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Article type : Letter to the Editor

Title page

***IL1RL1* gene variations are associated with asthma exacerbations in children and adolescents using inhaled corticosteroids**

F.N. Dijk, S.J. Vijverberg, N. Hernandez-Pacheco, K. Repnik, L. Karimi, M. Mitratza, N. Farzan, M.C. Nawijn, E.G. Burchard, M. Engelkes, K. M. Verhamme, U. Potočník, M. Pino-Yanes, D.S. Postma, A. Maitland-van der Zee,* and G.H. Koppelman*

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To the editor,

Asthma, one of the most common chronic diseases in childhood, is caused by interactions between genes and environmental factors. The mainstay of treatment is daily use of inhaled corticosteroids (ICS), which are the most effective medication for controlling asthma symptoms and preventing (severe) exacerbations. ICS use reduces both hospitalizations and mortality rates¹ and improves asthma control; reflected in forced expiratory volume in 1 second (FEV₁) levels and fraction of exhaled nitric oxide (FeNO). These effects are particularly observed in asthma patients with eosinophilic, type 2 airway inflammation.² However, responses to ICS are heterogeneous, which while controversial, possibly reflect genetic associations.⁴

Genome Wide Association studies (GWAS) have reproducibly found the *Interleukin 1 receptor like 1* (*IL1RL1*, *ST2*) gene to be associated with asthma susceptibility.⁵ *IL1RL1* single nucleotide polymorphisms (SNPs) and *IL1RL1* expression levels have been associated with blood eosinophils and markers of Th2 type inflammation.^{6,7} However, the influence of *IL1RL1* SNPs on the effectiveness of asthma treatment has not been investigated. Since the IL-33/*IL1RL1* pathway has been associated with eosinophilic, type 2, inflammation, we hypothesized that *IL1RL1* SNPs may affect corticosteroid treatment response in asthma patients. Since *IL1RL1* functions as a decoy receptor to dampen IL-33 induced signaling, genetically determined low levels of *IL1RL1* may predispose to enhanced IL-33 induced inflammation with consequently more exacerbations.

In the current study, we investigated whether *IL1RL1* gene variants are associated with asthma exacerbations (based on ER visits/hospitalizations and courses of oral corticosteroid (OCS) use), questionnaire-based asthma control and FeNO levels in asthma patients using ICS. Furthermore, we aimed to identify whether there is a pharmacogenetic effect of *IL1RL1* variants on change in FeNO levels and FEV₁%predicted in asthma patients after 4-6 weeks of ICS treatment.

After close inspection of the Linkage Disequilibrium structure of *IL1RL1*, we selected 6 *IL1RL1* SNPs that tag important LD blocks in *IL1RL1* ($r^2 > 0.8$) with SNPs previously found to be associated with asthma;⁵ rs13431828, rs1041973, rs1420101, rs1946131, rs1921622 and rs10204137 (Table S1). Cross-sectional *IL1RL1* SNP discovery analysis was performed in ICS treated asthmatic children, mainly of European ancestry, from the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) cohort (N=820) using logistic and linear regression models. We replicated FDR corrected significant findings ($P < 0.05$) in four different cohorts collaborating within the Pharmacogenomics in Childhood Asthma

(PiCA) consortium⁸, one Hispanic/Latino study; Genes-Environment and Admixture in Latino Americans (GALA II, N=876) study, one African American population; Study of African Americans, Asthma, Genes, and Environments (SAGE, N=525), and two European studies ($\geq 96\%$ European ancestry); the Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe, N=197) and SLOVENIA (N=104). In addition, we performed a meta-analysis (N= 2,412). The longitudinal effect of *IL1RL1* on FeNO levels and FEV₁% predicted upon ICS treatment in asthmatic children and adults was assessed in the SLOVENIA cohort. Conditional analysis was performed in PACMAN to assess the independent effects of the *IL1RL1* SNPs.

A detailed representation of the included cohorts and the allele frequencies of the *IL1RL1* SNPs is provided in Table S2 and Table S3. In PACMAN, we found a significant association between 4 of the 6 SNPs (rs13431828, rs1420101, rs1921622 and rs10204137) with ER visits and 'any exacerbation' (Table 1A-C), which were selected for the replication study. Sensitivity analyses on Dutch ethnicity, atopy and medication adherence did not change these results. We did not observe an association with questionnaire-based asthma control or FeNO measurements (Table S4A-B).

In GALA II, we replicated our findings with significant results with the same direction of effect for rs13431828, rs1420101, rs1921622 on ER visits/hospitalizations and 'any exacerbation'.

Rs10204137 showed a significant association with 'any exacerbation' (Table 1A-C). In SAGE, rs1921622 was associated with 'any exacerbation' but the direction of the effect differed when compared to PACMAN. No association between *IL1RL1* and questionnaire-based asthma control was found. In the smaller SLOVENIA and ESTATe studies no significant cross-sectional or longitudinal associations were found (Table S5).

Meta-analysis of the 4 *IL1RL1* SNPs carried through to replication showed statistically significant results for rs13431828. The C allele of rs13431828 was associated with ER visits/hospitalizations (OR=1.32, $P=0.02$) and increased risk of 'any exacerbations' (1.31, $P=0.02$) (Table 1A-C, Fig. 1). No evidence of heterogeneity was found ($Q=3.6$, $P=0.33$).

Conditional analysis in PACMAN on rs13431828, rs1420101, rs1921622 and rs10204137 for 'any exacerbation' indicated that rs13431828 was the most independently associated SNP (Table S6).

These results provide new evidence that children and adolescents with the *IL1RL1* risk alleles are prone to more exacerbations than children with the protective genotypes, while using ICS. This extends previous findings that SNPs in *IL1RL1* are important in different asthma phenotypes, with more prominent effect in studies investigating childhood onset asthma.⁵ Rs1420101 has been

specifically linked to the type 2-high asthma phenotype ⁶, as well as to increased eosinophil numbers in peripheral blood.⁹

We observed replicable associations of the same *IL1RL1* risk alleles in the Caucasian (PACMAN) and Hispanic/Latino (GALA II) population, but not in the African American study population (SAGE). This could be due to differences in ethnicity between study groups and LD patterns in this gene, suggested by the observed differences in allele frequency between the cohorts (see Table S3). It is possible that our results may have been influenced by factors other than currently included in the model such as inhalation technique or respiratory infections, but as such data was not available in all cohorts these were not considered.

Different mechanisms may explain our findings. Firstly, *IL1RL1* SNPs may modify the asthma phenotype into a more severe phenotype, with more severe exacerbations, which are insufficiently treated with the ICS dosages prescribed to the children in this study. The risk alleles described in our study for rs13431828 (C), rs1420101 (T), rs1921622 (A) and rs10204137 (A) were previously associated with lower *IL1RL1* blood methylation levels and lower serum IL1RL1-a levels⁷, indicating that the associated SNPs are important for regulation of *IL1RL1* expression. Another mechanism to explain our results is that *IL1RL1* may have a direct pharmacogenetic interaction with steroids resulting in reduced efficacy of the steroids. Rs10204137 is a missense mutation and has been associated with increased IL1RL1-a expression, which induces IL-33 expression and enhances IL-33 responsiveness.¹⁰ Moreover, rs10204137 tags an LD block that contains 5 nonsynonymous coding SNPs that result in changes to four amino acids in the intracellular domain of IL1RL1-b. These coding changes affect the Toll/interleukin-1 receptor (TIR) domain of the intracellular part of the *IL1RL1* protein, which plays an important role in IL-33 induced signal transduction by *IL1RL1*. This triggers a signaling cascade that eventually results in the activation of downstream mitogen-activated protein kinases and transcription factors, such as nuclear factor kB (NF-kB) and activator protein-1.⁵ Through this pathway, asthmatic children carrying the risk allele of rs10204137 may be more sensitive to IL-33. As *IL1RL1* is expressed on effector cells of the type-2 immune response such as mast cells, eosinophils, basophils, Th2 cells and ILC2 cells¹¹, an increased sensitivity to IL33 will contribute to an exaggerated type-2 inflammatory response after viral or allergen exposure.

Secondly, *IL1RL1* may have a direct pharmacogenetic interaction with steroids resulting in reduced efficacy of the steroids. A recent study on ulcerative colitis found an association between dexamethasone and upregulation of soluble *IL1RL1* transcription mediated via interaction of the steroid with the glucocorticoid-responsive element in the *IL1RL1* promotor patients carrying polymorphisms.¹² To gain more insight into the mechanism underlying our finding future studies

should be performed in larger cohorts or with the use of biobank data.

This study shows that an *IL1RL1* SNP effect is present in asthmatic children using ICS. This highlights the potential investigating if novel treatment strategies targeting the IL33/IL1RL1 pathway could be used as add-on asthma treatment in patients using ICS.

References

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Available from: www.ginasthma.org.
2. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper Type 2 – driven Inflammation Defines Major Subphenotypes of Asthma. *Am J Respir CritCare Med*. 2009;180:388-95.
3. Kersten ETG, Koppelman GH. Pharmacogenetics of asthma : toward precision medicine. *Curr Opin Pulm Med*. 2017;23:12–20.
4. Mosteller M, Hosking L, Murphy K, Shen J, Song K, Nelson M, et al. No evidence of large genetic effects on steroid response in asthma patients. *J Allergy Clin Immunol* [2017;139:797-803.e7.
5. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. *J Allergy Clin Immunol*. 2013;131:856–65.
6. Gordon ED, Palandra J, Wesolowska-Andersen A, Ringel L, Rios CL, Lachowicz-Scroggins ME, et al. IL1RL1 asthma risk variants regulate airway type 2 inflammation. *JCI Insight*. 2016;1:e87871..
7. Dijk FN, Xu C, Melén E, Carsin A-E, Kumar A, Nolte IM, et al. Genetic regulation of *IL1RL1* methylation and IL1RL1-a protein levels in asthma. *Eur Respir J*. 2018;51:1701377.
8. Farzan N, Vijverberg SJ, Andiappan AK, Arianto L, Berce V, Blanca-López N, et al. Rationale and design of the multiethnic Pharmacogenomics in Childhood Asthmaconsortium. *Pharmacogenomics*. 2017;18:931–43.
9. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadóttir A, Sulem P, Jonsdóttir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet*. 2009;41:342–7.
10. Brusselle GG, Maes T, Bracke KR. Eosinophils in the Spotlight: Eosinophilic airway inflammation in nonallergic asthma. *Nat Med*. 2013;19:977–9.
11. Saluja R, Ketelaar ME, Hawro T, Church MK, Maurer M, Nawijn MC. The role of the IL-33/IL-1RL1 axis in mast cell and basophil activation in allergic disorders. *Mol Immunol*. 2015;63:80–5.
12. Díaz-Jiménez D, Núñez L, De La Fuente M, Dubois-Camacho K, Sepúlveda H, Montecino M, et al. A functional IL1RL1 variant regulates corticosteroid-induced sST2 expression in ulcerative colitis. *Sci Rep*. 2017;7:1–14.

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Author contributions:

FND, SJV, AM and GHK were involved in the conception and design of the study. FND, SJV, NH, KR, LK, MM, ME participated in data collection and performed the analysis. FND, SJV, AM and GHK contributed to the interpretation of the data. FND and GHK drafted the manuscript for important intellectual content. All authors discussed the results and commented on the manuscript.

Conflict of Interest Statement:

N. Hernandez-Pacheco received a grant from Instituto de Salud Carlos III (ISCIII) and was co-funded by the European Social Funds from the European Union (ESF) “ESF invests in your future”. M.C. Nawijn received a grant from GSK during the conduct of this study and outside the submitted work. M.E. Engelkes received a grant from Zonmw. K. M. Verhamme received a grant from ZonMw and she works for a research group who in the past received unconditional grants from: Yamanouchi, Pfizer/Boehringer Ingelheim, Novartis and GSK. M Pino-Yanes received a grant from the Spanish Ministry of Economy, Industry and Competitiveness and a grant from Instituto de Salud Carlos III (ISCIII). A.H. Maitland-van der Zee received an unrestricted research grant from GSK, and Boehringer Ingelheim. She also received a grant from ERANET ERACOSYSMED, and she participated in an advisory board for Astra Zeneca. D.S. Postma declares that the University of Groningen has received money for D.S. Postma regarding a grant for research from Astra Zeneca, Chiesi, Genentec, GSK and Roche. Fees for consultancies were given to the University of Groningen by Astra Zeneca, Chiesi, and GSK. G.H. Koppelman received grants from the Lung Foundation of the Netherlands, the Ubbo Emmius Foundation, during the conduct of the study; and he received grants from Lung Foundation of the Netherlands, GSK, Tetri Foundation, Vertex, TEVA the Netherlands, outside the submitted work. GHK participated in an advisory board meeting of GSK. The rest of the authors declare that they have no relevant conflict of interests.

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Tables

TABLE 1. Results of associations of *IL1RL1* SNPs with ER visits/hospitalizations, OCS use and ‘any exacerbation per study and meta-analysis.

A

ER visits/hospitalisations															
PACMAN (n=698)			GALA II (n=876)			SAGE (n=525)			SLOVENIA (n=187)			ESTATe (n=104)			Meta-an
OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)
2.78 (1.11-6.94)	0.02	0.04	1.45 (1.04-2.03)	0.03	0.04	1.18 (0.88-1.58)	0.26	0.52	1.20 (0.62-2.35)	0.57	0.77	1.01 (0.18-5.72)	0.99	0.99	1.32 (1.08-1.62)
1.35 (0.77-2.37)	0.30	0.30													
1.61 (1.05-2.47)	0.02	0.04	1.28 (1.04-1.58)	0.02	0.04	0.90 (0.69-1.17)	0.45	0.60	1.09 (0.67-1.76)	0.72	0.77	1.35 (0.56-3.25)	0.51	0.94	1.16 (1.01-1.34)
1.47 (0.81-2.68)	0.20	0.24													
1.89 (1.18-3.03)	0.01	0.04	1.30 (1.06-1.59)	0.01	0.04	0.74 (0.55-0.99)	0.05	0.20	0.93 (0.57-1.51)	0.77	0.77				1.13 (0.97-1.31)
1.37 (0.87-2.16)	0.18	0.24	1.24 (0.99-1.56)	0.06	0.06	1.01 (0.76-1.35)	0.92	0.92	0.75 (0.46-1.22)	0.24	0.77	0.81 (0.34-1.94)	0.63	0.94	1.10 (0.95-1.29)

B.

OCS use															
PACMAN (n=720)			GALA II (n=876)			SAGE (n=525)			SLOVENIA (n=187)			ESTATe (n=104)			Meta-an
OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)
2.70 (1.08-6.79)	0.03	0.09	1.25 (0.87-1.79)	0.23	0.65	0.93 (0.68-1.27)	0.65	0.70	1.60 (0.57-4.47)	0.36	0.90	1.15 (0.46-2.85)	0.77	0.77	1.13 (0.91-1.41)
1.52 (0.86-2.66)	0.15	0.27													
1.32 (0.88-1.98)	0.18	0.27	1.10 (0.86-1.40)	0.49	0.65	0.78 (0.57-1.05)	0.09	0.20	0.95 (0.49-1.85)	0.89	0.90	0.68 (0.34-1.36)	0.28	0.77	0.98 (0.84-1.16)
1.08 (0.57-2.02)	0.83	0.83													
1.20 (0.78-1.86)	0.41	0.49	1.10 (0.88-1.38)	0.44	0.65	0.76 (0.54-1.08)	0.10	0.10	0.81 (0.41-1.59)	0.54	0.90				1.00 (0.84-1.18)
1.69 (1.05-2.73)	0.03	0.09	1.03 (0.81-1.32)	0.80	0.80	0.94 (0.69-1.28)	0.70	0.70	1.04 (0.52-2.05)	0.90	0.90	1.15 (0.58-2.27)	0.70	0.70	1.07 (0.90-1.27)

C.

Any exacerbation															
PACMAN (n=720)			GALA II (n=876)			SAGE (n=525)			SLOVENIA (n=187)			ESTATe (n=104)			Meta-an
OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)
2.63 (1.33-5.18)	0.006	0.03	1.63 (1.14-2.32)	0.009	0.01	1.04 (0.78-1.39)	0.80	0.80	1.19 (0.66-2.12)	0.73	0.83	1.09 (0.47-2.56)	0.83	0.86	1.31 (1.07-1.55)
1.28 (0.84-1.96)	0.26	0.26													
1.52 (1.08-2.13)	0.01	0.03	1.35 (1.06-1.72)	0.01	0.01	0.83 (0.63-1.10)	0.18	0.36	1.05 (0.65-1.67)	0.83	0.83	0.85 (0.48-1.53)	0.60	0.60	1.14 (0.98-1.32)
1.37 (0.83-2.25)	0.22	0.26													
1.45 (1.03-2.04)	0.03	0.04	1.35 (1.08-1.70)	0.009	0.01	0.67 (0.50-0.90)	0.009	0.03	0.84 (0.52-1.35)	0.47	0.83				1.08 (0.92-1.2)
1.52 (1.05-2.18)	0.02	0.04	1.29 (1.00-1.66)	0.04	0.04	0.91 (0.68-1.21)	0.50	0.66	0.81 (0.50-1.30)	0.38	0.83	0.95 (0.53-1.70)	0.86	0.86	1.11 (0.96-1.30)

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; ER, Emergency room; OCS, oral corticosteroid

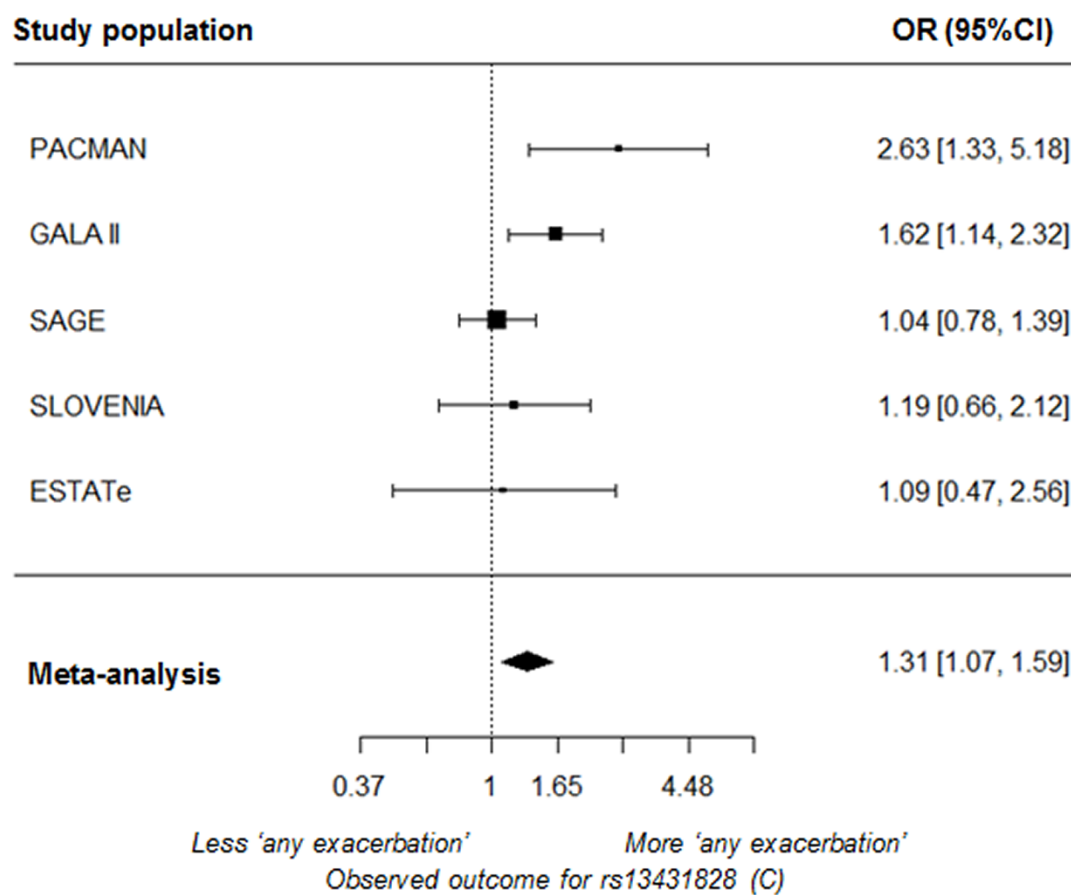
Bold faced results are FDR corrected significant results ($P < 0.05$). Missing values means the SNP was not present in the study.

* R = reference allele, E = effect allele.

† FDR corrected P value

‡ rs13431828 was not present in ESTATE, rs3771180 was used as a surrogate marker ($LD\ r^2 = 1$).

§ rs10204137 was not present in ESTATE, rs4988956 was used as a surrogate marker ($LD\ r^2 = 1$)



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1 **Supporting Information**

2

3 ***IL1RL1* gene variations are associated with asthma exacerbations in children and**
4 **adolescents using inhaled corticosteroids**

5

6 F.N. Dijk, S.J. Vijverberg, N. Hernández Pacheco, K. Repnik, L. Karimi, M. Mitratza, N.
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9

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11

Formattato: Inglese (Stati Uniti)

12 **Supporting methods**

13 **Original cohort**

14 *PACMAN*

15 The PACMAN cohort included children (4-12 years) with a reported regular use of any
16 asthma medication recruited through records of community pharmacies in the Netherlands.
17 Details of the study protocol have been described elsewhere.¹ Written informed consent was
18 obtained from all participants. Data on asthma symptoms, recent exacerbations and current
19 medication use over the preceding 12 months was obtained using a questionnaire during a
20 study visit in the community pharmacy . FeNO was measured in children with reported use of
21 asthma medication in the past 6 months. DNA was extracted from collected saliva samples
22 (Oragene DNA Self Collection kit, DNA Genotek, Inc., ON, Canada). The PACMAN study
23 was approved by the Medical Ethics Committee of the University Medical Centre Utrecht.
24

25 **Replication cohorts**

26 *GALA II*

27 The Genes-environments & Admixture in Latino Americans (GALA II) study is a case-control
28 study of asthma in Latinos recruited in five centers throughout the United States (Chicago,
29 Illinois; Bronx, New York; Houston, Texas; San Francisco Bay Area, California; and San
30 Juan, Puerto Rico). Participants were included if they were between 8 to 21 years old and
31 self-identified all four grandparents as Latino. Subjects were excluded if they reported any of
32 the following: (1) history of sickle cell disease, cystic fibrosis, sarcoidosis, cerebral palsy, or
33 history of heart or chest surgery; (2) 10 or more pack-years of smoking; (3) any smoking
34 within one year of recruitment date; or (4) pregnancy in the third trimester. Only children with
35 a physician-diagnosis of asthma that reported active symptoms and use of asthma
36 medication during the 12 months preceding the study enrollment were analyzed in the
37 current study. All local institutional review boards approved the study and all subjects/parents
38 provided written assent/consent, respectively.²⁻⁴
39

40 *SAGE*

41 The Study of African Americans, Asthma, Genes & Environments (SAGE) is an ongoing
42 study of asthma in children and young adults coordinated from the University of California
43 San Francisco. Recruitment protocols were similar to GALA II with the only differences being
44 that the recruitment was restricted to San Francisco Bay Area and participants self-identified
45 all four grandparents as African American.⁵
46

47 *SLOVENIA*

In the SLOVENIA cohort⁶, 224 children aged 5–18 years were selected. Asthma was diagnosed according to American Thoracic Society (ATS) criteria. Children were included with mild or moderate persistent asthma newly detected in this period. Data on asthma symptoms, exacerbations and clinical parameters were obtained during the physician visit over the preceding 12 months. The study was carried out in accordance with the Helsinki declaration of the World Medical Association and approved by the Slovenian National Medical Ethics Committee (KME 31/12/06). Parents signed informed consent for children under 15 years, whereas older children gave informed consent themselves.

ESTATe

The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) is a case-control study that includes children and young adults (4-19 years) with a physician diagnosis of asthma recruited from primary care practices in the Netherlands. Patients were selected from either IPCI (Interdisciplinary Processing of Clinical Information) database or the PHARMO Database Network. Both databases contain the electronic medical records of more than one million patients throughout the Netherlands with detailed information on patient diagnosis, patient prescription (IPCI) or patient dispensing (PHARMO). During the study period (2000 -2012) all children with asthma, aged 5 years and older and treated with asthma controller therapy were selected. Within this cohort, we selected cases with a asthma exacerbation (use of systemic corticosteroids, ER visit or hospitalization because of asthma) and matched each case to 4 controls on age, sex, general practice (GP) and type of asthma controller therapy. Next, all potential cases and controls were invited to participate via their respective GP. If patients agreed to participate, they provided written consent, completed a research questionnaire, and provided a saliva sample (for DNA extraction).

Treatment step

Categorization of asthma severity was modified from the British Thoracic Society (BTS) guidelines⁷ as follows in all studies: step 1: inhaled short-acting b2-agonists (SABA) as needed, step 2: step 1 plus use of inhaled SABA as needed plus regular ICS, step 3: step 2 plus regular long-acting inhaled b2-agonists (LABA) or plus leukotriene receptor antagonists (LTRA), and step 4: step 2 plus regular LABA and LTRA. For this study we included patients on treatment step 2 or higher.

Analyzed variables

In PACMAN, GALA II, SAGE, ESTATe and SLOVENIA data related to asthma exacerbations and questionnaire-based asthma control was available. FeNO levels were measured in PACMAN and SLOVENIA. Longitudinal data on FeNO and FEV₁ was only available in

85 SLOVENIA, with data being collected before start of ICS and 4-6 weeks after the start of
86 treatment.

87

88 **Definition of outcomes**

89 *Asthma exacerbations*

90 In PACMAN, asthma exacerbations were based on (1) emergency room (ER) visits and (2)
91 courses of OCS use in the 12 months preceding the study visit. In GALA II, SAGE, ESTATe
92 and SLOVENIA exacerbations were based on (1) ER visits and/or hospitalizations and (2) on
93 courses of OCS use in the past 12 months preceding the study visit. These separate
94 exacerbation conditions were combined additionally in one variable: 'any exacerbation'. In
95 PACMAN 'any exacerbation' was defined as ER visit and/or OCS use in the past 12 months.
96 In the studies included in the replication phase 'any exacerbation' was defined as ER visits
97 and/or hospitalizations and/or courses of OCS use in the past 12 months.

98

99 *Asthma control*

100 In PACMAN asthma control was evaluated using the Asthma Control Questionnaire score
101 (ACQ-6)⁸ in the past week; ACQ<0.75 = well controlled asthma, ACQ 0.75-1.5 = partly
102 controlled asthma and ACQ>1.5 = poor controlled asthma. In GALA II and SAGE asthma
103 control was based on a modified version of the 1978 American Thoracic Society-Division of
104 Lung Diseases (ATS-DLD) Epidemiology Questionnaire on symptoms, nighttime awakening,
105 interferences with normal activities, rescue medication use, and lung function measurements,
106 and defined using a modification of the NHLBI guidelines to fit our questionnaire as
107 controlled, partially controlled, or uncontrolled.⁹ In Slovenia asthma control was measured
108 using the Asthma Control Test (ACT) that contains 5 questions related to the frequency of
109 both asthma symptoms and required rescue medication use during the previous 4 weeks
110 after 6-month ICS treatment. Uncontrolled asthma was defined as ACT <15 points.¹⁰

111

112 *FeNO*

113 In PACMAN, a single-breath on-line measurement of FeNO (ppb) was carried out with a
114 hand-held electrochemical analyzer (NIOX Mino; Aerocrine, Solna, Sweden). In Slovenia
115 FeNO levels were measured by a Niox analyzer (Aerocrine Inc., USA), with usage as
116 specified in ERS (European Respiratory Society) and ATS guidelines.

117

118 *FEV₁*

119 FEV₁ values were adequately measured only in SLOVENIA using a Vitalograph 2150
120 spirometer (Compact, Buckingham, UK) according to ERS/ATS guidelines.

121

122 **SNP selection and genotyping**

123 LD pattern between the 6 selected *IL1RL1* SNPs (rs13431828, rs1041973, rs1420101,
124 rs1946131, rs1921622 and rs10204137) was assessed based on
125 data from 1000 Genomes Project Phase III¹¹ by means of LDLink.¹² Functional SNP
126 annotation was determined with HaploReg v4.1.¹³

128 **Genotyping**

129 *PACMAN*

130 In PACMAN, genotyping was performed at LGC Genomics (UK). Quality control (QC) was
131 performed by visual inspection of the genotypes, exclusion of individuals with missing
132 genotype call rate >0.50. SNPs were excluded with a missing genotype rate >0.01, a Hardy-
133 Weinberg equilibrium P-value <0.001 and a MAF <0.01.
134 Significant SNPs (p < 0.05) in PACMAN were selected for replication analysis. All genetic
135 data were aligned to assembly GRCh37/hg19.

137 *GALA II and SAGE*

138 Both GALA II and SAGE samples were genotyped with the Axiom® LAT1 array (Affymetrix
139 Inc.), and quality control of the data was carried out as described elsewhere.^{4,5} Imputation
140 was performed with Minimac¹⁴ by means of the Michigan Imputation Server¹⁵, using as
141 reference panel the second release of the Haplotype Reference Consortium (HRC) (r1.1
142 2016).¹⁶ For that, a prior step of haplotype reconstruction from genotype data was performed
143 with SHAPEIT.¹⁷

145 *SLOVENIA*

146 DNA samples were obtained from peripheral blood. First, peripheral blood lymphocytes
147 (PBMCs) were isolated using FicollPaque PLUS (GE Healthcare, Uppsala, Sweden) and
148 then DNA was isolated using TRI reagent (Sigma, Steinheim, Germany) according to
149 manufacturer instructions. SNPs rs13431828, rs1921622, rs1420101 and rs10204137 were
150 genotyped using high resolution melting analysis (HRMA) on LightCycler 480 instrument
151 (Roche, Germany) using LC480 HRM Master Mix (Roche, Germany). The following primers
152 were used for amplification:

153	rs13431828 (1)	AAGAGTATCACCAACTGCCTCA
154	rs13431828 (2)	GCAAGTATTGGTAACTCGTTGTTG
155	rs1921622 (1)	TGCCACTTCTTAATTCTGTCCA
156	rs1921622 (2)	GTACTTTTAAGGTATTTTCAGCTAGTGC
157	rs1420101 (1)	CTCGACAACATTTATGTACACCAT
158	rs1420101 (2)	TTGGTGTCTCAGAGTTTCTGCAA

159 rs10204137 (1) CTCTGAGCGAGCTGGACAT
160 rs10204137 (2) ATGTGGTCCTCCCTCCACTT

161
162 Amplification was performed using the following conditions: initial denaturation at 95°C for
163 10min, followed by 45 cycles of 95°C for 10s, 60°C for 15s and 72°C for 10s, followed by
164 HRM step of 95°C for 1min, 40°C for 1min and 60-90°C at 0,02°C/s. Genotypes were
165 determined using gene scanning analysis software.

166 167 *ESTATe*

168 Genotyping of *ESTATe* was performed with the Illumina Infinium CoreExome-24 BeadChip
169 (Illumina). SNPs and samples with a call rate <99%, SNPs with Hardy-Weinberg P-value
170 <10⁻⁴, and samples with excess autosomal heterozygosity were excluded. The genetic data
171 were aligned to assembly GRCh37/hg19.

172 173 **Statistical analyses**

174 Logistic regression models were used to assess the association between *IL1RL1* SNPs,
175 exacerbations and asthma control applying an additive genetic model. In PACMAN,
176 SLOVENIA and *ESTATe*, regression models were adjusted by age, gender and BTS
177 treatment step as described previously,¹⁸ while the first two principal components, obtained
178 with EIGENSOFT,¹⁹ were also included as covariates for GALA II and SAGE in order to
179 correct the ethnic admixture of these populations. Associations between genetic variants and
180 FeNO levels were evaluated by means of linear regressions using a logistic transformation of
181 the outcome variable. Age and gender were included as covariates in the regression models.

182 A-FDR corrected P-values were calculated by the method proposed by Benjamini and
183 Hochberg.²⁰ An FDR corrected P-value <0.05 was considered significant. Longitudinal
184 analyses were performed with linear regression models. The change in FeNO and FEV₁%
185 predicted values were calculated by subtracting the post ICS treatment value from the pre
186 ICS treatment value.

187 Sensitivity analyses in PACMAN were performed for Dutch ethnicity, atopy and medication
188 adherence. Atopy was based on the presence of eczema, food allergy or allergic rhinitis,
189 whereas medication adherence was assessed by using the Medication Adherence Report
190 Scale (MARS) comprising five questions on medication use behavior. Patients with a MARS
191 score ≥21 were considered to be highly adherent.²¹

192 We performed meta-analyses in METAL²² using a fixed effect model since there was no
193 evidence of heterogeneity.²³ Forest plots were made with the 'forestplot' package in R.²⁴ To
194 assess the independent effect of the *IL1RL* SNPs in the PACMAN cohort we performed

195 conditional analysis using a multivariate model with a backward step wise regression
196 analysis.
197 Statistical analyses were carried out using IBM SPSS 23.0 for Windows (SPSS, Inc.,
198 Chicago, IL, USA). In GALAI and SAGE, logistic regressions were performed by means of
199 the binary Wald test implemented in EPACTS 3.2.6.²⁵

200 **Supporting references**

- 201 1. Koster ES, Raaijmakers JAM, Koppelman GH, Postma DS, van der Ent CK,
 202 Koenderman L, et al. Pharmacogenetics of anti-inflammatory treatment in children with
 203 asthma: rationale and design of the PACMAN cohort. *Pharmacogenomics*.
 204 2009;10:1351–61.
- 205 2. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-Life
 206 air pollution and asthma risk in minority children the GALA II and SAGE II studies. *Am*
 207 *J Respir Crit Care Med*. 2013;188:309–18.
- 208 3. Thakur N, Oh SS, Nguyen EA, Martin M, Roth LA, Galanter J, et al. Socioeconomic
 209 status and childhood asthma in urban minority youths: The GALA II and SAGE II
 210 studies. *Am J Respir Crit Care Med*. 2013;188:1202–9.
- 211 4. Pino-Yanes M, Thakur N, Gignoux CR, Galanter JM, Roth LA, Eng C, et al. Genetic
 212 ancestry influences asthma susceptibility and lung function among Latinos. *J Allergy*
 213 *Clin Immunol*. 2015;135:228–35.
- 214 5. White MJ, Risse-Adams O, Goddard P, Contreras MG, Adams J, Hu D, et al. Novel
 215 genetic risk factors for asthma in African American children: Precision Medicine and
 216 the SAGE II Study. *Immunogenetics*. 2016;68:391–400.
- 217 6. Berce V, Kozmus CEP, Potočník U. Association among ORMDL3 gene expression,
 218 17q21 polymorphism and response to treatment with inhaled corticosteroids in
 219 children with asthma. *Pharmacogenomics J*. 2013;13:523–9.
- 220 7. Levy ML, Thomas M, Small I, Pearce L, Pinnock H, Stephenson P. Summary of the
 221 2008 BTS/SIGN British Guideline on the management of asthma. *Prim Care Respir J*.
 222 2009;18:Suppl 1:S1–16
- 223 8. Juniper EF, Bousquet J, Abetz L, Bateman ED; GOAL Committee. Identifying
 224 'wellcontrolled' and 'not well-controlled' asthma using the Asthma Control
 225 Questionnaire. *Respir Med*. 2006;100:616–21.
- 226 9. Borrell LN, Nguyen EA, Roth LA, Oh SS, Tcheurekdjian H, Sen S, et al. Childhood
 227 Obesity and Asthma Control in the GALA II and SAGE II Studies. *Am J Respir Crit*
 228 *Care Med*. 2013;187:697–702.
- 229 10. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma
 230 Control Test: Reliability, validity, and responsiveness in patients not previously
 231 followed by asthma specialists. *J Allergy Clin Immunol*. 2006;117:549–56.
- 232 11. 1000 Genomes Project Consortium, Auton A, Abecasis GR, Altshuler DM, Durbin RM,
 233 Abecasis GR, Bentley DR, et al. A global reference for human genetic variation.
 234 *Nature*. 2015;526:68–74.
- 235 12. Machiela MJ, Chanock SJ. LDassoc: an online tool for interactively exploring genome
 236 wide association study results and prioritizing variants for functional investigation.

- 237 Bioinformatics. 2018;34:887–9.
- 238 13. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation,
239 and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids*
240 *Res.* 2012;40:D930–4.
- 241 14. Fuchsberger C, Abecasis GR, Hinds DA. Minimac2: Faster genotype imputation.
242 *Bioinformatics.* 2015;31:782–4.
- 243 15. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation
244 genotype imputation service and methods. *Nat Genet.* 2016;48:1284–7.
- 245 16. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A
246 reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet.*
247 2016;48:1279–83.
- 248 17. Delaneau O, Coulonges C, Zagury JF. Shape-IT: New rapid and accurate algorithm
249 for haplotype inference. *BMC Bioinformatics.* 2008;9:1–14.
- 250 18. Koster ES, Maitland-Van Der Zee AH, Tavendale R, Mukhopadhyay S, Vijverberg
251 SJH, Raaijmakers JAM, et al. FCER2 T2206C variant associated with chronic
252 symptoms and exacerbations in steroid-treated asthmatic children. *Allergy.*
253 2011;66:1546–52.
- 254 19. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal
255 components analysis corrects for stratification in genome-wide association studies.
256 *Nat Genet.* 2006;38:904–9.
- 257 20. [Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful](#)
258 [approach to multiple testing. *J Royal Stat Soc Ser B.* 1995;57:289–300.](#)
- 259 21. Menckeberg TT. Adherence to Inhaled Corticosteroids and Patient Perception:
260 towards a better understanding and individualised care. Utrecht University, 2008.
261 (Thesis).
- 262 22. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide
263 association scans. *Bioinformatics.* 2010;26:2190–1.
- 264 23. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-
265 analyses. *BMJ Br Med J.* 2003;327:557–60.
- 266 24. (2015) RCT. R: A language and environment for statistical computing. R Foundation
267 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/> [Internet].
268 Vienna, Austria; Available from: <https://www.r-project.org>.
- 269 25. HM K. EPACTS (Efficient and Parallelizable Association Container Toolbox).
270 <http://genome.sph.umich.edu/wiki/EPACTS> (2016).

271 **Supporting Tables**

272

273 **TABLE S1. LD pattern of the six selected *IL1RL1* SNPS**

SNP	BP	Annotation	Position	LD values					
				rs13431828	rs1041973	rs1420101	rs1946131	rs1921622	rs10204137
rs13431828	102954653	5'-UTR	5'-UTR	1					
rs1041973	102955468	missense	Exon 3	0.61	1				
rs1420101	102957716	intronic	Intron 5	0.06	0.002	1			
rs1946131	102961929	intronic	Intron 8	0.02	0.06	0.03	1		
rs1921622	102966067	intronic	Intron 10	0.17	0.06	0.38	0.12	1	
rs10204137	102968212	missense	Exon 11	0.22	0.09	0.29	0.09	0.05	1

274

275 LD, linkage disequilibrium; BP, base pair; SNP, single nucleotide polymorphism

276 LD values (r^2) between the *IL1RL1* SNPS based on data originating from Phase 3 (Version 5) of the 1000 Genomes Project.¹¹

277 **TABLE S2. Clinical characteristics of the study population.**

Child Characteristics	PACMAN (n=720)	GALA II (n=876)	SAGE (n=525)	SLOVENIA (n=197)	ESTATe (n=104)
Age (years), mean (SD)	8.7 (2.3)	12.1 (3.18)	13.5 (3.41)	10.8 (3.31)	10.4 (4.3)
Male gender , % (n)	62.4 (449/720)	56.7 (497/876)	53.9 (283/525)	56.9 (112)	58.7 (67/104)
Asthma, % (n)	73.5 (525/714)	100.0 (876/876)	100.0 (525/525)	100.0 (197/197)	100.0 (104/104)
Atopy , % (n)					NA
Eczema	65.3 (466/714)	18.0 (158/876)	39.8 (209/525)	NA	NA
Food Allergy	50.8 (362/712)	NA	NA	NA	NA
Allergic Rhinitis	44.7 (315/705)	42.6 (373/876)	33.9 (178/525)	NA	
Family Ethnicity , % (n)					
European ancestry	91.3 (651/713)			100(197/197)	96.0 (98/102)
Mixed	6.7 (48/713)				1.0 (1/102)
Asian	0.6 (4/713)				1.0 (1/102)
African	1.1 (8/713)				2.0 (2/102)
Hispanic/Latino	0.3 (2/713)	100.0 (876/876)			
African American			100.0 (525/525)		
Asthma Exacerbation , % (n)					
ER visit/hospitalization*	6.3 (44/698)	55.1 (483/876)	40.8 (214/525)	27.4 (54/187)	12.5 (13/104)
OCS use	6.5 (47/720)	39.2 (343/876)	29.1 (153/525)	11.2 (22/187)	34.6 (36/104)
Any exacerbation	10.7 (77/720)	64.7 (567/876)	49.7 (261/525)	29.9 (59/187)	47.1 (49/104)
Uncontrolled Asthma, % (n)	19.4 (137/705)	41.5 (364/876)	45.1 (237/525)	1 (2/42)	NA
Adherence to Medication, % (n)	58.4 (398/682)	NA	NA	NA	NA
Treatment group, % (n)					
ICS + SABA	69.4 (500/720)	42.3 (371/876)	68.2 (258/525)	92.9 (183/197)	62.5 (65/104)
ICS + SABA + LABA or ICS + SABA + LTRA	24.3 (175/720)	42.7 (374/876)	25.3 (133/525)	7.1 (14/197)	35.6 (37/104)
ICS + SABA + LABA + LTRA	6.3 (45/720)	15.0 (131/876)	6.5 (34/525)		1.9 (2/104)
FeNo (ppb), mean (SD) (n)	23.02 (29.5) (596)	NA	NA	35.25 (25.3) (61)	NA
FEV ₁ % predicted, mean (SD) (n)	NA	NA	NA	94.4 (15.31) (183)	NA

278 SD, standard deviation, ER, emergency room; OCS, oral corticosteroid; ICS, inhalation corticosteroid; SABA, short-acting beta agonists; LABA, long-acting
279 beta agonists; LTRA, leukotriene receptor antagonists.
280

281 **TABLE S3. Allele frequency of the investigated SNPs per study.**

282

SNP	Allele (R/E)*	Allele Frequency (E)				
		PACMAN (n=720)	GALA II (n=876)	SAGE (n=525)	SLOVENIA (n=187)	ESTATe (n=104)
rs13431828†	T/C	0.88	0.87	0.75	0.83	0.87
rs1041973	A/C	0.78				
rs1420101	G/A	0.42	0.29	0.34	0.35	0.40
rs1946131	G/A	0.11				
rs1921622	G/A	0.56	0.36	0.27	0.42	
rs10204137‡	G/A	0.65	0.66	0.32	0.66	0.82

283 Allele frequency is displayed for the effect allele.

284 * R = reference allele, E = effect allele.

285 † rs13431828 was not present in ESTATe, rs3771180 was used as a surrogate marker (LD $r^2 = 1$)

286 ‡ rs10204137 was not present in ESTATe, rs4988956 was used as a surrogate marker (LD $r^2 = 1$)

287 **TABLE S4. Results of associations of *IL1RL1* SNPs with asthma control and FeNO levels per study and meta-analysis.**

288

289 **A.**

SNP	Allele (R/E)*	Asthma control														
		PACMAN (n=705)			GALA II (n=876)			SAGE (n=525)			SLOVENIA (n=54)			Meta-analysis (n=2148)		
		OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†	OR (95% CI)	P	P†
rs13431828‡	T/C	0.88 (0.60-1.29)	0.50	0.66	0.95 (0.69-1.31)	0.76	0.92	1.10 (0.83-1.46)	0.49	0.49	0.78 (0.03-16.58)	0.87	0.87	1.00 (0.83-1.20)	0.95	0.96
rs1041973	A/C	0.78 (0.58-1.04)	0.12	0.45												
rs1420101	G/A	0.93 (0.71-1.21)	0.55	0.66	0.94 (0.76-1.17)	0.57	0.92	1.15 (0.88-1.49)	0.33	0.44	2.47 (0.27-22.72)	0.42	0.87	0.99 (0.86-1.14)	0.96	0.96
rs1946131	G/A	1.23 (0.83-1.84)	0.32	0.64												
rs1921622	G/A	0.96 (0.74-1.26)	0.76	0.76	0.93 (0.76-1.13)	0.39	0.92	1.28 (0.97-1.69)	0.08	0.16	1.19 (0.13-10.83)	0.87	0.87	1.01 (0.88-1.16)	0.82	0.96
rs10204137§	G/A	0.83 (0.63-1.09)	0.15	0.45	1.01 (0.81-1.26)	0.92	0.92	1.34 (1.02-1.76)	0.03	0.12	0.52 (0.01-18.25)	0.72	0.87	1.03 (0.89-1.19)	0.65	0.96

290

291 **B.**

SNP	Allele (R/E)*	FeNO								
		PACMAN (n=596)			SLOVENIA (n=61)			Meta-analysis (n=657)		
		Beta (95% CI)	P	P†	Beta (95% CI)	P	P†	Beta (95% CI)	P	P†
rs13431828‡	T/C	0.08 (-0.08-0.24)	0.33	0.98	0.15 (-0.24-0.54)	0.44	0.57	*-0,09 (-0,24-0,06)	0.23	0.72
rs1041973	A/C	-0.02 (-0.15-0.11)	0.72	0.98						
rs1420101	G/A	0.02 (-0.08-0.12)	0.64	0.98	0.08 (-0.21-0.37)	0.57	0.57	0,03 (-0,06-0,12)	0.58	0.72
rs1946131	G/A	-0.001 (-0.17-0.17)	0.99	0.98						
rs1921622	G/A	-0.01 (-0.12-0.10)	0.82	0.98	0.23 (-0.05-0.53)	0.10	0.57	0,02 (-0,08-0,12)	0.72	0.72
rs10204137§	G/A	-0.04 (-0.15-0.07)	0.39	0.98	0.08 (-0.21-0.38)	0.55	0.57	*-0,03 (-0,13-0,07)	0.62	0.72

292

293 SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval

294 Missing values means the SNP was not present in the study.

295 * R = referece allele, E = effect allele.

296 †FDR corrected P value
297 ‡ rs13431828 was not present in ESTATE, rs3771180 was used as a surrogate marker (LD $r^2 = 1$).
298 § rs10204137 was not present in ESTATE, rs4988956 was used as a surrogate marker (LD $r^2 = 1$).

299 **TABLE S5. Pharmacogenetic effect of the *IL1RL1* SNPs on changes in FeNO and FEV₁% predicted values.**
300

SNP	Allele (R/E)*	ΔFeNO				ΔFEV1%			
		SLOVENIA (n=40)				SLOVENIA (n=183)			
		Beta	95% CI	P	P†	Beta	95% CI	P	P†
rs13431828	T/C	-10.10	-28.43-8.21	0.27	0.36	0.24	-3.35-3.83	0.89	0.95
rs1041973	A/C								
rs1420101	G/A	-12.10	-26.54-2.33	0.09	0.24	-0.08	-2.85-2.69	0.95	0.95
rs1946131	G/A								
rs1921622	G/A	2.75	-12.62-18.13	0.71	0.71	-1.46	-4.17-1.25	0.29	0.95
rs10204137	G/A	10.35	-3.04-23.76	0.12	0.24	0.16	-2.55-2.88	0.90	0.95

301
302 SNP, single nucleotide polymorphism; CI, confidence interval; AF, allele frequency
303 Missing values means the SNP was not present in the study.
304 * R = reference allele, E = effect allele
305 † FDR corrected *P* value.
306

307 **TABLE S6. Conditional analysis in PACMAN on rs13431828, rs142010, rs1921622 and rs10204137 for ‘any exacerbation’.**
308

Any exacerbation					
PACMAN (n=720)					
SNP	Allele (R/E)*	OR	95% CI	P	P†
rs13431828	T/C	2.46	1.06-5.73	0.03	0.12
rs1420101	G/A	1.25	0.69-2.24	0.45	0.90
rs1921622	G/A	0.98	0.56-1.70	0.95	0.95
rs10204137	G/A	1.03	0.63-1.70	0.88	0.95

309
310 SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval
311 * R = reference allele, E = effect allele
312 † FDR corrected *P* value.