%)	0.033
CT	24(12.9%)	40(23.8%)	0.404
CC	13(7%)	13(7.7%)	0.014

NC, normal control; n, number of subjects. *Each *P* value was calculated using the co-dominant, dominant and recessive models. Logistic regression analysis was applied to control for age and sex as covariates. Bold numbers implicate statistically significant *P* values.

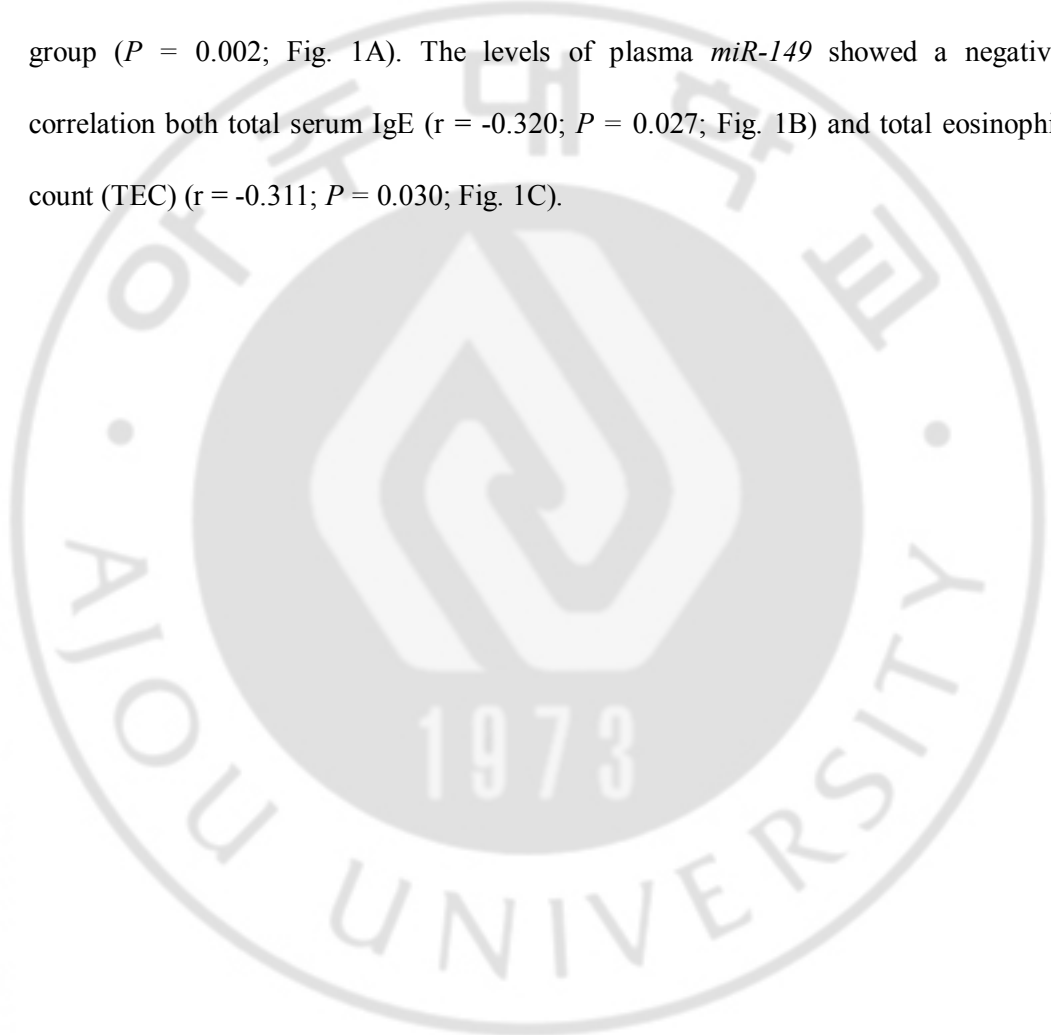
Table 3. Clinical characteristics of study subjects according to *miR-149* genotypes

	TT (n=264)	CT/CC (n=90)	P value
Age (year)†	36.25±14.06	35.31±14.78	0.279
Sex (Female/total)‡	106/197(53.8%)	77/187(42.5%)	0.029
Atopy (presence/total)‡	104/172(60.5%)	50/133(37.6%)	< 0.001
Total IgE (IU/L)†	494.48±1077.49	253.03±290.32	0.325
PC ₂₀ (mg/mL)†	9.09±15.23	11.72±16.55	0.360
Baseline FEV1(% Pred)†	88.96±20.51	90.04±24.9	0.602
IgE to D1 (KU/L)†	8.67±18.6	11.27±24.19	0.472
IgE to D2 (KU/L)†	11.29±23.44	15.97±31.9	0.949
Sputum eosinophil count (%)†	25.13±34	19.4±28.29	0.936
Sputum neutrophil count (%)†	59.44±33.98	64.57±31.05	0.503
Total eosinophil count (%)†	388.28±393.01	960.15±2978.23	0.836

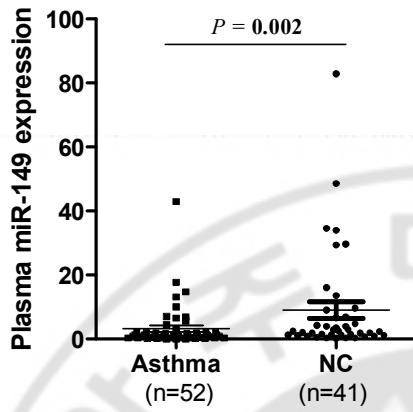
NC, normal control; n, number of subjects; NA, not applicable; PC₂₀: the provocative concentration of methacholine required to cause a 20% fall in FEV₁, FEV₁: forced expiratory volume in 1 second, D1: *Dermatophagoides pteronyssinus*, D2: *Dermatophagoides farinae*. † Data presented as mean ± SD. P values were obtained using Student's T test. ‡ Data presented as prevalence (%). P values were obtained using *Pearson's test*. Bold numbers implicate statistically significant P values.

Decreased expression of plasma miR-149 in asthmatic patients

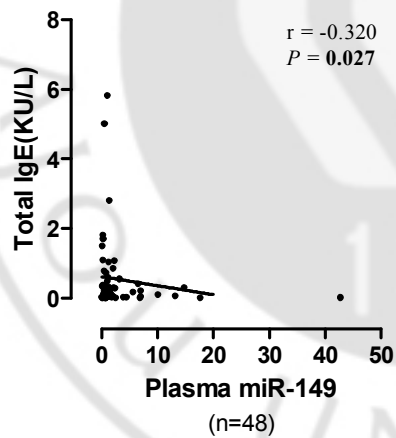
Fig. 1 shows the expression level of plasma *miR-149* in the study subjects and the correlation with two clinical parameters. Significantly lower levels of *miR-149* were found in the plasma from asthma patients compared to that in the plasma from the NC group ($P = 0.002$; Fig. 1A). The levels of plasma *miR-149* showed a negative correlation both total serum IgE ($r = -0.320$; $P = 0.027$; Fig. 1B) and total eosinophil count (TEC) ($r = -0.311$; $P = 0.030$; Fig. 1C).



(A)



(B)



(C)

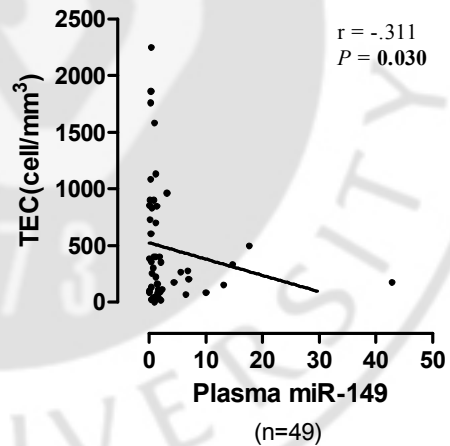
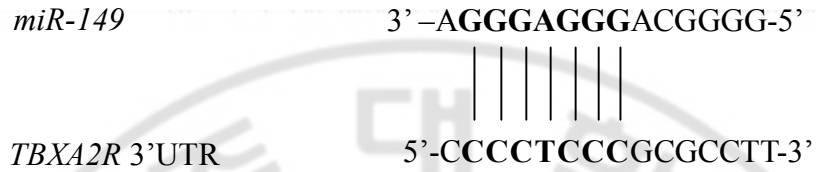


Fig 1. Expression level of *miR-149* in the plasma of study subjects. (A) Comparison of plasma *miR-149* expression levels between asthma and the NC group. Correlation of plasma *miR-149* expression level with (B) total serum IgE level and (C) total blood eosinophil count (TEC).

Enhanced expression of TBXA2R in asthmatics

The MicroRNA Target Prediction and Functional Study Database (<http://mirdb.org/miRDB/>) was used to predict which genes might be targeted by *miR-149*. We found that *miR-149* potentially targets a sequence in the 3' UTR of *thromboxane A2 receptor* mRNA (*TBXA2R*) as shown in Fig. 2A. This suggested that *miR-149* might regulate the expression levels of *TBXA2R* mRNA. Consequently, we investigated the expression levels of *TBXA2R* mRNA in PBMCs obtained from the study subjects. As shown in Fig. 2B *TBXA2R* mRNA levels were significantly higher in asthma patients compared to that in the NC group ($P = 0.024$).

(A)



(B)

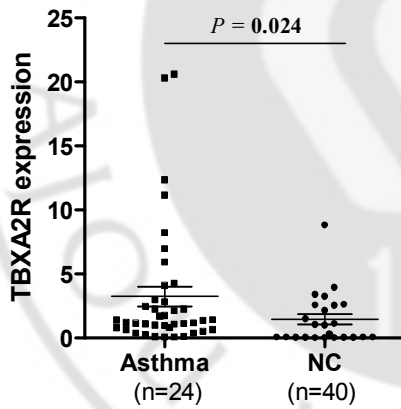
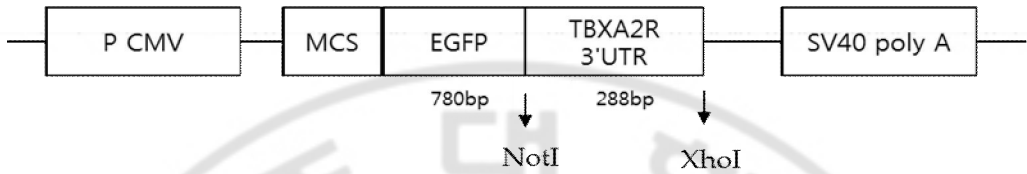


Fig 2. *MicroRNA-149* targets *TBXA2R*. (A) The predicted target sequence of *miR-149* in the 3' UTR of *TBXA2R*. (B) Expression levels of the *TBXA2R* mRNA in PBMCs between the asthma and NC groups.

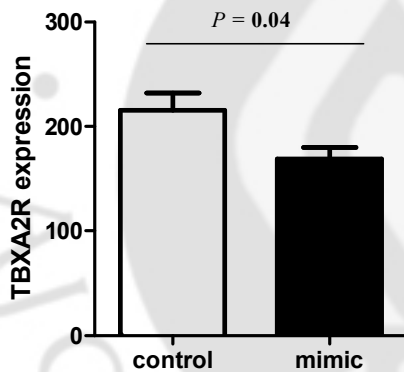
Negative regulation of TBXA2R expression by miR-149

To examine if *miR-149* could indeed target the *TBXA2R* mRNA the following experiment was conducted. A fragment containing the 3' UTR of the *TBXA2R* mRNA was cloned into the EGFP reporter-pcDNA3 plasmid to act as the 3' UTR of the EGFP gene (Fig. 3A). HEK293T cells were co-transfected with this EGFP-tagged *TBXA2R* 3' UTR construct, along with renilla luciferase plasmid DNA as a transfection control, a *miR-149* mimic/control (5 nM of each) or a *miR-149* inhibitor/control (50 nM of each). Transfection with the *miR-149* mimic significantly suppressed EGFP expression ($P = 0.04$) (Fig. 3B), whereas transfection with the *miR-149* inhibitor significantly increased expression ($P = 0.02$) (Fig. 3C). These results suggest that *miR-149* directly targeted the 3' UTR of *TBXA2R* to reduce the expression of EGFP.

(A)



(B)



(C)

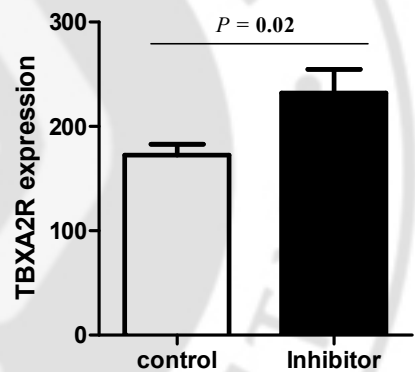


Fig 3. Targeting *TBXA2R* mRNA by *miR-149*. (A) Schematic representation of the EGFP-tagged *TBXA2R* 3' UTR construct. (B) Luciferase activity in HEK293T cells transfected with mimic and negative control. (C) Luciferase activity in HEK293T cells transfected with inhibitor and negative control. Transfection and luciferase assays were performed in triplicate. P values were obtained using a Mann-Whitney U -test.

IV. DISCUSSION

Current literature describes the potential importance of *miR-149* in human diseases both from a functional perspective, as well as from a biomarker perspective. It is suggested that *miR-149* exerts an inhibitory function to suppress cancer cell growth by targeting Akt1 and E2F1 thereby enhancing apoptotic signaling (Lin et al., 2010). In macrophages, *miR-149* suppresses the 3' UTR of *MyD88* and consequently suppresses the inflammatory response to TNF-alpha and IL-6 by attenuating the TLR/MyD88 signaling pathway (Xu et al., 2014). In endothelial cells, *miR-149* protects against pro-inflammatory action by negatively regulating several genes, including metalloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS) and interleukin-6 (IL-6) genes (Palmieri et al., 2014).

Considering the important role of *miR-149* in human diseases, the present study also suggests a potential genetic association of *miR-149* with asthma. Our findings imply that the C allele in *miR-149* is associated with a lower risk of asthma. In complete contrast, in a Chinese population, the T allele in *miR-149* was found to be associated with reduced asthma susceptibility (Su et al., 2011). This conflicting result may represent ethnic differences in susceptibility to asthma. Moreover, asthma is a heterogeneous and complex disease, comprising various phenotypes; therefore, this may reflect some underlying genetic predisposition. As our understanding of asthma as a disease with potentially many different subtypes evolves, further studies which stratify these distinct asthma phenotypes will be required to elucidate the association of

the *miR-149* genotypes with asthma.

Although the association between reduced *miR-149* expression and inflammatory diseases such as osteoarthritis have been described (Santini et al., 2014), there has been no study to date that examines expression of *miR-149* in plasma from asthma patients. This is, therefore, the first study to provide convincing evidence for different *miR-149* expression levels between asthma patients and NCs. Furthermore, the expression of plasma *miR-149* showed a significant negative correlation with total IgE and total eosinophil count. Mean total IgE level were significantly higher in asthmatic subjects than in non-asthmatic subjects (Gergen et al., 2009). Total eosinophil level was also higher in asthma patients compared to that in the NC group patients (Mehta et al., 2008). Taken together, these finding suggest that *miR-149* may be involved in pathogenesis of atopic and eosinophilic asthma.

In order to understand which gene might be targeted by *miR-149* we used miRDB and found that *miR-149* was predicted to bind to a sequence in the 3' UTR of *TBXA2R*. Upon examination of the levels of expression of *TBXA2R* mRNA in these study subjects it was found that asthma patients showed increased *TBXA2R* expression compared to that by the NC group.

To obtain evidence that the *TBXA2R* mRNA could be targeted by *miR-149*, HEK293T cells were transfected with an EGFP-tagged *TBXA2R* 3' UTR plasmid construct along with a *miR-149* mimic or a *miR-149* inhibitor. We found that the *miR-149* mimic could significantly suppress EGFP expression, whereas a *miR-149* inhibitor enhanced EGFP

expression. These findings imply that *miR-149* directly binds to the 3' UTR of *TBXA2R* mRNA leading to decreased expression of *TBXA2R*.

TBXA2R is a receptor for thromboxane A2 (TXA2) that could be involved in asthma pathogenesis in three different ways: (1) by inducing bronchoconstriction; (2) by increasing platelet activation; or (3) by recruiting inflammatory cells. TXA2 can induce bronchoconstriction through interaction with TBXA2R (Greenberg et al., 1982; Hernandez and Janssen, 2014). Along with TXA2, other bronchoconstrictors, including prostanoids and isoprostanes, exert their effects by binding to the TBXA2R, thereby demonstrating the important role of TBXA2R in the pathogenesis of asthma (Johnston et al., 1995; Rolin et al., 2006). TBXA2R antagonist inhibits eosinophil infiltration and modulate the expression of chemokines in bronchial tissues, suggesting an important role of TBXA2R in recruiting inflammatory cells to asthmatic airways (Hoshino et al., 1999).

Taken together these findings, *miR-149* could be a biomarker, as well as therapeutic target, to modulate atopic asthma by regulating the expression of *TBXA2R*.

V. CONCLUSION

We demonstrated the rs2292832 at *miR-149* as a genetic risk factor in asthma and the role of *miR-149* on *TBXA2R*. Thus, its modulation may provide a novel therapeutic strategy for asthma.



VI. REFERENCES

1. Chang HS, Heo J-S, Shin S-W, Bae D-J, Song HJ, Jun J, Kim JD, Park J-S, Park BL, Shin HD: Association between TAAR6 polymorphisms and airway responsiveness to inhaled corticosteroids in asthmatic patients. *Pharmacogenetics and genomics* 25: 334-342, 2015
2. Durham AL, Caramori G, Chung KF, Adcock IM: Targeted anti-inflammatory therapeutics in asthma and chronic obstructive lung disease. *Translational Research* 167: 192-203, 2016
3. Gergen PJ, Arbes SJ, Calatroni A, Mitchell HE, Zeldin DC: Total IgE levels and asthma prevalence in the US population: results from the National Health and Nutrition Examination Survey 2005-2006. *Journal of Allergy and Clinical Immunology* 124: 447-453, 2009
4. Greenberg R, Antonaccio MJ, Steinbacher T: Thromboxane A₂ mediated bronchoconstriction in the anesthetized guinea pig. *European Journal of Pharmacology* 80: 19-27, 1982
5. Haj-Salem I, Fakhfakh R, Bérubé JC, Jacques E, Plante S, Simard M, Bossé Y, Chakir J: MicroRNA-19a enhances proliferation of bronchial epithelial cells by targeting TGF β R2 gene in severe asthma. *Allergy* 70: 212-219, 2015
6. Hernandez JM, Janssen LJ: Revisiting the usefulness of thromboxane-A₂ modulation in the treatment of bronchoconstriction in asthma 1. *Canadian journal of physiology and pharmacology* 93: 111-117, 2014
7. Hinks TS, Brown T, Lau LC, Rupani H, Barber C, Elliott S, Ward JA, Ono J, Ohta S, Izuhara K: Multidimensional endotyping in patients with severe asthma reveals inflammatory heterogeneity in matrix metalloproteinases and chitinase 3-like protein 1. *Journal of Allergy and Clinical Immunology*, 2016

8. Hoshino M, Sim J, Shimizu K, Nakayama H, Koya A: Effect of AA-2414, a thromboxane A₂ receptor antagonist, on airway inflammation in subjects with asthma. *Journal of allergy and clinical immunology* 103: 1054-1061, 1999
9. Jiménez-Morales S, Gamboa-Becerra R, Baca V, Río-Navarro D, López-Ley D, Velázquez-Cruz R, Saldaña-Alvarez Y, Salas-Martínez G, Orozco L: MiR-146a polymorphism is associated with asthma but not with systemic lupus erythematosus and juvenile rheumatoid arthritis in Mexican patients. *Tissue antigens* 80: 317-321, 2012
10. Johnston S, Freezer N, Ritter W, O'Toole S, Howarth P: Prostaglandin D₂-induced bronchoconstriction is mediated only in part by the thromboxane prostanoid receptor. *European Respiratory Journal* 8: 411-415, 1995
11. Kim SH, Kang YM, Cho BY, Ye YM, Hur GY, Park HS: Histamine N-methyltransferase 939A> G polymorphism affects mRNA stability in patients with acetylsalicylic acid-intolerant chronic urticaria. *Allergy* 64: 213-221, 2009
12. Li JJ, Tay HL, Maltby S, Xiang Y, Evers F, Hatchwell L, Zhou H, Toop HD, Morris JC, Nair P: MicroRNA-9 regulates steroid-resistant airway hyperresponsiveness by reducing protein phosphatase 2A activity. *Journal of Allergy and Clinical Immunology* 136: 462-473, 2015
13. Li L-J, Gao L-B, Lv M-L, Dong W, Su X-W, Liang W-B, Zhang L: Association between SNPs in pre-miRNA and risk of chronic obstructive pulmonary disease. *Clinical biochemistry* 44: 813-816, 2011
14. Lim SY, Jo YJ, Chun EM: The correlation between the bronchial hyperresponsiveness to methacholine and asthma like symptoms by GINA questionnaires for the diagnosis of asthma. *BMC pulmonary medicine* 14: 1, 2014
15. Lin RJ, Lin YC, Yu AL: miR-149* induces apoptosis by inhibiting Akt1 and E2F1 in human cancer cells. *Molecular carcinogenesis* 49: 719-727, 2010

16. Lindsay MA: microRNAs and the immune response. *Trends in immunology* 29: 343-351, 2008
17. Liu Y, Yang K, Sun X, Fang P, Shi H, Xu J, Xie M, Li M: MiR-138 suppresses airway smooth muscle cell proliferation through the PI3K/AKT signaling pathway by targeting PDK1. *Experimental lung research* 41: 363-369, 2015
18. Mehta V, Campeau NG, Kita H, Hagan JB: Blood and sputum eosinophil levels in asthma and their relationship to sinus computed tomographic findings. In *Mayo Clinic Proceedings*, Elsevier, pp.671-678, 2008
19. Palmieri D, Capponi S, Geroldi A, Mura M, Mandich P, Palombo D: TNF α induces the expression of genes associated with endothelial dysfunction through p38MAPK-mediated down-regulation of miR-149. *Biochemical and biophysical research communications* 443: 246-251, 2014
20. Rolin S, Masereel B, Dogné J-M: Prostanoids as pharmacological targets in COPD and asthma. *European journal of pharmacology* 533: 89-100, 2006
21. Santini P, Politi L, Dalla Vedova P, Scandurra R, d'Abusco AS: The inflammatory circuitry of miR-149 as a pathological mechanism in osteoarthritis. *Rheumatology international* 34: 711-716, 2014
22. Shim E, Lee E, Yang S-I, Jung Y-H, Park GM, Kim HY, Seo J-H, Yu J: The association of lung function, bronchial hyperresponsiveness, and exhaled nitric oxide differs between atopic and non-atopic asthma in children. *Allergy, asthma & immunology research* 7: 339-345, 2015
23. Silkoff P, Laviolette M, Singh D, FitzGerald J, Kelsen S, Backer V, Porsbjerg C, Girodet P, Berger P, Kline J: Longitudinal stability of asthma characteristics and biomarkers from the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study. *Respiratory research* 17: 1, 2016
24. Simpson LJ, Patel S, Bhakta NR, Choy DF, Brightbill HD, Ren X, Wang Y, Pua HH, Baumjohann D, Montoya MM: A microRNA upregulated in asthma

- airway T cells promotes TH2 cytokine production. *Nature immunology* 15: 1162-1170, 2014
25. Song W-J, Kang M-G, Chang Y-S, Cho S-H: Epidemiology of adult asthma in Asia: toward a better understanding. *Asia Pacific Allergy* 4: 75-85, 2014
 26. Su X-W, Yang Y, Lv M-L, Li L-J, Dong W, Gao L-B, Luo H-B, Cong R-J, Liang W-B, Li Y-B: Association between single-nucleotide polymorphisms in pre-miRNAs and the risk of asthma in a Chinese population. *DNA and cell biology* 30: 919-923, 2011
 27. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, Solway J, Gern JE, Lemanske RF, Nicolae D: Allele-specific targeting of microRNAs to HLA-G and risk of asthma. *The American Journal of Human Genetics* 81: 829-834, 2007
 28. Wang R, Li M, Zhou S, Zeng D, Xu X, Xu R, Sun G: Effect of a single nucleotide polymorphism in miR-146a on COX-2 protein expression and lung function in smokers with chronic obstructive pulmonary disease. *International journal of chronic obstructive pulmonary disease* 10: 463, 2015
 29. Wei L, Zhou Q, Hou S, Bai L, Liu Y, Qi J, Xiang Q, Zhou Y, Kijlstra A, Yang P: MicroRNA-146a and Ets-1 gene polymorphisms are associated with pediatric uveitis. *PloS one* 9: e91199, 2014
 30. Xu G, Zhang Z, Xing Y, Wei J, Ge Z, Liu X, Zhang Y, Huang X: MicroRNA-149 Negatively Regulates TLR-Triggered Inflammatory Response in Macrophages by Targeting MyD88. *Journal of cellular biochemistry* 115: 919-927, 2014

- 국문요약 -

천식 환자에서 *Thromboxane A2 receptor* 유전자 발현에

미치는 *MicroRNA-149* 의 조절 작용

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연구배경 및 목적: 인간 질환의 염증 반응은 이에 관여하는 유전자들의 발현 수준에 따라 변화될 수 있고 이러한 유전자들은 마이크로알엔에이에 의해 조절 된다. 마이크로알엔에이는 표적 유전자의 3' 비해석부위 (3'UTR)에 결합하여 전사 후 단계에서 발현을 조절한다. 본 연구에서는 천식에서 *miR-149* 의 유전자 다형성이 미치는 유전적 영향을 밝히고 천식의 발병기전에서 *miR-149* 의 역할을 알아보려고 하였다.

재료 및 방법: 본 연구는 천식환자 184 명, 정상 대조군 168 명을 대상으로 하였다. *miR-149* 의 단일 염기 다형성은 TaqMan 대립 유전자 판별 분석을 이용하여 유전자 분석을 하였다. 천식환자와 정상 대조군에서 혈장에서 추출한 *miR-149* 의 발현을 TaqMan® MicroRNA Reverse Transcription 키트와 TaqMan MicroRNA Assays 키트를 사용하여 실시간 중합효소 연쇄반응으로

측정하였다. 말초혈액단핵구로부터 추출한 *TBXA2R* mRNA 발현은 실시간 중합효소 연쇄반응에 의해 측정 하였다. HEK293T 세포는 리포팩타민을 사용하여 EGFP 가 붙은 *TBXA2R* 3' 비해석부위가 삽입된 plasmid DNA 와 *mir-149* mimic 또는 *mir-149* 억제제를 세포 내로 감염시켰고, EGFP 발현은 형광 강도로 평가하였다.

결과: 천식 환자는 정상 대조군에 비해 *miR-149* 의 C 대립 유전자의 빈도가 유의하게 낮았다 ($P=0.014$). 혈장 *miR-149* 의 발현 정도는 천식환자가 정상대조군 보다 현저히 낮았다 ($P=0.002$). 혈장 *miR-149* 발현 정도는 총면역 글로불린 E ($P=0.027$) 및 말초혈액 총 호산구 수 ($P=0.030$)와 음의 상관관계를 나타내었다. 말초혈액단핵구로부터 추출한 *TBXA2R* 발현 정도는 천식 환자가 정상대조군 보다 유의하게 높았다 ($P=0.024$). *miR-149* mimic 을 세포 내로 감염 시킨 결과 *TBXA2R* 의 발현 정도가 유의하게 감소하였고 ($P=0.04$), *miR-149* 억제제를 세포 내로 감염 시킨 결과 *TBXA2R* 의 발현 정도가 유의하게 증가하였다 ($P=0.02$).

결론: 본 연구에서는 *miR-149* 유전자에 있는 C 대립 유전자가 천식의 표현형과 연관 있고, *miR-149* 가 표적 유전자인 *TBXA2R* 의 발현을 감소시킨 것을 보여주었다. 이 연구를 통하여 *miR-149* 가 천식의 발병기전에 기여할 것이라고 생각한다.

핵심어: 천식, *miR-149*, *TBXA2R*