associated with asthma susceptibility

Miao miao Zhang\textsuperscript{1*}, Guo Chen\textsuperscript{2,3*}, Yu Wang\textsuperscript{1}, Shou quan Wu\textsuperscript{1}, Andrew J Sandford\textsuperscript{4}

Jian qing He\textsuperscript{1**}

1. Department of Respiratory and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China
2. Department of Geriatrics, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, Sichuan, China
3. Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, Sichuan, China
4. Centre for Heart Lung Innovation, University of British Columbia and St. Paul’s Hospital, Vancouver, BC, Canada

* These authors contributed equally.

** Corresponding author: Dr. Jian qing He, PhD, MD

No. 37, Guo Xue Alley, Chengdu, Sichuan, PR China 610041

Phone: 01186-18980602293 Fax: 01186-85422571

E-mail:

Miao miao Zhang: Zhangmiaomiao0522@126.com

Guo Chen: 5503850@163.com

Yu Wang: 390628840@qq.com

Shou quan Wu: 76201313@qq.com

Andrew J Sandford: Andrew.Sandford@hli.ubc.ca

Jian qing He: jianqing_he@scu.edu.cn; jianqhe@gmail.com
Abstract

Background: As a main line of defense of the respiratory tract, the airway epithelium plays an important role in the pathogenesis of asthma. CDHR3 and EMSY were reported to be expressed in the human airway epithelium. Although previous genome-wide association studies found that the two genes were associated with asthma susceptibility, similar observations have not been made in the Chinese Han population.

Methods: A total of 300 asthma patients and 418 healthy controls who were unrelated Chinese Han individuals were enrolled. Tag-single nucleotide polymorphisms (Tag-SNPs) were genotyped and the associations between SNPs and asthma risk were analyzed by binary logistic regression analysis.

Results: After adjusting for confounding factors, the A allele of rs3847076 in CDHR3 was associated with increased susceptibility to asthma (OR = 1.407, 95% CI: 1.030-1.923). For the EMSY gene, the T alleles of both rs2508746 and rs12278256 were related with decreased susceptibility to asthma (additive model: OR = 0.718, 95% CI: 0.536-0.961; OR = 0.558, 95% CI: 0.332-0.937, respectively). In addition, the GG genotype of rs1892953 showed an association with increased asthma risk under the recessive model (OR = 1.667, 95% CI: 1.104-2.518) and the GATCTGAGT haplotype in EMSY was associated with reduced asthma risk (P = 0.037).
Conclusions: This study identified novel associations of rs3847076 in *CDHR3*, as well as rs1892953, rs2508746 and rs12278256 in *EMSY* with adult asthma susceptibility in the Chinese Han population. Our observations suggest that *CDHR3* and *EMSY* may play important roles in the pathogenesis of asthma in Chinese individuals. Further study with larger sample size is needed.

Trial Registration: Not applicable.

**Key Words:** *CDHR3*; *EMSY*; asthma; polymorphism; susceptibility
Introduction

Asthma is a chronic airway inflammatory disease that affects populations throughout the world. A World Health Organization report [1] predicted that the number of asthma patients would increase to 400 million by 2025 and 250,000 patients may die from this disease each year. A recent survey indicated that the prevalence of asthma among individuals aged >14 years was 1.24% and there are approximately 30 million asthmatic patients in China [2]. The pathogenesis of asthma is still incompletely understood but it is known that genetic factors play a significant part in asthma susceptibility. The heritability of asthma was estimated to be 60% to 70% in an Australian twin study [3]. Genetic factors contributed to 90% of the variance in the susceptibility to asthma in a 5-year-old twin pair study [4].

As the first barrier between the human body and the environment, the airway epithelium has an important role in regulating the inflammation, immunity and tissue repair in the pathogenesis of asthma [5]. One genome-wide association study (GWAS) of a Danish population identified Cadherin related family member 3 (CDHR3), which is highly expressed in human airway epithelium, as a susceptibility locus for childhood asthma with severe exacerbations [6]. A GWAS in 2017 demonstrated that Chromosome 11 open reading frame 30 (C11orf30), also called EMSY or BRCA2-interacting transcriptional repressor, another gene expressed in airway epithelium, was a risk locus for food allergy in a Canadian population [7] and this gene has been shown to be involved in the epigenetic regulation of gene expression...
However, there have been few studies of these two genes in Chinese asthmatics. Therefore, this study aimed to investigate the association of common variants in CDHR3 and EMSY with adult asthma in the Chinese population.

Materials and Methods

Study population

The asthmatic cases were diagnosed by at least three respiratory physicians from the West China Hospital according to the criteria of the Global Strategy for Asthma Management and Prevention [9]. The healthy controls were collected from the physical examination center in the same hospital. Subjects were excluded if any of the following conditions were present: chronic obstructive pulmonary disease, diabetes, tumors, any immune disease, and immune deficiency. Use of hormones or immunosuppressive drugs were also exclusion criteria. Cases and controls were unrelated Chinese Han individuals. After signing the informed consent, 3 ml of venous blood were drawn from every subject and stored in a -80°C freezer. All blood specimens were collected from September 2013 to September 2016. The study was approved by the ethical committee of the West China Hospital of Sichuan University (Protocol No. 23).

SNP Selection and Genotyping

Single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) $\geq 0.05$ and $r^2 \geq 0.64$, located in the region 3000 base pairs upstream to 300 base pairs
downstream of CDHR3 were downloaded from the Han Chinese in Beijing database of the Genome Variation Server 147 (http://gvs.gs.washington.edu/GVS/), which is an online resource based on dbSNP. The final selections were 23 tag-SNPs including rs3887998, rs12155008, rs41267, rs3892893, rs10270308, rs34426483, rs193795, rs2526978, rs381188, rs10241452, rs3847076, rs11981655, rs10808147, rs193806, rs2528883, rs41269, rs2526979, rs2526976, rs41262, rs41266, rs6967330, rs41270 and rs448024. The selection of SNPs in EMSY was based on the tag-SNP strategy and literature review [10-13]. The tag-SNP selection strategy was the same as above except for $r^2 \geq 0.80$. The 17 SNPs selected were rs3753051, rs7125744, rs7926009, rs4945087, rs2508740, rs1939469, rs7115331, rs1044265, rs12278256, rs2513513, rs2508755, rs2155219, rs2513525, rs2508746, rs1892953, rs7130588 and rs10899234.

Genomic DNA was extracted from the blood samples using a genomic DNA purification kit (Axygen Scientific Inc, Union City, CA, USA). SNPs were genotyped by Genesky Bio-Tech Co., Ltd (http://geneskybiotech.com/index.html) using the SNPscanTM multiplex SNP genotyping technique based on double ligation and multiplex fluorescence polymerase Chain Reaction (PCR) [14]. As a quality control measure, 5% of random samples were repeat genotyped with a concordance rate of 100%.

**Data Analyses**

Statistical tests were performed using the Statistical Package for the Social Sciences
(SPSS, SPSS Inc., Chicago, IL, USA), version 21.0. A $p$ value <0.05 was considered to be statistically significant. Continuous and categorical variables were analyzed by student’s t-test and chi-square test, respectively. Genotype distributions under additive, dominant and recessive models were calculated by binary logistic regression analysis. Hardy-Weinberg equilibrium (HWE) among the controls was tested using plink software. Haploview and SHEsis software (http://analysis.bio-x.cn) were combined to perform linkage disequilibrium (LD) and haplotype analysis. Statistically significant SNPs were predicted by the software RegulomeDB (http://www.regulomedb.org/) and Haploreg v4 (http://compbio.mit.edu/HaploReg).

**Results**

**Subject characteristics**

A total of 300 asthma patients and 418 healthy controls were enrolled. The average ages of asthma patients and controls were 43.6 ± 13.48 and 44.09 ± 13.75 years, respectively. No significant differences in sex, body mass index (BMI) and smoking history were observed between case and control groups (Table 1). Late-onset asthma (age of asthma onset $\geq 18$ years) accounted for 74.3% in the case group.

**Association analyses between CDHR3, EMSY SNPs and asthma susceptibility**

The characteristics of the selected SNPs are listed in Table S1 and S2. Rs10899234 in EMSY and rs6967330 in CDHR3 were excluded due to their deviation from HWE in the control subjects ($P<0.05$). The genotyping assays failed for rs12155008, rs41270 and rs448024 in CDHR3.
After adjusting for confounding factors including age, sex, BMI and smoking history, four SNPs were found to be associated with asthma susceptibility (Table 2). The A allele of rs3847076 in CDHR3 was associated with increased susceptibility to asthma under the additive model ($P = 0.032$, OR = 1.407, 95% CI: 1.030-1.923). For EMSY, both the TC/TT genotype and T allele of rs2508746 were associated with decreased risk of asthma (dominant model: $P = 0.019$, OR = 0.660, 95% CI: 0.465-0.935; additive model: $P = 0.026$, OR = 0.718, 95% CI: 0.536-0.961). The TG/TT genotype and T allele of rs12278256 were associated with reduced asthma risk (dominant model: $P = 0.033$, OR = 0.563, 95% CI: 0.332-1.953; additive model: $P = 0.027$, OR = 0.558, 95% CI: 0.332-0.937). Finally, the GG genotype of rs1892953 showed an association with increased asthma risk under the recessive model ($P = 0.015$, OR = 1.667, 95% CI: 1.104-2.518).

Stratified analysis results by gender, smoking status, BMI status and onset age of asthma were shown in Table 3. Allele A of rs3847076 was associated with increased susceptibility to asthma in male subgroup, smoking subgroup, non-overweight subgroup and late onset asthma subgroup ($P=0.023$, OR=1.869; $P=0.009$, OR=2.168; $P=0.005$, OR=1.835 and $P=0.023$, OR=1.457, respectively). Similarly, rs2508746 TC+TT was related with decreased asthma susceptibility in the non-smoking subgroup, non-overweight subgroup, and late-onset asthma subgroup in dominant model ($P=0.014$, OR=0.618; $P=0.027$, OR=0.612 and $P=0.016$, OR=0.637, respectively). Meanwhile, rs1892953 GG shown increased risk of asthma in the
female subgroup, non-smoking subgroup, non-overweight subgroup, and late onset asthma subgroup in recessive model ($P=0.038$, OR=1.738; $P=0.04$, OR=1.615; $P=0.017$, OR=1.910 and $P=0.017$, OR=1.680, respectively). Rs12278256 T was still associated with decreased asthma susceptibility in female subgroups, non-smoking subgroups, and non-overweight subgroups in additive model ($P=0.032$, OR=0.465; $P=0.02$, OR=0.508 and $P=0.028$, OR=0.481, respectively).

**Haplotype and LD analysis**

The LD between SNPs of *CDHR3* and *EMSY* was low and those SNPs were divided into eight haplotype blocks with Haploview software (Figures 1 and 2). Only the haplotype consisting of GATCTGAGT in block 1 of *EMSY* was associated with decreased risk of asthma ($P = 0.037$, OR = 0.615, 95% CI: 0.388-0.975) (Table 4).

**Functional prediction results**

Four statistically significant SNPs were predicted using the software RegulomeDB and Haploreg v4 (Table S3). Rs144934374 is strongly linked to rs12278256 and its RegulomeDB scores is lower than that of rs12278256, suggesting that it may be the functional site represented by rs12278256. Acting as promoter histone marks or enhancer histone marks, or affecting DNAse is suggested to be associated with chromatin status, and binding proteins or altering regulatory motifs in ChIP-Seq suggest that transcription levels may be affected. It seems that these four SNPs may have certain effects on chromatin status and transcription level. Rs1892953 appears as an expression quantitative trait loci (eQTL) SNP in thyroid tissue [12].
Discussion

In this group of Chinese Han adults, the relationship between two airway epithelial-related genes *EMSY* and *CDHR3* and risk of asthma were investigated, and four polymorphisms related to asthma susceptibility were obtained, which were rs3847076 of *CDHR3* and rs2508746, rs1892953 and rs12278256 of *EMSY*. A further subgroup analysis of these four variants revealed that their association with asthma was present in the subgroup.

*CDHR3*, located on chromosome 7, is specifically expressed in ciliated airway epithelial cells which are the targets of Rhinovirus C (RV-C) infection, and its expression was positively associated with RV-C binding, replication and entry into the host cells [15,16]. There are only a few studies describing the relationship between *CDHR3* polymorphisms and asthma, and the results were inconsistent in different populations. The A allele of rs6967330 in *CDHR3* increased the risk of wheezing illnesses and hospitalizations for childhood asthma in a Danish study [6]. Rs17152490, in LD with rs6967330 was reported to affect asthma risk through *cis*-regulation of its gene expression in cells from human bronchial epithelial biopsy [17]. However, rs6967330 was only related to early-onset asthma in a Japanese population [18] and no association between rs6967330 and asthma was found in Chinese children [19]. In the present study, rs6967330 was not in HWE and our data suggest that rs3847076 may increase the risk of asthma in adults, which were inconsistent with the previous studies. The potential reasons for this discrepancy are as follows: Firstly, the
susceptibility to asthma may differ in different populations, and secondly, late-onset asthma patients accounted for the majority of the case group in this study, in contrast to the above Japanese study which reported the positive relationship between rs6967330 and early-onset asthma in children. A future study of different asthma phenotypes would be beneficial to the accurate prevention and treatment of asthma.

EMSY, located on chromosome 11q13.5, is expressed in the human airway epithelium and encoded by the EMSY protein. GWAS studies showed that EMSY was involved in allergic diseases including atopic dermatitis and food allergy [20,21]. Several SNPs, rs7130588, rs10899234, rs6592657, as well as we studied SNPs rs2508746 and rs1892953 were associated with total serum immunoglobulin E (IgE) levels in non-Hispanic Caucasian asthmatic patients [10]. In an eQTL analysis, Li et al [17] reported that rs2508740, rs2513525, rs4300410 (in complete LD with rs7926009), rs10793169 (in complete LD with rs7926009), rs2513513 and rs4245443 were significantly correlated with mRNA expression levels of EMSY in human bronchial alveolar lavage. Another GWAS study reported that rs7130588 in EMSY was associated with asthma [22]. A meta-analysis demonstrated that rs2155219 in EMSY increased the risk of allergic sensitization [11]. In the present study, three SNPs (rs2508746, rs1892953 and rs12278256) were related to asthma susceptibility in the Chinese Han population, of which rs12278256 has not been reported in previous studies. As a variant located in the upstream region of EMSY, rs12278256 might
affect the regulatory motifs and chromatin status of this gene and further study is needed to verify this hypothesis.

Studies in the twin population have shown that susceptibility to asthma can be attributed to genetic factors [3,4]. Although current genome-wide association studies have identified numerous polymorphisms associated with asthma susceptibility, the odds ratio (OR) is around 1.2, and only a small percentage of asthma prevalence can be contributed to them. Some experts have proposed to study the interaction between genes and environment [23,24]. It is well known that environmental factors such as smoking and obesity are susceptibility factors for asthma, but the specific mechanism is not clear. A number of studies have shown that smoking is associated with increased risk of asthma, reduced efficacy of inhaled corticosteroids treatment, acute exacerbations, and airway remodeling in asthma [25-29]. Mechanisms of asthma in the obese may include mechanical factors and inflammatory immunity [30]. Studies have shown that the SNPs at 17q21.2 is associated with BMI levels in asthmatic patients [31]. In our study, further analysis of the interaction between rs3847076 and environmental factors (smoking, BMI) revealed negative (Table S4). Functional prediction suggests that rs3847076 may affect the motif TCF4, and further investigation is needed.

Recently, genetic studies have detected a lot of susceptibility genes for asthma. This study was the first attempt to investigate the association between CDHR3, EMSY and adult asthma susceptibility in the Chinese Han population. We found rs3847076 in
CDHR3, rs2508746, rs1892953 and rs12278256 in EMSY were associated with the risk of adult asthma. However, there were some limitations to this study. Adjustment was not performed to correct the results for multiple testing, due to the weak effect of each single polymorphism on asthma susceptibility. In addition, the allergic phenotypes of the asthma patients were not clear and serum IgE levels were not analyzed in the study. Lastly, CDHR3 is a huge gene spanning over 159kb and the strategy of tag-SNPs selection with \( r^2 > 0.64 \) in this study may have missed some SNPs associated with the disease.

Conclusions

In conclusion, this study is the first to identify that the airway epithelium related genes EMSY and CDHR3 were associated with adult asthma susceptibility in the Chinese Han population. The CDHR3 rs3847076 allele A and EMSY rs1892953 genotype GG may increase the risk of asthma. The EMSY rs2508746 and rs12278256 allele T may decrease asthma risk. A population with a larger sample size is needed for further exploration of the association.

List of abbreviations

Tag-SNPs, tag-single nucleotide polymorphisms

GWAS, genome-wide association study

CDHR3, cadherin related family member 3
**C11orf30**, chromosome 11 open reading frame 30

SNPs, single nucleotide polymorphisms

MAF, minor allele frequency

PCR, polymerase chain reaction

SPSS, statistical package for the social sciences

HWE, Hardy-Weinberg equilibrium

LD, linkage disequilibrium

BMI, body mass index

RV-C, rhinovirus C

IgE, immunoglobulin E

eQTL, expression quantitative trait loci

OR, odds ratio

**Declarations**

**Ethics approval and consent to participate**

All protocols for this study were reviewed and approved by the Institutional Review Board of the West China Hospital of Sichuan University (Protocol No. 23). Written informed consent was obtained from all the study participants.

**Acknowledgements**

We thank everyone who provided blood samples and consent for genetic analysis. And we thank all of the clinicians, nurses and study coordinators for their contributions to the work.

**Funding**
This work was supported by the National Natural Science Foundation of China [Grant No. 81370121]; the Health and Family Planning Commission of Sichuan Province Project [Grant No. 16PJ413]; and the Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital Project [Grant No. 2017QN11]; and the Sichuan Provincial Cadre Health Research Project [Grant No. 2018-211].

**Author Contributions**

Conceptualization, Jianqing He; Data curation, Yu Wang and Shou-Quan Wu; Formal analysis, Yu Wang and Shou-Quan Wu; Project administration, Miao-miao Zhang, Guo Chen and Jianqing He; Supervision, Jianqing He; Writing – original draft, Miao-miao Zhang and Guo Chen; Writing – review & editing, Andrew J Sandford.

**Competing interests**

The Authors declare that there is no conflict of interest.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

**References**

1. WHO. Global surveillance, prevention and control of chronic respiratory diseases: WHO; 2007

   [http://www.who.int/respiratory/publications/global_surveillance/en/ (accessed April 23, 2018)]. 2007.
2. Lin, J.; Wang, W.; Chen, P.; Zhou, X.; Wan, H.; Yin, K.; Ma, L.; Wu, C.; Li, J.; Liu, C., et al. Prevalence and risk factors of asthma in mainland China: The CARE study. *Respir Med* **2018**, *137*, 48-54, doi:10.1016/j.rmed.2018.02.010.

3. Duffy, D.L.; Martin, N.G.; Battistutta, D.; Hopper, J.L.; Mathews, J.D. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* **1990**, *142*, 1351-1358, doi:10.1164/ajrccm/142.6 Pt_1.1351.

4. van Beijsterveldt, C.E.; Boomsma, D.I. Genetics of parentally reported asthma, eczema and rhinitis in 5-yr-old twins. *Eur Respir J* **2007**, *29*, 516-521, doi:10.1183/09031936.00065706.

5. Lambrecht, B.N.; Hammad, H. The airway epithelium in asthma. *Nat Med* **2012**, *18*, 684-692, doi:10.1038/nm.2737.

6. Bonnelykke, K.; Sleiman, P.; Nielsen, K.; Kreiner-Moller, E.; Mercader, J.M.; Belgrave, D.; den Dekker, H.T.; Husby, A.; Sevelsted, A.; Faura-Tellez, G., et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet* **2014**, *46*, 51-55, doi:10.1038/ng.2830.

7. Asai, Y.; Eslami, A.; van Ginkel, C.D.; Akhabir, L.; Wan, M.; Ellis, G.; Ben-Shoshan, M.; Martino, D.; Ferreira, M.A.; Allen, K., et al.
Genome-wide association study and meta-analysis in multiple populations identifies new loci for peanut allergy and establishes C11orf30/EMSY as a genetic risk factor for food allergy. *J Allergy Clin Immunol* 2018, 141, 991-1001, doi:10.1016/j.jaci.2017.09.015.

8. Varier, R.A.; Carrillo de Santa Pau, E.; van der Groep, P.; Lindeboom, R.G.; Matarese, F.; Mensinga, A.; Smits, A.H.; Edupuganti, R.R.; Baltissen, M.P.; Jansen, P.W., et al. Recruitment of the Mammalian Histone-modifying EMSY Complex to Target Genes Is Regulated by ZNF131. *J Biol Chem* 2016, 291, 7313-7324, doi:10.1074/jbc.M115.701227.

9. Reddel, H.K.; Bateman, E.D.; Becker, A.; Boulet, L.P.; Cruz, A.A.; Drazen, J.M.; Haahtela, T.; Hurd, S.S.; Inoue, H.; de Jongste, J.C., et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015, 46, 622-639, doi:10.1183/13993003.00853-2015.

10. Li, X.; Ampleford, E.J.; Howard, T.D.; Moore, W.C.; Li, H.; Busse, W.W.; Castro, M.; Erzurum, S.C.; Fitzpatrick, A.M.; Gaston, B., et al. The C11orf30-LRRC32 region is associated with total serum IgE levels in asthmatic patients. *J Allergy Clin Immunol* 2012, 129, 575-578, 578 e571-579, doi:10.1016/j.jaci.2011.09.040.
11. Bonnellykke, K.; Matheson, M.C.; Pers, T.H.; Granell, R.; Strachan, D.P.; Alves, A.C.; Linneberg, A.; Curtin, J.A.; Warrington, N.M.; Standl, M., et al. Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. *Nat Genet* 2013, 45, 902-906, doi:10.1038/ng.2694.

12. Consortium, G.T. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015, 348, 648-660, doi:10.1126/science.1262110.

13. Weidinger, S.; Willis-Owen, S.A.; Kamatani, Y.; Baurecht, H.; Morar, N.; Liang, L.; Edser, P.; Street, T.; Rodriguez, E.; O’Regan, G.M., et al. A genome-wide association study of atopic dermatitis identifies loci with overlapping effects on asthma and psoriasis. *Hum Mol Genet* 2013, 22, 4841-4856, doi:10.1093/hmg/ddt317.

14. Du, W.; Cheng, J.; Ding, H.; Jiang, Z.; Guo, Y.; Yuan, H. A rapid method for simultaneous multi-gene mutation screening in children with nonsyndromic hearing loss. *Genomics* 2014, 104, 264-270, doi:10.1016/j.ygeno.2014.07.009.

15. Bochkov, Y.A.; Watters, K.; Ashraf, S.; Griggs, T.F.; Devries, M.K.; Jackson, D.J.; Palmenberg, A.C.; Gern, J.E. Cadherin-related family member 3, a childhood asthma susceptibility gene product,
mediates rhinovirus C binding and replication. *Proc Natl Acad Sci U S A* 2015, 112, 5485-5490, doi:10.1073/pnas.1421178112.

16. Griggs, T.F.; Bochkov, Y.A.; Basnet, S.; Pasic, T.R.; Brockman-Schneider, R.A.; Palmenberg, A.C.; Gern, J.E. Rhinovirus C targets ciliated airway epithelial cells. *Respir Res* 2017, 18, 84, doi:10.1186/s12931-017-0567-0.

17. Li, X.; Hastie, A.T.; Hawkins, G.A.; Moore, W.C.; Ampleford, E.J.; Milosevic, J.; Li, H.; Busse, W.W.; Erzurum, S.C.; Kaminski, N., et al. eQTL of bronchial epithelial cells and bronchial alveolar lavage deciphers GWAS-identified asthma genes. *Allergy* 2015, 70, 1309-1318, doi:10.1111/all.12683.

18. Kanazawa, J.; Masuko, H.; Yatagai, Y.; Sakamoto, T.; Yamada, H.; Kaneko, Y.; Kitazawa, H.; Iijima, H.; Naito, T.; Saito, T., et al. Genetic association of the functional CDHR3 genotype with early-onset adult asthma in Japanese populations. *Allergol Int* 2017, 66, 563-567, doi:10.1016/j.alit.2017.02.012.

19. Chen, J.; Zhang, J.; Hu, H.; Jin, Y.; Xue, M. Polymorphisms of RAD50, IL33 and IL1RL1 are associated with atopic asthma in Chinese population. *Tissue Antigens* 2015, 86, 443-447, doi:10.1111/tan.12688.
20. Marenholz, I.; Grosche, S.; Kalb, B.; Ruschendorf, F.; Blumchen, K.; Schlags, R.; Harandi, N.; Price, M.; Hansen, G.; Seidenberg, J., et al. Genome-wide association study identifies the SERPINB gene cluster as a susceptibility locus for food allergy. *Nat Commun* 2017, 8, 1056, doi:10.1038/s41467-017-01220-0.

21. Esparza-Gordillo, J.; Weidinger, S.; Folster-Holst, R.; Bauerfeind, A.; Ruschendorf, F.; Patone, G.; Rohde, K.; Marenholz, I.; Schulz, F.; Kerscher, T., et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. *Nat Genet* 2009, 41, 596–601, doi:10.1038/ng.347.

22. Ferreira, M.A.; Matheson, M.C.; Duffy, D.L.; Marks, G.B.; Hui, J.; Le Souef, P.; Danoy, P.; Baltic, S.; Nyholt, D.R.; Jenkins, M., et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet* 2011, 378, 1006–1014, doi:10.1016/S0140-6736(11)60874-X.

23. Moffatt, M.F.; Gut, I.G.; Demenais, F.; Strachan, D.P.; Bouzigon, E.; Heath, S.; von Mutius, E.; Farrall, M.; Lathrop, M.; Cookson, W., et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010, 363, 1211–1221, doi:10.1056/NEJMoa0906312.
24. Ober, C. Asthma Genetics in the Post-GWAS Era. *Ann Am Thorac Soc* 2016, 13 Suppl 1, S85–90, doi:10.1513/AnnalsATS.201507-459MG.

25. Nakamura, K.; Nagata, C.; Fujii, K.; Kawachi, T.; Takatsuka, N.; Oba, S.; Shimizu, H. Cigarette smoking and the adult onset of bronchial asthma in Japanese men and women. *Ann Allergy Asthma Immunol* 2009, 102, 288–293, doi:10.1016/S1081-1206(10)60333-X.

26. Shimoda, T.; Obase, Y.; Kishikawa, R.; Iwanaga, T. Influence of cigarette smoking on airway inflammation and inhaled corticosteroid treatment in patients with asthma. *Allergy Asthma Proc* 2016, 37, 50–58, doi:10.2500/aap.2016.37.3944.

27. Heijink, I.; van Oosterhout, A.; Kliphuis, N.; Jonker, M.; Hoffmann, R.; Telenga, E.; Klooster, K.; Slebos, D. J.; ten Hacken, N.; Postma, D., et al. Oxidant-induced corticosteroid unresponsiveness in human bronchial epithelial cells. *Thorax* 2014, 69, 5–13, doi:10.1136/thoraxjn1-2013-203520.

28. Silverman, R.A.; Boudreaux, E.D.; Woodruff, P.G.; Clark, S.; Camargo, C.A., Jr. Cigarette smoking among asthmatic adults presenting to 64 emergency departments. *Chest* 2003, 123, 1472–1479, doi:10.1378/chest.123.5.1472.

29. Fattahi, F.; Hylkema, M.N.; Melgert, B.N.; Timens, W.; Postma, D.S.; ten Hacken, N.H. Smoking and nonsmoking asthma: differences in
clinical outcome and pathogenesis. *Expert Rev Respir Med* **2011**, *5*, 93-105, doi:10.1586/ers.10.85.

30. Dixon, A.E.; Holguin, F.; Sood, A.; Salome, C.M.; Pratley, R.E.; Beuther, D.A.; Celedon, J.C.; Shore, S.A.; American Thoracic Society Ad Hoc Subcommittee on, O.; Lung, D. An official American Thoracic Society Workshop report: obesity and asthma. *Proc Am Thorac Soc* **2010**, *7*, 325-335, doi:10.1513/pats.200903-013ST.

31. Wang, L.; Murk, W.; DeWan, A.T. Genome-Wide Gene by Environment Interaction Analysis Identifies Common SNPs at 17q21.2 that Are Associated with Increased Body Mass Index Only among Asthmatics. *PLoS One* **2015**, *10*, e0144114, doi:10.1371/journal.pone.0144114.
