Novel genetic variants in long non-coding RNA MEG3 are associated with the risk of asthma

Kuo-Liang Chiu [1, 2], **Wen-Shin Chang** [3, 4], **Chia-Wen Tsai** [3, 4], **Mei-Chin Mong** [5], **Te-Chun Hsia** [Corresp., 4], **Da-Tian Bau** [Corresp. 3, 4, 6]

[1] Division of Chest Medicine, Department of Internal Medicine, Taichung Tzu Chi Hospital, Taichung, Taiwan

[2] School of Post-Baccalaureate Chinese Medicine, Tzu Chi University, Hualien, Taiwan

[3] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

[4] Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan

[5] Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan

[6] Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan

Corresponding Authors: Te-Chun Hsia, Da-Tian Bau
Email address: derrick.hsia@msa.hinet.net, artbau2@gmail.com

Background: Asthma is the most common chronic inflammatory airway disease worldwide. Asthma is a complex disease whose exact etiologic mechanisms remain elusive; however, it is increasingly evident that genetic factors play essential roles in the development of asthma. The purpose of this study is to identify novel genetic susceptibility loci for asthma in Taiwanese. We selected a well-studied long non-coding RNA (lncRNA), *MEG3*, which is involved in multiple cellular functions and whose expression has been associated with asthma. We hypothesize that genetic variants in *MEG3* may influence the risk of asthma. **Methods:** We genotyped four single nucleotide polymorphisms (SNPs) in *MEG3*, rs7158663, rs3087918, rs11160608, and rs4081134, in 198 patients with asthma and 453 healthy controls and measured serum *MEG3* expression level in a subset of controls. **Results:** The variant AG and AA genotypes of *MEG3* rs7158663 were significantly over-represented in the patients compared to the controls ($P = 0.0024$). In logistic regression analyses, compared with the wild-type GG genotype, the heterozygous variant genotype (AG) was associated with a 1.62-fold [95% confidence interval (CI) = 1.18-2.32, $P = 0.0093$] increased risk and the homozygous variant genotype (AA) conferred a 2.68-fold (95% CI = 1.52-4.83, $P = 0.003$) increased risk of asthma. The allelic test showed the A allele was associated with a 1.63-fold increased risk of asthma (95% CI = 1.25-2.07, $P = 0.0004$). The AG plus AA genotypes were also associated with severe symptoms ($P = 0.0148$). Furthermore, the AG and AA genotype carriers had lower serum MEG3 expression level than the GG genotype carriers, consistent with the reported downregulation of MEG3 in asthma patients. **Conclusion:** *MEG3* SNP rs7158663 is a genetic susceptibility locus for asthma in Taiwanese. Individuals carrying the variant genotypes have lower serum MEG3

level and are at increased risks of asthma and severe symptoms.

Novel genetic variants in long non-coding RNA MEG3 are associated with the risk of asthma

Kuo-Liang Chiu[1,2], Wen-Shin Chang[3,4], Chia-Wen Tsai[3,4], Mei-Chin Mong[5], Te-Chun Hsia[4*] and Da-Tian Bau[3,4,6*]

[1]Division of Chest Medicine, Department of Internal Medicine, Taichung Tzu Chi Hospital, Taichung, Taiwan

[2]School of Post-Baccalaureate Chinese Medicine, Tzu Chi University, Hualien, Taiwan

[3]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

[4]Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan

[5]Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan

[6]Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan

Corresponding Authors:

Da-Tian Bau and Te-Chun Hsia

Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan

Email address: artbau2@gmail.com (D.-T.B.); derrick.hsia@msa.hinet.net (T.-C.H.)

Short title: MEG3 Genotypes in Asthma

Abstract

Background: Asthma is the most common chronic inflammatory airway disease worldwide. Asthma is a complex disease whose exact etiologic mechanisms remain elusive; however, it is increasingly evident that genetic factors play essential roles in the development of asthma. The purpose of this study is to identify novel genetic susceptibility loci for asthma in Taiwanese. We selected a well-studied long non-coding RNA (lncRNA), *MEG3*, which is involved in multiple cellular functions and whose expression has been associated with asthma. We hypothesize that genetic variants in *MEG3* may influence the risk of asthma.

Methods: We genotyped four single nucleotide polymorphisms (SNPs) in *MEG3*, rs7158663, rs3087918, rs11160608, and rs4081134, in 198 patients with asthma and 453 healthy controls and measured serum *MEG3* expression level in a subset of controls.

Results: The variant AG and AA genotypes of *MEG3* rs7158663 were significantly over-represented in the patients compared to the controls ($P = 0.0024$). In logistic regression analyses, compared with the wild-type GG genotype, the heterozygous variant genotype (AG) was associated with a 1.62-fold [95% confidence interval (CI) = 1.18-2.32, $P = 0.0093$] increased risk and the homozygous variant genotype (AA) conferred a 2.68-fold (95% CI = 1.52-4.83, $P = 0.003$) increased risk of asthma. The allelic test showed the A allele was associated with a 1.63-fold increased

risk of asthma (95% CI = 1.25-2.07, P = 0.0004). The AG plus AA genotypes were also associated with severe symptoms (P = 0.0148). Furthermore, the AG and AA genotype carriers had lower serum MEG3 expression level than the GG genotype carriers, consistent with the reported downregulation of MEG3 in asthma patients. **Conclusion:** *MEG3* SNP rs7158663 is a genetic susceptibility locus for asthma in Taiwanese. Individuals carrying the variant genotypes have lower serum MEG3 level and are at increased risks of asthma and severe symptoms.

Keywords: asthma, genotype, MEG3, polymorphism, SNP, non-coding RNA, lncRNA

Introduction

Asthma is the most common chronic lung disease characterized by airway obstruction, airway inflammation, and airway hyperresponsiveness. Globally, approximately 300 million people are affected by asthma annually, and its prevalence is continuously increasing (*Mattiuzzi & Lippi, 2020; Global Initiative for Asthma, 2022*). There are large geographical differences in the incidence of asthma and developed countries generally have higher incidences of asthma than developing countries largely due to higher environmental exposures including smog and air particles (*Mulgirigama et al., 2019*). Asthma is a complex disease whose etiology involves both environmental exposures and genetic susceptibility. It was estimated that asthma has a genetic heritability of up to 60%–80% (*Ober & Yao, 2011; Kabesch & Tost, 2020; Bonnelykke & Ober, 2016*). An animal disease model mimicking human asthma suggested that approximately 200 genes may contribute to the etiology of asthma (*Temesi et al., 2014*). Candidate gene studies focusing on biologically relevant genes such as inflammatory and immunological genes and genome-wide association studies (GWAS) have identified a number of genetic susceptibility loci for asthma (*Bonnelykke & Ober, 2016; Liu et al., 2022; Shen et al., 2017; Hsia et al., 2015; García-Sánchez et al., 2015; Shi et al., 2022; Ranjbar et al. 2022*). However, the identified asthma susceptibility loci to date only explain a small portion of the genetic heritability of asthma and additional genetic

susceptibility loci to asthma remains to be uncovered.

Long noncoding RNAs (lncRNAs) are defined as RNAs longer than 200 nucleotides that are not translated into functional proteins (*Orom & Shiekhattar, 2013*). In recent years, it has been increasingly evident that lncRNAs are important regulators of gene expression in diverse cellular processes, thus are essential for maintaining normal physiology, and are often dysregulated in various diseases including asthma (*Melissari et al. 2016; Rafiee et al. 2018; Statello et al. 2021*; *Gysens et al., 2022; Chen & Deng, 2022*). A number of studies have compared the expression of different lncRNAs in blood cells between asthma patients and healthy controls and identified several differentially expressed lncRNAs (*Tsitsiou et al., 2012; Booton &Lindsay, 2014; Feng et al., 2020; Chen & Deng, 2022*), among which maternally expressed gene 3 (MEG3) is of particular interest. The *MEG3* gene is located in chromosome 14q32.3 and encodes a lncRNA of approximately 1.6 kb (Zhang et al., 2010). MEG3 is abundantly expressed in normal tissues and plays an essential role in cell growth and organ development (Da Rocha et al., 2008). Earlier studies found reduced expression of MEG3 in the circulating CD8+ T cells of patients with severe asthma (*Tsitsiou et al., 2012; Booton &Lindsay, 2014*). A recent study (*Feng et al., 2020*) reported that serum MEG3 level was significantly lower in asthma patients than in healthy controls and was the lowest in the most severe asthma patients. Furthermore, there was a significant inverse correlation between serum

MEG3 expression and the course of asthma ($r = -0.666$, $P<0.001$) (Feng et al., 2020). *In vitro* experiments also provide indirect evidence supporting that low MEG3 expression is linked to asthma development: treating human bronchial epithelial cells with cigarette smoke condensate (Hu et al. 2009) or an environmental carcinogen nickel (Zhou et al., 2017) caused marked downregulation of MEG3 expression.

Given that aberrant MEG3 expression is linked to asthma development, we hypothesize that genetic variants that affect MEG3 expression may be associated with the risk of developing asthma. Several SNPs in MEG3 have been reported in literature that may affect MEG3 expression and influence the risks of various diseases including inflammatory response, diabetes, stroke, and cancer (*Wallace, 2010; Han et al., 2018; Ghaedi et al., 2018; Ghafouri-Fard & Taheri, 2019; Gao et al., 2021; Zhu et al., 2021; Zhong et al., 2022*). We therefore perform the first study to evaluate the associations of SNPs in MEG3 with the risks of asthma using a case control study design. In addition, we determine whether the selected SNPs influence the expression level of MEG3 in serum samples and provide strong genotype-gene expression correlation that explains the observed significant association between SNPs and asthma risk.

Materials & Methods

Recruitment of asthmatic cases and non-asthmatic healthy controls

A total of 198 patients with asthma and 453 non-asthmatic healthy controls were recruited from China Medical University Hospital (CMUH) as previously described (*Hsia et al., 2015; Li et al., 2021*). The diagnosis of asthma was based on the following inclusion criteria: 1) more than two or three episodes of wheezing and shortness of breath during the past year; 2) diagnosis of asthma by pulmonologists together with the demonstration of reversible and variable airflow obstruction by spirometry; 3) symptoms; and 4) prescription of medications for asthma. No children were recruited (the youngest being 25 years old) and there was no sex restriction. The controls were selected by frequency-matching to cases by age and sex after initial random sampling from the Health Examination Cohort of CMUH. The inclusion criteria for controls were: 1) no past or present physician's diagnosis of asthma and other pulmonary diseases; 2) no history of wheezing, shortness of breath, or other symptoms of allergic diseases such as nasal and skin symptoms; 3) no use of medications for asthma; 4) absence of first-degree relatives with a history of asthma; and 5) older than 25 years. Those with chronic inflammatory responses, diabetes, stroke and cancer were also excluded for both cases and controls, the flow chart of participant recruitment scheme was shown in Figure 1. The symptom severity for each asthma case was verified by two experienced pulmonologists according to the Global Initiative for Asthma (GINA) guidelines (*Global Initiative*

for Asthma, 2022). Specifically, symptom severities were classified into 4 groups based on the treatment to control the symptoms and exacerbations. Group 1 (mildest): treated with as-needed inhaled corticosteroid (ICS)-formoterol alone; Group 2, treated with low-intensity maintenance controller treatment of ICS-formoterol, leukotriene receptor antagonists or chromones; Group 3, treated with low dose ICS-long acting β2 agonist (LABA); and Group 4, treated (severest) with high dose ICS-LABA. Peripheral blood was collected from all subjects and genomic DNA was extracted and stored until genotyping (*Yang et al., 2017b*). This study was approved by the Research Ethics Committee of the China Medical University Hospital (CMUH106-REC1-004). All protocols were conducted in accordance with relevant guidelines. All patients provided written informed consent at the time of recruitment.

Genotyping methodology for *MEG3* genotypes

We selected 4 SNPs (Figure 2) that were either correlated with MEG3 expression or associated with other diseases in previous publications (*Han et al., 2018; Ghaedi et al., 2018; Ghafouri-Fard & Taheri, 2019; Gao et al., 2021; Zhu et al., 2021; Zhong et al., 2022*). The *MEG3* rs7158663, rs3087918, rs11160608, and rs4081134 genotypes were determined using TaqMan assay with an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as previously described (*Pei*

et al., 2022).

Transcriptional expression of MEG3 in serum

To evaluate the correlation between MEG3 RNA expression and *MEG3* rs7158663 genotype, 46 serum samples were randomly selected from the controls and subjected to extraction of total RNA using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). They were randomly selected from the pool of controls, and there were no significant differences between these samples and the remaining controls in terms of age, sex and smoking status. The expression levels of MEG3 were measured by real-time quantitative reverse transcription-PCR (RT-PCR) using an FTC-3000 real-time PCR instrument series (Funglyn Biotech Inc., Canada). GAPDH was used as an internal control (*Liao et al., 2020; Huang et al., 2018*). MEG3 primers were purchased from Qiagen (UniGene No. Hs.654863, Catalog No. 4331182, LPH02974A-200). For GAPDH, the forward and reverse primers were 5'-GAAATCCCATCACCATCTTCCAGG-3' and 5'-GAGCCCCAGCCTTCTCCATG-3', respectively. During the analysis, the levels of MEG3 RNA were normalized to those of GAPDH expression. Each sample was measured three times.

Statistical methodology

The goodness-of-fit chi-square test was used to examine the fitness of the Hardy-Weinberg equilibrium in the control group. The *Student's t*-test was used to examine the differential distributions of age between the case and control groups. Pearson's chi-squared test was used to examine the differential distribution of various *MEG3* genotypes and the interaction between *MEG3* genotypes and symptom severity. The association of *MEG3* genotypes with asthma risk was evaluated by calculating the odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) using multivariable logistic regression analyses adjusting for age, sex, and smoking status. The differential expression levels of MEG3 shown in Figure 3 were evaluated with unpaired *Student's t*-test. The statistical significance level was set at $P < 0.05$.

Results

Demographics of asthmatic and non-asthmatic subjects

The age, sex, smoking, and clinical characteristics such as pulmonary function and symptom severity of the 198 patients and 453 controls are shown in Table 1. The patients and controls were frequency-matched on age and sex ($P = 0.2972$ and 0.9956, respectively). There were slightly more ever-smokers among cases than controls (29.8% vs. 28%), but the difference was not statistically significant ($P = 0.7161$). Regarding pulmonary function, the average ratio of forced expiratory volume in the first second to forced vital capacity (FEV1/FVC, %) and the percentage of predicted FEV1 (FEV1%) was significantly lower in the asthmatic

group than in the control group (both $P < 0.0001$). The percentage of patients within symptom severity group 1 (mild), 2, 3, and 4 (severe) was 30.3, 32.8, 17.2, and 19.7, respectively (Table 1).

Associations of *MEG3* genotypes with asthma risk

The distributions of the genotypes of *MEG3* rs7158663, rs3087918, rs11160608, and rs4081134 in the cases and controls are shown in Table 2. First, the genotype frequencies of the four SNPs in the control group all fit well with Hardy-Weinberg equilibrium (all $P > 0.05$). Second, the genotype frequencies of rs7158663 were differentially distributed between the cases and controls with the heterozygous variant AG and homozygous variant AA genotypes over-represented in the cases compared to controls (44.5% vs. 36.4% for AG and 12.1% vs. 6.6% for AA genotype). In logistic regression analyses, the AG and AA genotypes were both associated with increased risks of asthma (adjusted ORs = 1.62 and 2.68, 95% CIs = 1.18-2.32 and 1.52-4.83, $P = 0.0093$ and 0.0030, respectively) compared with the wild-type GG genotype. In the dominant model, individuals carrying the variant genotypes (AG+AA) exhibited a 1.75-fold increased risk of asthma (adjusted OR = 1.75, 95% CI = 1.29-2.48, $P = 0.0015$). The other three SNPs, rs3087918, rs11160608, and rs4081134, were not significantly associated with the risks of asthma (Table 2).

222

223 **Allelic frequency distribution analysis**

224 The allelic frequencies of rs7158663, rs3087918, rs11160608, and rs4081134 SNPs
225 among cases and controls are shown in Table 3. Consistent with the findings in Table
226 2, individuals with the variant A allele at rs7158663 were at a higher risk of asthma
227 than those with the wild-type G allele after adjusting for age, sex, and smoking status
228 (adjusted OR = 1.63, 95% CI = 1.25-2.07, $P = 0.0004$). In contrast, the variant alleles
229 of the other three SNPs were not associated with asthma risks (Table 3).

230

231 ***MEG3* rs7158663 genotypes are associated with the severity of asthma symptom**

232 We then analyzed the distributions of *MEG3* genotypes among asthma patients
233 within different symptom groups (Table 4). The variants genotypes (AG or AA) of
234 rs7158663 were over-represented in patients with more severe symptoms: the
235 frequencies of the variant genotypes and wild-type (GG) genotype was 35% and
236 65% in patients with the mildest symptom (Group 1), 44.6% and 55.4% in Group 2,
237 55.9% and 44.1% in Group 3, and 69.2% and 30.8%, respectively, in patients with
238 the most severe symptom (Group 4) ($P = 0.0148$) (Table 4). In contrast, the other
239 three SNPs were not associated with asthma symptoms.

240

241 To determine whether the association of rs7158663 with asthma differs between

patients with mild and severe symptoms, we next performed stratified analyses. To increase statistical power, we combined the two milder symptom groups and two severer symptom groups. As shown in Table 5, the association of the rs7158664 variant genotypes with asthma risk was only significant in patients with severer symptoms (adjusted OR=2.48, 95% CI=1.44-4.26, $P = 0.0024$), but not in patients with milder symptoms (adjusted OR = 0.91, 95% CI=0.61-1.43, $P = 0.6115$) (Table 5).

The variant genotypes of rs7158663 correlated with lower serum MEG3 level

Since *MEG3* rs7158663 variant genotypes are associated with increased asthma risks, and asthma patients have lower serum MEG3 level than controls (Feng et al., 2020), we next determine whether there is a correlation between rs7158663 genotypes and serum MEG3 level. We randomly selected 46 healthy controls and measured serum MEG3 expression level by quantitative PCR (one sample was somehow technically undetectable). Compared to the wild-type GG genotype carriers, the serum MEG3 expression level was significantly lower for the GA genotype carriers ($P < 0.0001$) and the lowest in AA genotype carriers ($P < 0.0001$ vs. GG genotype carriers and $P = 0.0081$ vs. AG genotype carriers) (Figure 3).

Discussion

In the current study, we found significant associations between *MEG3* rs7158663 SNP and the risk of asthma and symptom severity. Specifically, the variant genotypes (AG and AA) of rs7158663 were associated with increased risks of asthma and severe asthma symptoms. The variant genotype carriers had significantly lower serum MEG3 expression (Figure 3), consistent with prior reports of downregulation of MEG3 expression in asthma patients and providing biological explanation for the observed associations between variant genotypes and increased asthma risks. To our knowledge, the present study is the first to report the associations of *MEG3* genotypes with asthma risk and symptom severity.

MEG3 is a lncRNA that is abundantly expressed in most normal tissues but downregulated in a variety of tumor tissues including lung cancer (*Ghafouri-Fard & Taheri, 2019*). There have been strong evidences supporting that MEG3 plays a role in asthma development. An early study compared the profile of lncRNA expression in circulating $CD8^+$ T cells from asthma patients and healthy controls and found MEG3 expression was significantly lower in patients with severe asthma than healthy controls (*Tsitsiou et al., 2012*). A recent study (Feng et al., 2020) compared serum MEG3 expression level in 119 asthma patients and 125 healthy controls and found significantly lower serum MEG3 level in asthma patients than in controls and the expression level was the lowest in mixed granulocytic asthma (the subtype with

the most severe symptoms). Furthermore, serum MEG3 level was negatively correlated with the course of disease ($r = -0.666$, $P < 0.001$). Logistic regression analysis showed that inflammatory phenotype, the course of disease, and serum MEG3 level were independent prognostic factors for the recurrence of asthma (Feng et al., 2020). Supporting these direct evidences of human studies linking low MEG3 expression to asthma development, an early *in vitro* experiment tested the effects of cigarette smoke condensate (CSC) treatment on gene expression of human bronchial epithelial cells (HBEC) and found MEG3 was down-regulated in CSC-treated cells (*Hu et al., 2009*). Another *in vitro* study showed MEG3 expression was suppressed upon nickel (an environmental toxigenic molecule) treatment of HBEC (*Zhou et al., 2020*). All these evidences suggest that abundant MEG3 expression is important to maintain normal lung function and physiology, whereas reduced MEG3 expression favors proliferation and promotes the pathogenesis of common lung diseases such as asthma and lung cancer.

Our findings in this current study supports the notion that reduced MEG3 expression favors asthma development. We found the variant genotypes (AG and AA) of *MEG3* rs7158663 were associated with increased risks of asthma and severe symptoms. Furthermore, the variant genotypes of rs7158663 correlated with lower serum MEG3 expression. The rs7158663 genotypes-MEG3 expression-asthma

302 relationship is therefore biologically meaningful and plausible. Consistent with our
303 rs7158663 genotype-MEG3 expression correlation, a previous study also found the
304 variant A allele of rs7158663 was associated with significantly decreased serum
305 MEG3 level compared to the wild type G allele ($P < 0.0001$) (Ali et al., 2020).
306
307 The exact biological mechanisms underlying the roles of MEG3 in asthma
308 pathogenesis remains unclear. MEG3 is a multi-faceted molecule involved in many
309 microRNA and protein interactions, signal pathways, and cellular processes
310 (Ghafouri-Fard & Taheri, 2019; Chen & Deng, 2022). MEG3 activates transcription
311 factor and tumor suppressor TP53 by stabilizing TP53 protein and/or augmenting its
312 transcriptional activity, thereby induces TP53-dependent target gene expression and
313 inhibits cell growth (*Zhou et al., 2007; Ghafouri-Fard & Taheri, 2019*). MEG3
314 interacts with the polycomb repressive complex 2 (PRC2), which catalyzes the
315 methylation of histone H3 lysine 27 (H3K27) and functions as a key epigenetic
316 regulator for normal development (*Simon & Kingston, 2013*). MEG3 interacts with
317 PRC2 and its cofactor JARID2 to regulate TGF-β signaling pathway genes (*Kaneko,*
318 *et al., 2014; Mondal et al., 2019*). MEG3 also contributes to the regulation of several
319 other important cellular signaling pathways, including PI3K/AKT/mTOR, Wnt/β-
320 catenin, JAK/STAT, and Notch, and these pathways have been implicated in asthma
321 (*Wang et al., 2014; Ghafouri-Fard & Taheri, 2019*; *Athari 2019; Zhao et al., 2020)*.

More recently, increasing studies have shown another important function of MEG3: serving as a competing endogenous RNA (ceRNA) and sponge for various miRNAs, and counteracting their regulatory effects on target genes (*Moradi et al., 2019; Li et al., 2016; Zhang et al., 2021; Li et al., 2018; Luo et al., 2021; Qiu et al., 2019; Dong et al., 2022; Sun et al., 2021; Wang et al. 2021*). In this regard, it is worth noting that MEG3 could sponge microRNA-125a-5p (*Li et al. 2016*) and miRNA-17 (*Qiu et al. 2019*) as a ceRNA, relieve their inhibition on orphan receptor γt (RORγt), which is a regulator of regulatory T (Treg) cells and helper T cell-17 (Th17). Mounting evidences suggest that the balance of Treg and Th17 (Treg/Th17) plays a critical role in asthma development (*Tao et al., 2015; Zhu et al., 2017; Jing et al., 2019; Zhou et al., 2022*. Therefore, the microRNA sponge effect of MEG3 may be one of the biological mechanisms responsible for the involvement of MEG3 in asthma development. Better understanding of the biology behind the roles of MEG3 in asthma warrants further investigation.

MEG3 SNPs are associated with multiple diseases including asthma, inflammatory response, diabetes, stroke, and cancer (*Wallace, 2010; Han et al., 2018; Ghaedi et al., 2018; Ghafouri-Fard & Taheri, 2019; Gao et al., 2021; Zhu et al., 2021; Zhong et al., 2022*). Pleiotropy (i.e., shared genetic predisposition loci to different human diseases and traits) is pervasive in human genome (*Sivakumaran et*

342 *al., 2011; Pickrell et al., 2016; Tian et al., 2022*). For example, a missense SNP
343 (rs13107325) the zinc transporter SLC39A8 influences the risks of at least 7
344 different diseases and genetic traits, including schizophrenia, Parkinson's disease,
345 Crohn's disease, allergy, height, nearsightedness, and HDL (*Pickrell et al., 2016*);
346 and SNPs in the TERT-CLPTM1L locus have been associated with more than 12
347 different cancer and non-cancer diseases (*Tian et al., 2022*).
348
349 The present study has several limitations. First, we only selected one candidate
350 gene and the sample size was modest. Larger scale studies are needed to have a more
351 complete understanding of the genetic susceptibility to asthma in Taiwan. Second,
352 we had missing or incomplete information on some known environmental risk
353 factors and lacked longitudinal data of the dynamic environmental exposures and
354 could not perform gene-environment interaction analyses. Lastly, the mRNA levels
355 of MEG3 are lower in asthma patients than in healthy controls (*Feng et al., 2020*).
356 As a common practice, we performed the genotype-expression correlation in a subset
357 of healthy controls. It is worthwhile to validate that the variant genotypes also
358 correlate with lower MEG expression in asthma patients.
359

360 ## Conclusions

This study provides the first evidence that the variant genotypes at *MEG3* rs7158663 are associated with increased asthma risk and symptom severity. Moreover, there is a significant association between the variant genotype and lower MEG3 expression in serum. *MEG3* rs7158663 is a novel genetic susceptibility locus for asthma.

Figure legends

Figure 1. The proposed flow chart of participant recruitment.

Figure 2. The map of polymorphic sites of *MEG* rs7158663, rs3087918, rs11160608, and rs4081134.

Figure 3. Correlation between *MEG3* rs7158663 genotype and MEG3 expression in the serum of healthy controls. *Statistically significantly different from GG genotype; # Statistically significantly different from AG genotype.

Acknowledgments

We thank Yu-Ting Chin and Tai-Lin Huang for technical assistance in DNA extraction and serum MEG3 measurement.

Additional information and declarations

Funding

This study was supported by grants from the Taichung Tzu Chi Hospital (TTCRD111-15), China Medical University Hospital, and Asia University (CMU111-ASIA-02). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

Taichung Tzu Chi Hospital in Taichung, Taiwan: TTCRD111-15.

China Medical University Hospital, and Asia University in Taichung, Taiwan: CMU111-ASIA-02.

Competing Interests

The authors declare no competing interests.

Author Contributions

Kuo-Liang Chiu conceived and designed the experiments, analyzed the data, and approved the final draft.

Wen-Shin Chang performed the experiments, analyzed the data, and approved the final draft.

Chia-Wen Tsai performed the experiments, analyzed the data, prepared figures and tables, and approved the final draft.

Mei-Chin Mong conceived and designed the experiments, authored and reviewed drafts of the paper, and approved the final draft.

Te-Chun Hsia analyzed the data, authored and reviewed drafts of the paper, and approved the final draft.

Da-Tian Bau conceived and designed the experiments, authored and reviewed drafts of the paper, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The study was approved by the Ethics Review Committee of the China Medical University Hospital (CMUH106-REC1-004).

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

References

Ali MA, Shaker OG, Alazrak M, AbdelHafez MN, Khalefa AA, Hemeda NF, Abdelmoktader A, Ahmed FA. 2020. Association analyses of a genetic variant in long non-coding RNA MEG3 with breast cancer susceptibility and serum MEG3 expression level in the Egyptian population. *Cancer Biomark* **28(1)**:49-63. DOI 10.3233/CBM-191072.

Athari SS. 2019. Targeting cell signaling in allergic asthma. *Signal Transduction and Targeted Therapy* **4**:45. DOI 10.1038/s41392-019-0079-0.

Balik V, Srovnal J, Sulla I, Kalita O, Foltanova T, Vaverka M, Hrabalek L, Hajduch M. 2013. MEG3: a novel long noncoding potentially tumour-suppressing RNA in meningiomas. *Journal of Neuro-oncology* **112(1)**:1-8. DOI 10.1007/s11060-012-1038-6.

Bayarmaa B, Wu Z, Peng J, Wang Y, Xu S, Yan T, Yin W, Lu J, Zhou L. 2019. Association of LncRNA MEG3 polymorphisms with efficacy of neoadjuvant chemotherapy in breast cancer. *BMC Cancer* **19(1)**:877. DOI 10.1186/s12885-019-6077-3.

Bhat AA, Younes SN, Raza SS, Zarif L, Nisar S, Ahmed I, Mir R, Kumar S, Sharawat SK, Hashem S, Elfaki I, Kulinski M, Kuttikrishnan S, Prabhu KS, Khan AQ, Yadav SK, El-Rifai W, Zargar MA, Zayed H, Haris M, Uddin S. 2020. Role of non-coding RNA networks in leukemia progression, metastasis and drug resistance. *Molecular Cancer* **19(1)**:57. DOI 10.1186/s12943-020-01175-9.

Bonnelykke K, Ober C. 2016. Leveraging gene-environment interactions and endotypes for asthma gene discovery. *The Journal of Allergy and Clinical Immunology* **137(3)**:667-679. DOI 10.1016/j.jaci.2016.01.006.

Booton R, Lindsay MA. 2014. Emerging role of MicroRNAs and long noncoding RNAs in respiratory disease. *Chest* **146(1)**:193-204. DOI 10.1378/chest.13-2736.

Braconi C, Kogure T, Valeri N, Huang N, Nuovo G, Costinean S, Negrini M, Miotto E, Croce CM, Patel T. 2011. microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. *Oncogene* **30(47)**:4750-4756. DOI 10.1038/onc.2011.193.

Chak WP, Lung RW, Tong JH, Chan SY, Lun SW, Tsao SW, Lo KW, To KF. 2017. Downregulation of long non-coding RNA MEG3 in nasopharyngeal carcinoma. *Molecular Carcinogenesis* **56(3)**:1041-1054. DOI 10.1002/mc.22569.

Chen XF, Deng JM. 2022. Emerging role of long noncoding RNAs in asthma. *Combinatorial Chemistry & High Throughput Screening* **26(2)**:247-255. DOI 10.2174/1386207325666220825152237.

da Rocha ST, Edwards CA, Ito M, Ogata T, Ferguson-Smith AC. 2008. Genomic imprinting at the mammalian Dlk1-Dio3 domain. *Trends in Genetics* **24(6)**:306-316. DOI 10.1016/j.tig.2008.03.011.

Dong J, Xia R, Zhang Z, Xu C. 2021. lncRNA MEG3 aggravated neuropathic pain and astrocyte overaction through mediating miR-130a-5p/CXCL12/CXCR4 axis. *Aging (Albany NY)* **13(19)**:23004-23019. DOI 10.18632/aging.203592.

Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. 1990. Genetics of asthma and hay fever in Australian twins. *The American Review of Respiratory Disease* **142(6 Pt 1)**:1351-1358. DOI 10.1164/ajrccm/142.6_Pt_1.1351.

Edfors-Lubs ML. 1971. Allergy in 7000 twin pairs. *Acta Allergologica* **26(4)**:249-285. DOI 10.1111/j.1398-9995.1971.tb01300.x.

Feng Y, Yang C, Yan W. 2020. Expression of lncRNA MEG3 in asthma with different phenotypes and its relationship with course of disease. *Experimental and Therapeutic Medicine* **19(3)**:2211-2217. DOI 10.3892/etm.2020.8414.

Gao X, Li X, Zhang S, Wang X. 2021 The Association of MEG3 Gene rs7158663

474 Polymorphism With Cancer Susceptibility. *Frontiers in Oncology* **11**:796774
475 DOI 10.3389/fonc.2021.796774.
476 **Garcia-Sanchez A, Isidoro-Garcia M, Garcia-Solaesa V, Sanz C, Hernandez-**
477 **Hernandez L, Padron-Morales J, Lorente-Toledano F, Davila I. 2015.**
478 Genome-wide association studies (GWAS) and their importance in asthma.
479 *Allergologia et Immunopathologia (Madr)* **43(6)**:601-608. DOI
480 10.1016/j.aller.2014.07.004.
481 **Geisler S, Coller J. 2013.** RNA in unexpected places: long non-coding RNA
482 functions in diverse cellular contexts. *Nature reviews. Molecular Cell Biology*
483 **14(11)**:699-712. DOI 10.1038/nrm3679.
484 **Ghaedi H, Zare A, Omrani MD, Doustimotlagh AH, Meshkani R, Alipoor S,**
485 **Alipoor B. 2018.** Genetic variants in long noncoding RNA H19 and MEG3
486 confer risk of type 2 diabetes in an Iranian population. *Gene* **675**:265-271.
487 DOI 10.1016/j.gene.2018.07.002.
488 **Ghafouri-Fard S, Taheri M. 2019.** Maternally expressed gene 3 (MEG3): A tumor
489 suppressor long non coding RNA. *Biomedicine & Pharmacotherapy*
490 **118**:109129. DOI 10.1016/j.biopha.2019.109129.
491 **Global Initiative for Asthma (2022)** Global Initiative for Asthma (GINA)
492 guidelines. Global strategy for asthma management and prevention (Update
493 2022). Available from: https://ginasthma.org/ gina-reports/.
494 **Gysens F, Mestdagh P, de Bony de Lavergne E, Maes T. 2022.** Unlocking the
495 secrets of long non-coding RNAs in asthma. *Thorax* **77(5)**:514-522. DOI
496 10.1136/thoraxjnl-2021-218359.
497 **Han X, Zheng Z, Wang C, Wang L. 2018.** Association between MEG3/miR-181b
498 polymorphisms and risk of ischemic stroke. *Lipids in Health and Disease*
499 **17(1)**:292. DOI 10.1186/s12944-018-0941-z.
500 **Hsia TC, Chang WS, Wang S, Shen TC, Hsiao WY, Liu CJ, Liang SJ, Chen**

WC, Tu CY, Tsai CW, Hsu CM, Bau DT. 2015. The Contribution of Interleukin-10 Promoter Genotypes to Susceptibility to Asthma in Adults. *In Vivo* **29(6)**:695-699.

Hu YC, Yang ZH, Zhong KJ, Niu LJ, Pan XJ, Wu DC, Sun XJ, Zhou PK, Zhu MX, Huo YY. 2009. Alteration of transcriptional profile in human bronchial epithelial cells induced by cigarette smoke condensate. *Toxicology Letters* **190(1)**:23-31. DOI 10.1016/j.toxlet.2009.06.860.

Huang CY, Chang WS, Tsai CW, Hsia TC, Shen TC, Bau DT, Shui HA. 2018. The contribution of interleukin-8 genotypes and expression to nasopharyngeal cancer susceptibility in Taiwan. *Medicine (Baltimore)* **97(36)**:e12135. DOI 10.1097/MD.0000000000012135.

Jing W, Wang W, Liu Q. 2019. Passive smoking induces pediatric asthma by affecting the balance of Treg/Th17 cells. *Pediatric Research* **85(4)**:469-476. DOI 10.1038/s41390-019-0276-0.

Kabesch M, Tost J. 2020. Recent findings in the genetics and epigenetics of asthma and allergy. *Seminars in Immunopathology* **42(1)**:43-60. DOI 10.1007/s00281-019-00777-w.

Kaneko S, Bonasio R, Saldana-Meyer R, Yoshida T, Son J, Nishino K, Umezawa A, Reinberg D. 2014. Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin. *Molecular Cell* **53(2)**:290-300. DOI 10.1016/j.molcel.2013.11.012.

Li CH, Chiu KL, Hsia TC, Shen TC, Chen LH, Yu CC, Mong MC, Chang WS, Tsai CW, Bau DT. 2021. Significant Association of Cyclin D1 Promoter Genotypes With Asthma Susceptibility in Taiwan. *In Vivo* **35(4)**:2041-2046. DOI 10.21873/invivo.12473.

Li JQ, Hu SY, Wang ZY, Lin J, Jian S, Dong YC, Wu XF, Dai L, Cao LJ. 2016. Long non-coding RNA MEG3 inhibits microRNA-125a-5p expression and

induces immune imbalance of Treg/Th17 in immune thrombocytopenic purpura. *Biomedicine & Pharmacotherapy* **83**:905-911. DOI 10.1016/j.biopha.2016.07.057.

Li ZY, Yang L, Liu XJ, Wang XZ, Pan YX, Luo JM. 2018. The Long Noncoding RNA MEG3 and its Target miR-147 Regulate JAK/STAT Pathway in Advanced Chronic Myeloid Leukemia. *EBioMedicine* **34**:61-75. DOI 10.1016/j.ebiom.2018.07.013.

Liao CH, Chang WS, Liao JM, Wu HC, Shen TC, Yang JS, Tsai FJ, Tsai CW, Yu CC, Bau DT. 2020. The role of genotype/phenotype at apurinic/apyrimidinic endonuclease Rs1130409 in renal cell carcinoma. *The Chinese Journal of Physiology* **63(1)**:43-49. DOI 10.4103/CJP.CJP_72_19.

Liu T, Yin W, Luo L, Wu Y, Qin S, Qin X. 2022. Association between Interleukin-4-590C>T Polymorphism and the Susceptibility to Asthma: A Meta-Analysis of Case-Control Study. *Journal of Healthcare Engineering* **2022**:1712715. DOI 10.1155/2022/1712715.

Luo Z, Gong T, Li W, Tao W. 2022. LncRNA Meg3 promotes oxygen and glucose deprivation injury by decreasing angiogenesis in hBMECs by targeting the miR1225p/NDRG3 axis. *Experimental and Therapeutic Medicine* **24(4)**:622. DOI 10.3892/etm.2022.11559.

Mattiuzzi C, Lippi G. 2020. Worldwide asthma epidemiology: insights from the Global Health Data Exchange database. *International Forum of Allergy & Rhinology* **10(1)**:75-80. DOI 10.1002/alr.22464.

Melissari MT, Grote P. 2016. Roles for long non-coding RNAs in physiology and disease. *Pflugers Archiv* **468(6)**:945-958. DOI 10.1007/s00424-016-1804-y.

Mondal T, Subhash S, Vaid R, Enroth S, Uday S, Reinius B, Mitra S, Mohammed A, James AR, Hoberg E, Moustakas A, Gyllensten U, Jones SJ, Gustafsson CM, Sims AH, Westerlund F, Gorab E, Kanduri C. 2015.

MEG3 long noncoding RNA regulates the TGF-beta pathway genes through formation of RNA-DNA triplex structures. *Nature Communications* **6**:7743. DOI 10.1038/ncomms8743.

Moradi MT, Fallahi H, Rahimi Z. 2019. Interaction of long noncoding RNA MEG3 with miRNAs: A reciprocal regulation. *Journal of Cellular Biochemistry* **120(3)**:3339-3352. DOI 10.1002/jcb.27604.

Mulgirigama A, Barnes N, Fletcher M, Pedersen S, Pizzichini E, Tsiligianni I. 2019. A review of the burden and management of mild asthma in adults - Implications for clinical practice. *Respiratory Medicine* **152**:97-104. DOI 10.1016/j.rmed.2019.04.024.

Ober C, Yao TC. 2011. The genetics of asthma and allergic disease: a 21st century perspective. *Immunological Reviews* **242(1)**:10-30. DOI 10.1111/j.1600-065X.2011.01029.x.

Orom UA, Shiekhattar R. 2013. Long noncoding RNAs usher in a new era in the biology of enhancers. *Cell* **154(6)**:1190-1193. DOI 10.1016/j.cell.2013.08.028.

Pei JS, Chang WS, Chen CC, Mong MC, Hsu SW, Hsu PC, Hsu YN, Wang YC, Tsai CW, Bau DT. 2022. Novel Contribution of Long Non-coding RNA MEG3 Genotype to Prediction of Childhood Leukemia Risk. *Cancer Genomics Proteomics* **19(1)**:27-34. DOI 10.21873/cgp.20301.

Pickrell JK, Berisa T, Liu JZ, Tung JY. 2016. Detection and interpretation of shared genetic influences on 42 human traits. *Nature Genetics* **48(7)**:709-717. DOI 10.1038/ng.3570.

Qiu YY, Wu Y, Lin MJ, Bian T, Xiao YL, Qin C. 2019. LncRNA-MEG3 functions as a competing endogenous RNA to regulate Treg/Th17 balance in patients with asthma by targeting microRNA-17/ RORgammat. *Biomedicine & Pharmacotherapy* **111**:386-394. DOI 10.1016/j.biopha.2018.12.080.

582 **Rafiee A, Riazi-Rad F, Havaskary M, Nuri F. 2018.** Long noncoding RNAs:
583 regulation, function and cancer. *Biotechnology and Genetic Engineering*
584 *Reviews* **34(2)**:153-180. DOI 10.1080/02648725.2018.1471566.
585 **Ranjbar M, Whetstone CE, Omer H, Power L, Cusack RP, Gauvreau GM.**
586 **2022.** The Genetic Factors of the Airway Epithelium Associated with the
587 Pathology of Asthma. *Genes (Basel)* **13(10)**:1870. DOI
588 10.3390/genes13101870.
589 **Shen TC, Tsai CW, Chang WS, Wang S, Chao CY, Hsiao CL, Chen WC, Hsia**
590 **TC, Bau DT. 2017.** Association of Interleukin-12A rs568408 with
591 Susceptibility to Asthma in Taiwan. *Scientific Reports* **7(1)**:3199. DOI
592 10.1038/s41598-017-03523-0.
593 **Shi F, Zhang Y, Qiu C. 2022.** Gene polymorphisms in asthma: a narrative review.
594 *Annals of Translational Medicine* **10(12)**:711. DOI 10.21037/atm-22-2170.
595 **Simon JA, Kingston RE. 2013.** Occupying chromatin: Polycomb mechanisms for
596 getting to genomic targets, stopping transcriptional traffic, and staying put.
597 *Molecular Cell* **49(5)**:808-824. DOI 10.1016/j.molcel.2013.02.013.
598 **Sivakumaran S, Agakov F, Theodoratou E, Prendergast JG, Zgaga L, Manolio**
599 **T, Rudan I, McKeigue P, Wilson JF, Campbell H. 2011.** Abundant
600 pleiotropy in human complex diseases and traits. *American Journal of Human*
601 *Genetics* 89(5):607-618. DOI 10.1016/j.ajhg.2011.10.004.
602 **Statello L, Guo CJ, Chen LL, Huarte M. 2021.** Gene regulation by long non-
603 coding RNAs and its biological functions. *Nature Reviews Molecular Cell*
604 *Biology* **22(2)**:96-118. DOI 10.1038/s41580-020-00315-9.
605 **Sun HJ, Zhang FF, Xiao Q, Xu J, Zhu LJ. 2021.** lncRNA MEG3, Acting as a
606 ceRNA, Modulates RPE Differentiation Through the miR-7-5p/Pax6 Axis.
607 *Biochemical Genetics* **59(6)**:1617-1630. DOI 10.1007/s10528-021-10072-9.
608 **Tao B, Ruan G, Wang D, Li Y, Wang Z, Yin G. 2015.** Imbalance of Peripheral

Th17 and Regulatory T Cells in Children with Allergic Rhinitis and Bronchial Asthma. *Iranian Journal of Allergy, Asthma and Immunology* **14(4)**:273-279.

Temesi G, Virag V, Hadadi E, Ungvari I, Fodor LE, Bikov A, Nagy A, Galffy G, Tamasi L, Horvath I, Kiss A, Hullam G, Gezsi A, Sarkozy P, Antal P, Buzas E, Szalai C. 2014. Novel genes in Human Asthma Based on a Mouse Model of Allergic Airway Inflammation and Human Investigations. *Allergy Asthma Immunology Reseasrch* **6(6)**:496-503. DOI 10.4168/aair.2014.6.6.496.

Terashima M, Tange S, Ishimura A, Suzuki T. 2017. MEG3 Long Noncoding RNA Contributes to the Epigenetic Regulation of Epithelial-Mesenchymal Transition in Lung Cancer Cell Lines. *The Journal of Biological Chemistry* **292(1)**:82-99. DOI 10.1074/jbc.M116.750950.

Tian J, Wang Y, Dong Y, Chang J, Wu Y, Chang S, Che G. 2022. Cumulative Evidence for Relationships Between Multiple Variants in the TERT and CLPTM1L Region and Risk of Cancer and Non-Cancer Disease. *Frontiers in Oncology* **12**:946039. DOI 10.3389/fonc.2022.946039.

Tsitsiou E, Williams AE, Moschos SA, Patel K, Rossios C, Jiang X, Adams OD, Macedo P, Booton R, Gibeon D, Chung KF, Lindsay MA. 2012. Transcriptome analysis shows activation of circulating CD8+ T cells in patients with severe asthma. *Journal of Allergy and Clinical Immunology* **129(1)**:95-103. DOI 10.1016/j.jaci.2011.08.011.

Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG. 2010. The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. *Nature Genetics* **42(1)**:68-71. DOI 10.1038/ng.493.

Wang G, Ye Q, Ning S, Yang Z, Chen Y, Zhang L, Huang Y, Xie F, Cheng X, Chi J, Lei Y, Guo R, Han J. 2021. LncRNA MEG3 promotes endoplasmic

reticulum stress and suppresses proliferation and invasion of colorectal carcinoma cells through the MEG3/miR-103a-3p/PDHB ceRNA pathway. *Neoplasma* **68(2)**:362-374. DOI 10.4149/neo_2020_200813N858.

Wang J, Li F, Yang M, Wu J, Zhao J, Gong W, Liu W, Bi W, Dong L. 2014. FIZZ1 promotes airway remodeling through the PI3K/Akt signaling pathway in asthma. *Experimental and Therapeutic Medicine* **7(5)**:1265-1270. DOI 10.3892/etm.2014.1580.

Yan J, Guo X, Xia J, Shan T, Gu C, Liang Z, Zhao W, Jin S. 2014. MiR-148a regulates MEG3 in gastric cancer by targeting DNA methyltransferase 1. *Medical Oncology* **31(3)**:879. DOI 10.1007/s12032-014-0879-6.

Yang IV, Lozupone CA, Schwartz DA. 2017a. The environment, epigenome, and asthma. *The Journal of Allergy and Clinical Immunology* **140(1)**:14-23. DOI 10.1016/j.jaci.2017.05.011.

Yang MD, Lin KC, Lu MC, Jeng LB, Hsiao CL, Yueh TC, Fu CK, Li HT, Yen ST, Lin CW, Wu CW, Pang SY, Bau DT, Tsai FJ. 2017b. Contribution of matrix metalloproteinases-1 genotypes to gastric cancer susceptibility in Taiwan. *Biomedicine (Taipei)* **7(2)**:10. DOI 10.1051/bmdcn/2017070203.

Ying L, Huang Y, Chen H, Wang Y, Xia L, Chen Y, Liu Y, Qiu F. 2013. Downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer. *Molecular Biosystems* **9(3)**:407-411. DOI 10.1039/c2mb25386k.

Zhang S, Ji WW, Wei W, Zhan LX, and Huang X. 2021. Long noncoding RNA Meg3 sponges miR-708 to inhibit intestinal tumorigenesis via SOCS3-repressed cancer stem cells growth. *Cell Death & Disease* **13(1)**:25. DOI 10.1038/s41419-021-04470-5.

Zhang W, Nie Y, Chong L, Cai X, Zhang H, Lin B, Liang Y, Li C. 2013. PI3K and Notch signal pathways coordinately regulate the activation and

proliferation of T lymphocytes in asthma. *Life Sciences* **92(17-19)**:890-895. DOI 10.1016/j.lfs.2013.03.005.

Zhang X, Rice K, Wang Y, Chen W, Zhong Y, Nakayama Y, Zhou Y, Klibanski A. 2010. Maternally expressed gene 3 (MEG3) noncoding ribonucleic acid: isoform structure, expression, and functions. *Endocrinology* **151(3)**:939-947. DOI 10.1210/en.2009-0657.

Zhao Y, Liu S, Li X, Xu Z, Hao L, Cui Z, Bi K, Zhang Y, Liu Z. 2020. Cross-talk of Signaling Pathways in the Pathogenesis of Allergic Asthma and Cataract. *Protein & Peptide Letters* **27(9)**:810-822. DOI 10.2174/0929866527666200207113439.

Zheng Y, Wang M, Wang S, Xu P, Deng Y, Lin S, Li N, Liu K, Zhu Y, Zhai Z, Wu Y, Dai Z, Zhu G. 2020. LncRNA MEG3 rs3087918 was associated with a decreased breast cancer risk in a Chinese population: a case-control study. *BMC Cancer* **20(1)**:659. DOI 10.1186/s12885-020-07145-0.

Zhong C, Yao Q, Han J, Yang J, Jiang F, Zhang Q, Zhou H, Hu Y, Wang W, Zhang Y, Sun Y. 2022. SNP rs322931 (C>T) in miR-181b and rs7158663 (G>A) in MEG3 aggravate the inflammatory response of anal abscess in patients with Crohn's disease. *Aging (Albany NY)* **14(7)**:3313-3324. DOI 10.18632/aging.204014.

Zhou C, Huang C, Wang J, Huang H, Li J, Xie Q, Liu Y, Zhu J, Li Y, Zhang D, Zhu Q, Huang C. 2017. LncRNA MEG3 downregulation mediated by DNMT3b contributes to nickel malignant transformation of human bronchial epithelial cells via modulating PHLPP1 transcription and HIF-1alpha translation. *Oncogene* **36(27)**:3878-3889. DOI 10.1038/onc.2017.14.

Zhou Y, Zhao H, Wang T, Zhao X, Wang J, Wang Q. 2022. Anti-Inflammatory and Anti-asthmatic Effects of TMDCT Decoction in Eosinophilic Asthma Through Treg/Th17 Balance. *Frontiers in Pharmacology* **13**:819728. DOI

10.3389/fphar.2022.819728.

Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, Ansell PJ, Zhao J, Weng C, Klibanski A. 2007. Activation of p53 by MEG3 non-coding RNA. *Journal of Biological Chemistry* **282(34)**:24731-24742. DOI 10.1074/jbc.M702029200.

Zhu J, Liu X, Wang W, Ouyang X, Zheng W, Wang Q. 2017. Altered expression of regulatory T and Th17 cells in murine bronchial asthma. *Experimental and Therapeutic Medicine* **14(1)**:714-722. DOI 10.3892/etm.2017.4519.

Zhu R, Xiao T, Wang Q, Zhao Y, Liu X. 2021. Genetic polymorphisms in lncRNAs predict recurrence of ischemic stroke. *Metabolic Brain Disease* **36(6)**:1353-1359. DOI 10.1007/s11011-021-00725-4.

Table 1(on next page)

Table 1

1 **Table 1.** Distributions of baseline characteristics among the 198 asthmatic patients and 453 controls.

Index		Controls (n=453)		Cases (n=198)		*P*-value[a]
		n	%	n	%	
Age (years)	25-40	285	63.4%	133	67.2%	
	>40	168	36.6%	65	32.8%	0.2972
Gender	Male	190	41.9%	83	41.9%	
	Female	263	58.1%	115	58.1%	0.9956
Smoking status	Non-smoker	326	72.0%	139	70.2%	
	Smoker	127	28.0%	59	29.8%	0.7161
Pulmonary functions	(mean ± SD)					
FEV1/FVC (%)		80.8 ± 8.1		62.0 ± 13.0		<0.0001
FEV1%		92.9 ± 5.8		69.1 ± 12.9		<0.0001
Symptoms severity						
1 (mild)				60	30.3%	
2				65	32.8%	
3				34	17.2%	
4 (severe)				39	19.7%	

2Abbreviation: FEV1, forced expiratory volume in first second; FVC, forced vital capacity; FEV1%,
3percent of predicted FEV1; [a]Chi-square without Yate's correction test or *Student's* t-test.

Table 2(on next page)

Table 2

Table 2. Distributions of *MEG* various genotypes among asthmatic patients and non-asthmatic controls

Genotype	Asthmatic cases, n (%)	Non-asthmatic controls, n (%)	Adjusted OR (95% CI)[a]	*P*-value[b]
rs7158663				
GG	86 (43.4)	258 (57.0)	1.00 (Reference)	
AG	88 (44.5)	165 (36.4)	**1.62 (1.18-2.32)**	**0.0093***
AA	24 (12.1)	30 (6.6)	**2.68 (1.52-4.83)**	**0.0030***
AG+AA	112 (56.6)	195 (43.0)	**1.75 (1.29-2.48)**	**0.0015***
P_{trend}				**0.0024***
P_{HWE}				0.6039
rs3087918				
TT	74 (37.4)	183 (40.4)	1.00 (Reference)	
GT	94 (47.5)	215 (47.5)	1.12 (0.79-1.65)	0.6732
GG	30 (15.1)	55 (12.1)	1.37 (0.85-2.31)	0.2588
GT+GG	124 (62.6)	240 (59.6)	1.29 (0.87-1.81)	0.1650
P_{trend}				0.5285
P_{HWE}				0.5014
rs11160608				
AA	65 (32.8)	136 (30.0)	1.00 (Reference)	
AC	98 (49.5)	232 (51.2)	0.85 (0.62-1.31)	0.5221
CC	35 (17.7)	85 (18.8)	0.87 (0.55-1.43)	0.5527

AC+CC	133 (67.2)	317 (70.0)	0.89 (0.62-1.28)	0.4758
P_{trend}				0.7710
P_{HWE}				0.4256
rs4081134				
GG	104 (52.5)	254 (56.0)	1.00 (Reference)	
AG	82 (41.4)	172 (38.0)	1.18 (0.84-1.68)	0.3915
AA	12 (6.1)	27 (6.0)	1.07 (0.55-2.27)	0.8226
AG+AA	94 (47.5)	199 (44.0)	1.11 (0.87-1.64)	0.4029
P_{trend}				0.6920
P_{HWE}				0.7657

OR: Odds ratio; CI: confidence interval; HWE: Hardy-Weinberg Equilibrium; P_{trend}, *P*-value for trend analysis; P_{HWE}, *P*-value for Hardy-Weinberg equilibrium analysis; [a]Data has been adjusted for confounding factors for asthma including age, gender and smoking. [b]Based on Chi-square test with Yates' correction; *Statistically significant.

Table 3(on next page)

Table 3

Table 3. Distribution of *MEG* allelic frequencies among asthmatic patients and non-asthmatic controls

Allelic type	Asthmatic cases, n (%)	Non-asthmatic controls, n (%)	Adjusted OR (95% CI)[a]	*P*-Value[b]
rs7158663				
Allele G	260 (65.7)	681 (75.2)	1.00 (Reference)	
Allele A	136 (34.3)	225 (24.8)	**1.63 (1.25-2.07)**	**0.0004***
rs3087918				
Allele T	242 (61.1)	581 (64.1)	1.00 (Reference)	
Allele G	154 (38.9)	325 (35.9)	1.14 (0.85-1.48)	0.2990
rs11160608				
Allele A	228 (57.6)	504 (55.6)	1.00 (Reference)	
Allele C	168 (42.4)	402 (44.4)	0.89 (0.74-1.19)	0.5148
rs4081134				
Allele G	290 (73.2)	680 (75.1)	1.00 (Reference)	
Allele A	106 (26.8)	226 (24.9)	1.08 (0.84-1.47)	0.4875

OR: Odds ratio; CI: confidence interval. [a]Data has been adjusted for confounding factors for asthma including age, gender and smoking. [b]Based on Chi-square test with Yates' correction; *Statistically significant.

Table 4(on next page)

Table 4

Table 4. Association of *MEG* polymorphisms with the symptoms severity among asthmatic patients.

Genotype	Symptom severity, n (%)				*P*-value[a]
	1 (mild)	2	3	4 (severe)	
rs7158663					
Wild-type genotype	39 (65.0)	36 (55.4)	15 (44.1)	12 (30.8)	
Variant genotypes	21 (35.0)	29 (44.6)	19 (55.9)	27 (69.2)	**0.0148***
rs3087918					
Wild-type genotype	33 (55.0)	31 (47.7)	17 (50.0)	18 (46.2)	
Variant genotypes	27 (45.0)	34 (52.3)	17 (50.0)	21 (53.8)	0.8941
rs11160608					
Wild-type genotype	31 (51.7)	33 (50.8)	16 (47.1)	17 (43.6)	
Variant genotypes	29 (48.3)	32 (49.2)	18 (52.9)	22 (56.4)	0.9452
rs4081134					
Wild-type genotype	34 (56.7)	32 (49.2)	17 (50.0)	18 (46.2)	
Variant genotypes	26 (43.3)	33 (50.8)	17 (50.0)	21 (53.8)	0.8526

[a]Chi-square without Yate's correction test; The significant *p*-value and odds ratio are bolded and marked with a star.

Table 5(on next page)

Table 5

Table 5. Association of *MEG* rs7158663 genotypes with the symptoms severity among stratified asthmatic patients.

Genotype	Asthmatic cases, n (%)	Non-asthmatic controls, n (%)	Adjusted OR (95% CI)[a]	*P*-value[b]
Milder symptom severity				
Wild-type genotype	75 (60.0)	258 (57.0)	1.00 (Ref)	
Variant genotypes	50 (40.0)	195 (43.0)	0.91 (0.61-1.43)	0.6115
Severer symptom severity				
Wild-type genotype	27 (37.0)	258 (57.0)	1.00 (Ref)	
Variant genotypes	46 (63.0)	195 (43.0)	**2.48 (1.44-4.26)**	**0.0024***

OR: Odds ratio; CI: confidence interval. [a]Data has been adjusted for confounding factors for asthma including age, gender and smoking. [b]Based on Chi-square test with Yates' correction; The significant *p*-value and odds ratio are bolded and marked with a star.

Figure 1

Figure 1

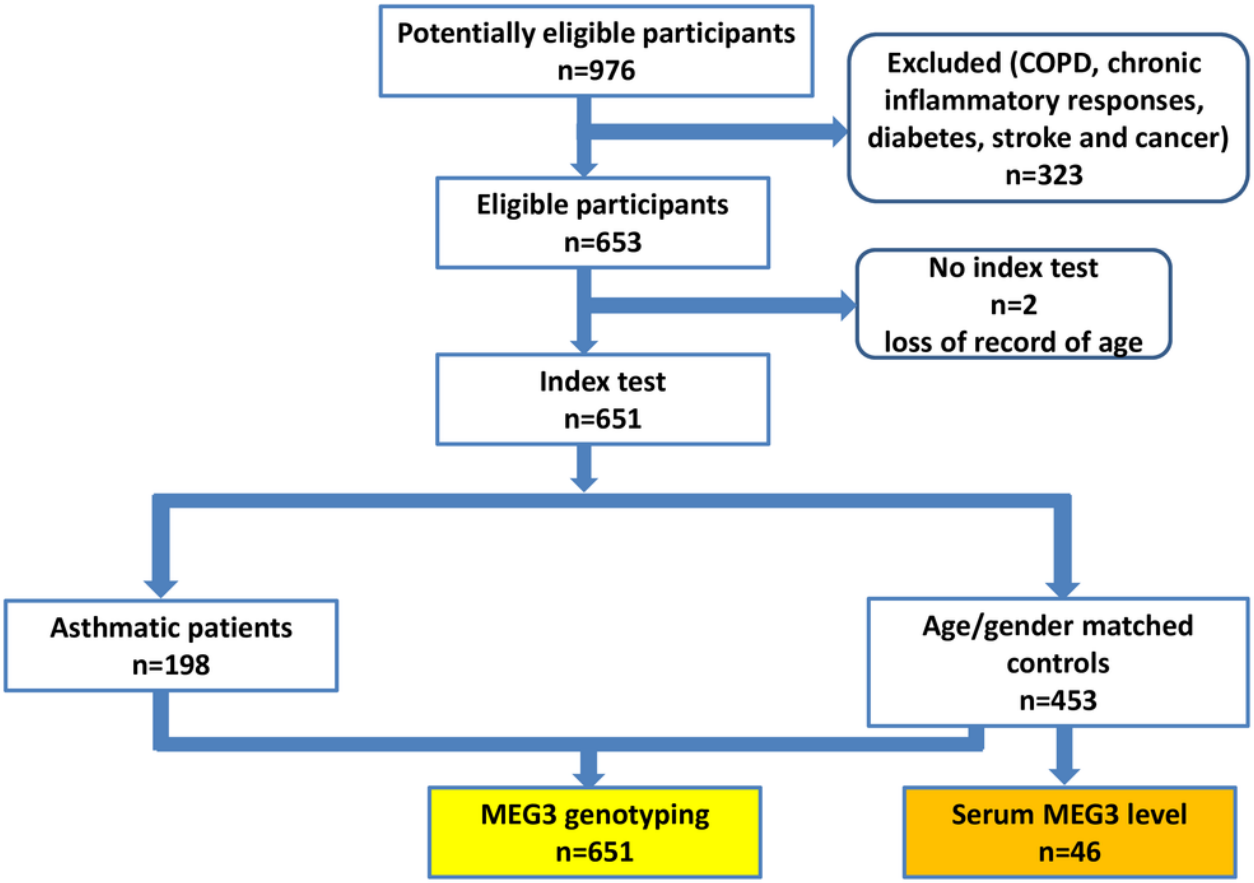

Figure 2

Figure 2

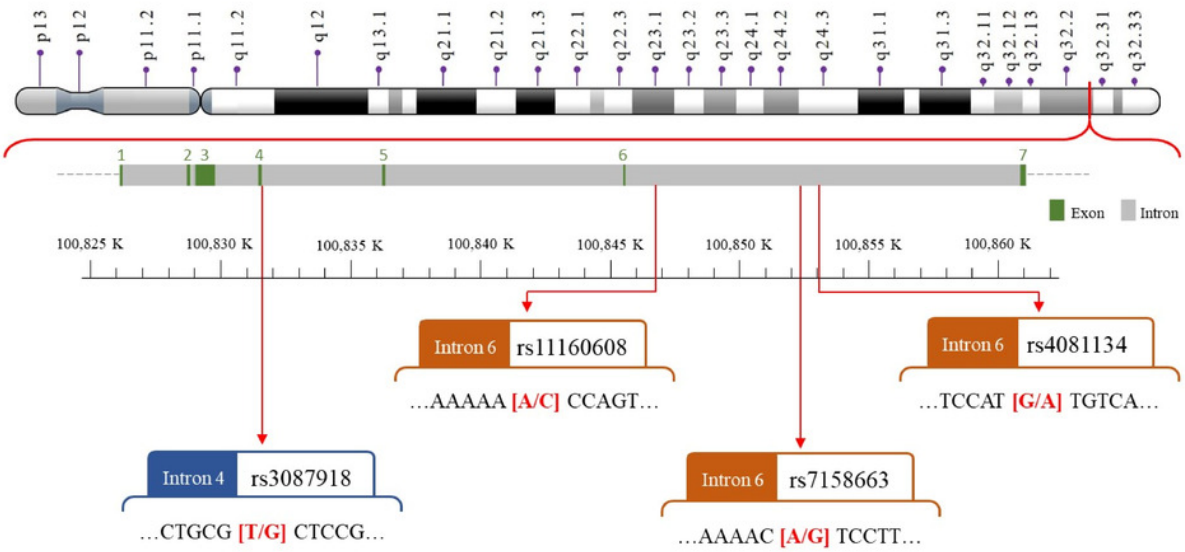

Figure 3
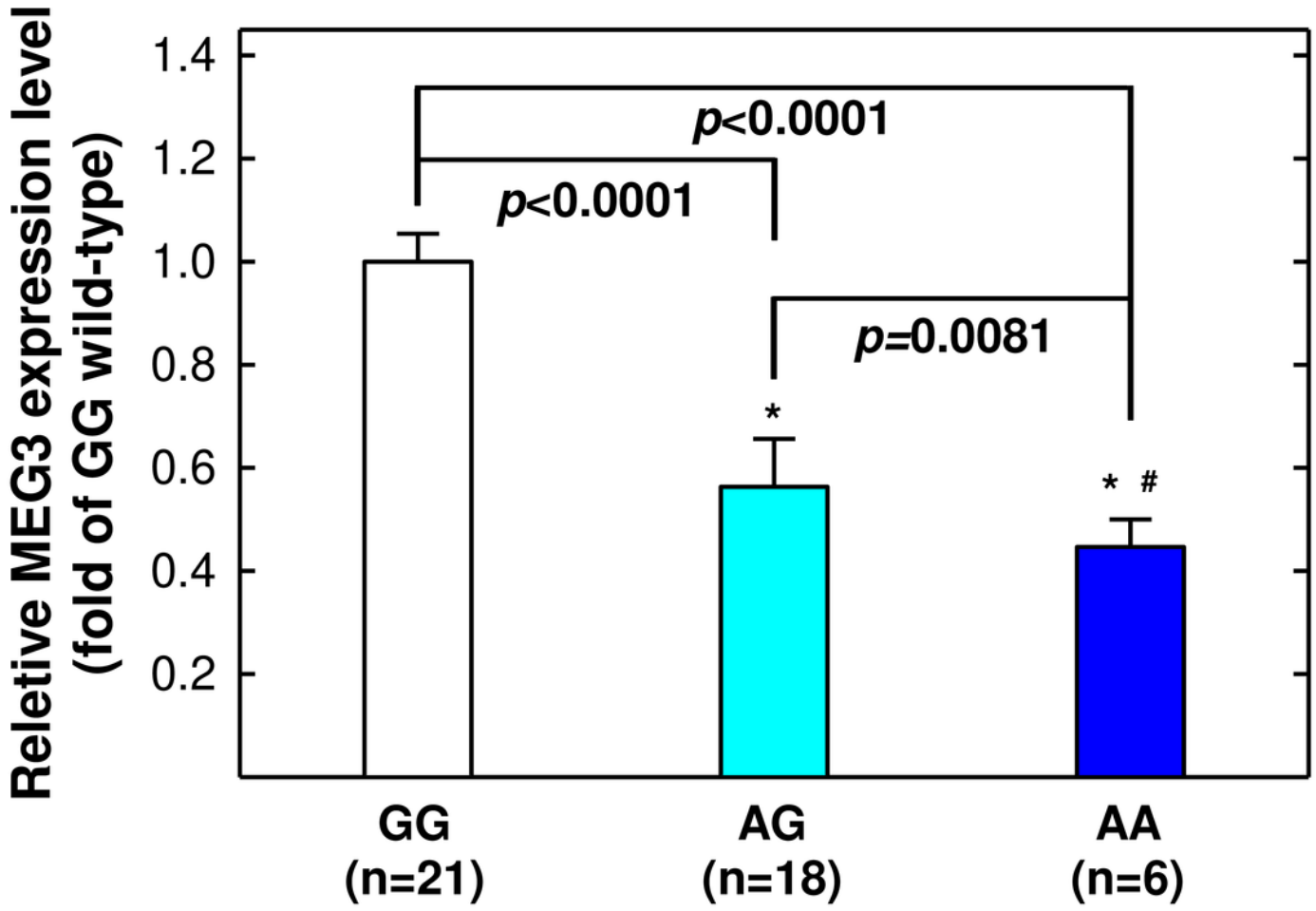
Figure 3