Rationale and design of the multi-ethnic Pharmacogenomics in Childhood Asthma (PiCA) consortium

Farzan N [1,2], Vijverberg SJ[1,2], Andiappan AK[3], Arianto L[4], Blanca-López N[5], Bisgaard H[4], Bønnelykke K[4], Burchard EG[6], Campo P[7], Canino G[8], Carleton B[9,10], Celedón JC[11], Chew FT[12], Chiang WC[12], Cloutier MM[13], Daley D[14], Den Dekker HT [15,16], Dijk FN[17,18], Duijts L[15,19], Flores C[20,21], Forno E[11], Hawcutt DB[22,23], Hernandez-Pacheco N[21], de Jongste JC[15], Kabesch M[24], Koppelman GH[17,18], Manolopoulos VG[25], Melén E[26,27], Mukhopadhyay S[28,29], Nilsson S[26,27], Palmer CN[29], Pino-Yanes M[20,21], Pirmohamed M[30], Potočnik U[31,32], Raaijmakers JA[1], Repnik K[31,32], Schieck M[24,33], Sio YY[12], Smyth RL[34], Szalai C[35,36], Tantisira KG[37,38], Turner S[39], van der Schee MP[40], Verhamme KM[41], Maitland-van der Zee AH[1,2]

[1]Division of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht University, Utrecht, Netherlands, [2]Department of Respiratory Medicine, Academic Medical Center (AMC). University of Amsterdam, Amsterdam, the Netherlands, [3]Singapore Immunology Network, Agency for Science, Technology and Research, Singapore 138648, Singapore, [4]Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark, [5]Allergy Service, Infanta Leonor Hospital, Madrid, Spain, [6]Departments of Medicine, Bioengineering and Therapeutic Sciences University of California, San Francisco, USA, [7]Allergy Unit, IBIMA, Regional University Hospital of Malaga, Malaga, Spain, [8]Behavioral Sciences institute, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico, [9]Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, Canada, [10]Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, Canada, [11]Division of Pulmonary Medicine, Allergy, and Immunology, Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center, University of Pittsburgh, Pittsburgh, Pennsylvania, [12]Department of Biological Sciences, National University of Singapore, Singapore, and the Allergy and Immunology Division, Department of Paediatric Medicine, KK Children's Hospital, Singapore, [13]University of Connecticut Health Center, Asthma Center, Connecticut Children's Medical Center, Connecticut, United States of America, [14]Respiratory Division, Department of Medicine, University of British Columbia, Vancouver, Canada, [15]Department of Pediatrics, Division of Respiratory Medicine and Allergology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands, [16] Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands, [17]University of Groningen, University Medical Center Groningen , Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, Groningen, Netherlands, [18]Groningen Research Institute for Asthma and COPD, University of Groningen, University Medical Center Groningen, Groningen, [19]Department of Pediatrics, Division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands, [20]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, [21]Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Santa Cruz de Tenerife, Spain, [22]Alder Hey Children's Hospital, Liverpool, UK, [23]Department of Women's and Children's Health, University of Liverpool, Liverpool, UK, [24] Department of Pediatric Pneumology and Allergy, University Children's Hospital Regensburg (KUNO), Regensburg, Germany, [25]Laboratory of Pharmacology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece, [26]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [27]Centre of Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden, [28]Academic Department of Paediatrics, Brighton and

Sussex Medical School, Royal Alexandra Children's Hospital, Brighton, United Kingdom, [29]Population Pharmacogenetics Group, Biomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom, [30]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom, [31]Centre for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, University of Maribor, Maribor, Slovenia, [32]Faculty for Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia, [33]Department of Human Genetics, Hannover Medical School, Hannover, Germany, [34] Great Ormond Street Institute of Child Health, University College London, London, United Kingdom, [35]Department of Genetics, Cell and Immuno-biology, Semmelweis University, Budapest, Hungary, [36]Central Laboratory, Heim Pal Children Hospital, Budapest, Hungary, [37]the Channing Division of Network Medicine, Dept. of Medicine, Brigham and Women's hospital and Harvard Medical School, Boston, United States of America, [38]Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States of America, [39]Child Health, University of Aberdeen, Aberdeen, United Kingdom, [40]Department of Respiratory Medicine, Academic Medical Centre, University of Medical Centre Amsterdam, Amsterdam, the Netherlands, [41]Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, the Netherlands.

Abstract

Aim: International collaboration is needed to enable large-scale pharmacogenomics studies in childhood asthma. Here, we describe the design of the Pharmacogenomics in Childhood Asthma [PiCA] consortium.

Material & Methods: Investigators of each study participating in PiCA provided data on the study characteristics by answering an online questionnaire.

Results: Twenty-one studies, including 14,227 children/young persons (58% male), from 12 different countries are currently enrolled in the PiCA consortium. Fifty-six percent of the patients are Caucasians. In total 7,619 were inhaled corticosteroid [ICS] users. Among patients from 13 studies with available data on asthma exacerbations, one third reported exacerbations despite ICS use. In the future pharmacogenomics studies within the consortium, the pharmacogenomics analyses will be performed separately in each center and the results will be meta-analyzed.

Conclusions: PiCA is a valuable platform to perform pharmacogenetics studies within a multi-ethnic pediatric asthma population.

***Keywords**: asthma, children, consortium, genetics, pharmacogenomics, treatment*

Introduction

Asthma is the most common chronic disease in childhood. Although it cannot be cured, effective treatments are available to decrease the symptoms, maintain lung function and prevent future exacerbations (1). Standard treatment regimens for persistent asthma include regular use of inhaled corticosteroids [ICS] combined with long-acting β2 agonists [LABA] and short-acting β2 agonists [SABA] as needed (2). There is heterogeneity in response to treatment; approximately 30-40% of the patients receiving ICS, do not show an improvement lung function and remain uncontrolled(3–6). Uncontrolled asthma is associated with low quality of life for patients and can be life threatening (7,8). Furthermore, unscheduled physician visits and hospital admissions due to exacerbations are responsible for almost half of the costs of asthma management (9,10).

Poor adherence to medication, continuous environmental exposures, disease severity and misdiagnosis influence response to treatment in asthmatic patients. In addition, it has been shown that genetic variation contributes to the heterogeneity in treatment response (11). To date, a large number of candidate gene studies and several genome-wide association studies [GWAS] have been conducted to study the pharmacogenomics of childhood asthma (12,13). However, one of the main unmet needs for pediatric asthma management is the lack of clinically available biomarkers (for example pharmacogenetic markers) to guide asthma treatment. Genetic associations have been reported with three commonly used outcome measures (i.e. asthma exacerbations, asthma symptoms and lung function) (14,15). Different outcomes might reflect different aspects of asthma control and the heterogeneity in the outcome measures complicates the comparison of study results. In addition, most studies have been performed in relatively small study populations. There is a need for international collaboration in the field of pharmacogenomics of asthma to obtain large sample sizes of well-phenotyped asthmatic children to perform large scale meta-analysis to assess the clinical value of genetic markers for asthma management and identify markers that can guide asthma treatment. (16,17). There have been successful efforts to establish consensus on diagnosis and management of asthma (18,19). The Pharmacogenomics in Childhood Asthma [PiCA] consortium was initiated in December 2013 and brings together asthma studies that have genetic data and treatment outcome measures. The main goals of the PiCA consortium are to create a platform to identify new pharmacogenomic markers in asthma by conducting GWAS meta-analyses. To replicate these new and also previously identified loci that are associated with treatment response, and finally, to develop pharmacogenetics-guided (PG) algorithms to guide asthma therapy to improve symptoms and reduce/prevent future exacerbations. This is the first consortium that focuses on

pharmacogenomics in childhood asthma. In this study, we describe the characteristics of the study populations currently included in the PiCA consortium, assess the outcome measures that can be used to study treatment response within the consortium and describe the design of the pharmacogenomics studies that will be performed within PiCA

Methods

PiCA consortium

The PiCA consortium was established in December 2013 by the pharmacogenomics research group of Prof. dr. AH Maitland van der Zee (Utrecht University, The Netherlands) by expanding existing and new collaborations. Studies were identified from the literature, at conferences and by references of other PiCA collaborators. Studies were eligible to participate in the PiCA consortium if:

- Data of asthmatic children or young persons were collected;
- DNA samples were collected or could be collected;
- Data were collected on asthma drug use;
- Data were collected on treatment outcome.

PiCA is a growing consortium and new studies can join the consortium if they meet the inclusion criteria (www.pica-consoortium.org).

Data collection:

An online questionnaire (created using www.surveymonkey.com) was sent to the investigators of each cohort to collect information about the patients and design of the studies.

Characteristics of the studies and study populations

Information was collected on the following characteristics of the studies: study design (i.e. asthma cohort, clinical trial and (high risk) birth cohort), country where the study was conducted and location of patient enrollment (type of health care centers: primary, secondary or tertiary care). Per study, the following data were collected on the study populations: the age range (in yrs.), number of male asthmatics, and the number of patients in distinct ethnic groups (i.e. Caucasians, African-Americans, Hispanics and Asians). In order to assess the potential of PiCA to perform pharmacogenomics studies, the numbers of patients with

a reported use of asthma medication (ICS, SABA, LABA, Leukotriene modifiers [LTMs], Anti-IgE and Oral corticosteroids [OCS]) were collected per study. It was also ascertained whether data regarding environmental exposures and atopy were collected. The source for the DNA collection (i.e. blood, saliva) and availability of whole genome genotyping data was assessed.

Outcome measures and treatment response

The presence of information on exacerbations, asthma symptoms and lung function was assessed for each study. A severe exacerbation was considered as a short course (3-5 days) OCS use or a hospitalization/emergency room [ER] visit according to the American Thoracic Society/European Respiratory Society [ATS/ERS] 2009 statement (20). The presence of information on unscheduled General Practitioner [GP] visits or asthma-related absences from school was also assessed. The two outcomes have been used as indicators of exacerbations in several pharmacogenomics studies. For asthma symptoms, presence of information on validated asthma symptom questionnaires (asthma control questionnaire [ACQ] or Asthma Control Test [ACT]) was assessed within the studies. The comparability of the results of these two questionnaires has been shown previously (21). Patients with ACQ scores ≥0.75 and ACT scores <20 were considered to have poor asthma control. In addition, availability of information on asthma symptoms based on guidelines (i.e. Global initiative for Asthma [GINA] and ATS/ERS) was also assessed. According to the availability of data in each study, the number of patients with exacerbations despite regular use of ICS was collected. For observational studies, the presence of any of these outcomes in the preceding six or twelve months was gathered. Asthma diagnosis is difficult in infants and pre-school children. Hence from birth cohorts within the PiCA consortium, we collected outcomes of children ≥ 6 years of age with physician-diagnosed asthma. Cohen's Kappa statistic was calculated per study, to show the overlap between patients experiencing exacerbations and asthma symptoms (22). This was calculated for those studies in which both outcomes were available. The analysis was performed in R (Package 'irr')(23).

Furthermore, since lung function measures are widely used as a response outcome in asthma, it was ascertained whether data regarding lung function measurements, especially changes in FEV_1 from baseline over time (before and after treatment) and changes in FEV_1 after SABA use were also collected within the studies included in the consortium.

Results

Baseline characteristics of the studies and patients

Currently, 21 asthma studies from 12 different countries are enrolled in the PiCA consortium. PiCA includes 15 asthma cohorts, three birth cohorts, two high-risk birth cohorts (inclusion of infants based on allergic history of the mother) and one clinical trial (Table 1).

In total, PiCA includes data of 14,227 asthmatic patients up to 25 years of age. In 17 studies (80%), asthma was based on physician-diagnosis and/or hospital records. For three studies asthma diagnosis was based on parental-reported asthma diagnosis. PACMAN included children with a regular use of asthma medication. Analysis of PiCA children showed that 58% are male. From almost all patients within PiCA (97%) information was available on ethnic background. The majority of the asthmatic patients in PiCA are Caucasian (56%), 12% are Asian, 22% are Hispanic, and 8% have an African/African-American background and the remaining (2%) has mixed/other ethnic backgrounds (Figure 1). In the PiCA consortium studies, data on medication use was collected based on parental/patient reports (17 studies), pharmacy records (nine studies), and physician's prescriptions (five studies). Medication data was available for 12,736 patients. Most of the patients in the studies were treated with ICS (n=7,619) and SABA (n=8,571). Furthermore, 2,050 patients received LABA, and 2,132 used LTRA. OCS as a maintenance medication was used in 568 patients (Figure 2). In line with clinical asthma guidelines, most patients were treated with a combination of different asthma medications.

Outcome measures and treatment response

Thirteen studies had information on exacerbations and approximately one third of the patients had severe exacerbations despite ICS treatment. In eleven studies (including 5,769 patients) data were available on OCS use as rescue medication despite ICS treatment. The prevalence of OCS use ranged between 7-67% in different studies, and in total 1,929 (33%) PiCA patients on ICS had received rescue OCS in the preceding 6-12 months of the study visit. Thirteen studies had data available on asthma-related ER visits or hospitalizations despite ICS (n=6,095). The prevalence of ER visits/hospitalizations ranged between 7-67%. In total 1,806 (29%) patients reported asthma-related ER visits or hospitalizations. Data on asthma-related school absences despite ICS use were available for 2,587 patients in six studies. Furthermore, data on unscheduled general practitioner (GP) visits were available for 1,479 patients in six studies (Figure 3). The total number of patients experiencing exacerbations in each study is shown in supplementary table 1.

Validated scaled questionnaires to assess current asthma symptoms (ACQ and ACT) were used in five studies (DUCHA, ESTATE, PACMAN, PAGES and Singapore Cross Sectional Genetic Epidemiology Study) (in a total of 2,070 patients). In this population, 37% (n=766) of the patients had ACQ scores ≥ 0.75 or ACT scores <20 indicating poor asthma control. Furthermore, a modified version of the 1978 American Thoracic Society–Division of Lung Diseases Epidemiology Questionnaire (24) was used to assess current asthma control in GALA II and SAGE II in 1,725 patients; 41% had uncontrolled asthma symptoms based on this questionnaire. In addition to these scaled questionnaires, several other categorical measures of symptoms were used in studies. Modified GINA definition for long-term asthma control was used in BAMSE (n=226), with 34% of the patients having poor asthma symptoms. In the PIAMA study (n=110), 43% of the patients using inhaled steroids had uncontrolled asthma at age eight. Guidelines of the Dutch Pediatric Society (NVK), which follow the GINA guidelines, were used to define uncontrolled asthma(25).
Regarding lung function measurements, changes in FEV_1 after bronchodilator were measured in seven studies and changes in FEV_1 from baseline were measured in four studies.
Information on asthma severity was available for 5,608 PiCA patients. The number of severe asthmatics according to ATS/ERS, GINA and British Thoracic Society/Scottish Intercollegiate Guidelines Network [BTS/SIGN] (step 4 or higher) guidelines was 838.

Overlap between exacerbations and asthma symptoms:

In three studies (GALA II, PACMAN and SAGE II), we could assess the overlap between exacerbations (defined by OCS use) and patients with asthma symptoms. In all three patient populations, there was only a slight to fair agreement between these two outcomes (kappa: 0.03-0.21); 46-72% of the patients with reported OCS use as a rescue medication also had uncontrolled asthma symptoms according to the asthma questionnaire. The overlap between patients with ER visits/hospitalizations in the past 6/12 months and uncontrolled asthma symptoms in four studies (BAMSE, GALA II, PACMAN, and SAGE II) was also poor (Kappa: 0.03 to 0.22); 41-55% of the patients with ER visits/hospitalizations had uncontrolled asthma symptoms (supplementary table 2).

Pharmacogenomic studies in PiCA:

DNA samples have been collected in 20 studies, and for one study the DNA collection is still ongoing. The source of DNA per study is shown in table 1.

Design of the Pharmacogenomics in Childhood Asthma consortium

A protocol written by the research center interested in a specific research question will be sent to the Principal investigators [PI] of the consortium for review. Next, the protocol will be sent to all PiCA studies. Centers that are willing to participate will perform the association analysis and the results will be sent to research center that initially initiated the research proposal. In case, individual study lack resources or expertise to perform the analyses, other PiCA collaborators will help to perform the analysis.

GWAS in PiCA:

Currently GWAS data is available for 13 studies (n= 6,743) (table 2). In addition, 1,967 DNA samples from 5 studies will be genotyped; BAMSE (n=400), BREATHE (n=92), PAGES (n=514), GoShare (n=561) and SLOVENIA (n=400).

In the discovery phase of the GWAS, genotyped samples will be imputed with the Michigan imputation server (Available at: https://imputationserver.sph.umich.edu). After imputation and quality check association analysis will be performed with EPACS (efficient and parallelizable association container toolbox. Available at: http://genome.sph.umich.edu/wiki/EPACTS). Principal component analysis and adjustment for gender and age will be performed when necessary. GWAS meta-analysis will be performed by METASOFT (26). In the replication phase, association analysis will be performed for the top hits identified in the discovery phase.

Candidate gene approach in PiCA:

Candidate gene studies will be conducted for newly identified SNPs from GWAS meta-analyses and for previously identified SNPs in GWAS of childhood asthma onset and pharmacogenomics of asthma and SNPs that might associate with treatment response based on biological pathways.

Association analysis will be performed in the studies that have genotype or imputed data with high quality. The results of the association analysis will be meta-analyzed.

Discussion

The PiCA consortium is a unique initiative that brings together data from 14,227 asthmatic children/young adults from 12 different countries worldwide. In genetic association studies, replication of the results across populations with different ethnic backgrounds is of high importance in order to support the findings of the pharmacogenomics analysis (27). The PiCA consortium is a novel platform to study the pharmacogenomics of uncontrolled childhood asthma despite asthma treatment.

It is important to study pharmacogenomics of childhood asthma in addition to adult asthma, since asthma phenotypes differ between children and adults and findings in adult studies cannot be translated directly to the pediatric asthma population (28). For example, a genetic variant influencing *FBXL7* expression has been found by the CAMP group to associate with improvement in asthma symptoms in response to ICS in two pediatric populations, but it failed to replicate in adults (13). Several GWAS of response to asthma medication have been published by the CAMP study group (29–31) and they can be found in the National Human Genome Research Institute [NHGRI] and the European Bioinformatics Institute [EMBL-EBI] GWAS catalog (32). In addition, variation in the *ADRB2* gene has been associated with altered LABA response, but mainly in pediatric populations (33–36). Hence, it is important to study treatment response in asthmatic children. However, assessing treatment response in asthmatic children remains a challenging subject, as symptoms may vary over time. Different measures of uncontrolled asthma (i.e. exacerbations, symptoms, or lung function) might reflect distinct dimensions of the disease. It has been previously shown that demographic characteristics and biomarker profiles of children with severe exacerbations were different from children with persistent symptoms (15), and children without asthma symptoms can be prone to severe exacerbations(37). Furthermore, It has been shown that the definition of treatment response influences the genetic risk profile associated with drug response (38,29,39). Calculated Kappa values showed only minimal to moderate agreements between asthma symptoms and exacerbations. Since different dimensions of uncontrolled asthma include different patient populations and overlap only partly, distinct outcome measures need to be studied separately. An important strength of PiCA is the collection of well-defined asthma outcomes in > 14.000 individuals for future pharmacogenomics studies within the PiCA consortium, we will perform analyses using distinct measures of poor treatment response that reflect different dimensions of asthma.

Within the PiCA consortium, we included study designs such as observational asthma cohorts and (high risk) birth cohorts. An observational study (cohort or case-control) is a common approach to assess pharmacogenomics and should not be undervalued. Observational studies can provide valuable evidence

for clinically relevant pharmacogenomics markers. Once identified, the next step would be further replication and developing a prognostic biomarker test with additional replication for generalizability and investigating the functional biology to interrogate the mechanistic aspect of the replicated findings.

Major strengths of the design of the PiCA consortium are inclusion of patients from mild to severe asthmatics with thoroughly investigated outcome and phenotype data (i.e. exacerbations and asthma symptoms), and the coverage of the broad spectrum of pediatric asthmatic medication users, which will make it possible to assess the value of pharmacogenetics for subgroups of patients. Study heterogeneity makes it possible to assess the generalizability of findings across multiple designs and/or multiple ethnicities. Sensitivity analyses can be used to assess for which group a certain marker might have the highest clinical value.

In addition to large-scale pharmacogenomics studies, which are the main goal of this consortium, PiCA also has potential to study other factors influencing treatment outcomes, such as continued exposure to allergens or epigenomics. However, obtaining additional biological samples or data might be complicated for some PiCA studies, this might only be possible in part of the PiCA population. Several potential limitations of this consortium should be acknowledged. One of the limitations of PiCA could be population stratification. However, this heterogeneity will help us to identify different genetic markers associated with the treatment response in patients with different ethnicities. Furthermore, it will help us to discover pharmacogenomics markers that are associated with the treatment response in asthmatics regardless of the ethnic background of the patients. In genome-wide association analyses, we will adjust the results of each cohort by principal components when necessary. In candidate gene studies, the analyses will be performed separately for each study and the results will be meta-analyzed. Furthermore, we will also perform sensitivity analysis by conducing separate analysis for patients with different ethnic backgrounds. The results of these analyses will be compared and in the presence of a significant difference, they will be reported. Another limitation could be the wide age range of the patients included in PiCA, although this does reflect the general asthma population in clinical practice, infant onset asthma might be a different phenotype from asthma in teenagers (40). In addition, asthma diagnosis is complicated at a young age, and infants and pre-school children can suffer from symptoms (such as wheezing) similar to those caused by asthma. In PiCA we will only children include that were still suffering from asthma symptoms at ≥ 6 years of age. In the majority of the PiCA studies (17 out of 21), asthma was based on physician-diagnosis and/or hospital records. Although criteria for physician-diagnosis might differ between countries, this difference reflects current clinical practice.

Design of the Pharmacogenomics in Childhood Asthma consortium

This is the first large effort to unite childhood asthma studies with a common interest in pharmacogenetics. Various studies within PiCA have collected detailed information on asthmatic children and followed children prospectively, making PiCA a unique platform for collaboration and validation. Several other studies (Asthma Genetics in Hungary [AGH], EUROPA from the Netherlands, GoShare from the UK and the Canadian asthma cohort) are still in the stage of recruiting patients, data and genotyping DNA samples, and will participate in the future projects of the PiCA consortium. In other fields, such as in cardiovascular pharmacogenomics, large research consortia have delivered key discoveries (41–44). PiCA is a growing consortium and it provides the opportunity to study pharmacogenetics on a large scale, paving the way for precision medicine in asthma.

References

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: www.ginasthma.org.
2. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004 May;59(5):469–78.
3. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. J Allergy Clin Immunol. 2002 Mar;109(3):410–8.
4. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol. 2005 Feb;115(2):233–42.
5. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Piñeiro A, Wei LX, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. Ann Intern Med. 1999 Mar 16;130(6):487–95.
6. Langmack EL, Martin RJ. Heterogeneity of response to asthma controller therapy: clinical implications. Curr Opin Pulm Med. 2010 Jan;16(1):13–8.
7. Bahadori K, Doyle-Waters MM, Marra C, Lynd L, Alasaly K, Swiston J, et al. Economic burden of asthma: a systematic review. BMC Pulm Med. 2009 Jan;9:24.
8. Chapman KR, Boulet LP, Rea RM, Franssen E. Suboptimal asthma control: prevalence, detection and consequences in general practice. Eur Respir J. 2008 Feb;31(2):320–5.
9. Williams AE, Lloyd AC, Watson L, Rabe KF. Cost of scheduled and unscheduled asthma management in seven European Union countries. Eur Respir Rev. 2006 Jun 1;15(98):4–9.
10. Barnett SBL, Nurmagambetov TA. Costs of asthma in the United States: 2002-2007. J Allergy Clin Immunol. 2011 Jan;127(1):145–52.
11. Fleming L, Wilson N, Bush A. Difficult to control asthma in children. Curr Opin Allergy Clin Immunol. 2007 Apr;7(2):190–5.
12. Pijnenburg MW, Szefler S. Personalized medicine in children with asthma. Paediatr Respir Rev. 2015 Mar;16(2):101–7.
13. Park H-W, Dahlin A, Tse S, Duan QL, Schuemann B, Martinez FD, et al. Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids. J Allergy Clin Immunol. 2014 Mar;133(3):664–9.e5.
14. Rogers AJ, Tantisira KG, Fuhlbrigge AL, Litonjua AA, Lasky-Su JA, Szefler SJ, et al. Predictors of poor response during asthma therapy differ with definition of outcome. Pharmacogenomics. 2009 Aug;10(8):1231–42.
15. Wu AC, Tantisira K, Li L, Schuemann B, Weiss ST, Fuhlbrigge AL. Predictors of symptoms are different from predictors of severe exacerbations from asthma in children. Chest. 2011 Jul;140(1):100–7.
16. Evans WE, Relling M V. Moving towards individualized medicine with pharmacogenomics. Nature. 2004 May 27;429(6990):464–8.
17. Yip VLM, Hawcutt DB, Pirmohamed M. Pharmacogenetic Markers of Drug Efficacy and Toxicity. Clin Pharmacol Ther. 2015 Jul;98(1):61–70.
18. Custovic A, Ainsworth J, Arshad H, Bishop C, Buchan I, Cullinan P, et al. The Study Team for Early Life Asthma Research (STELAR) consortium "Asthma e-lab": team science bringing data, methods and investigators together. Thorax. 2015 Aug;70(8):799–801.
19. Bacharier LB, Boner A, Carlsen K-H, Eigenmann PA, Frischer T, Götz M, et al. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy. 2008 Jan;63(1):5–34.
20. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009 Jul 1;180(1):59–99.
21. Khalili B, Boggs PB, Shi R, Bahna SL. Discrepancy between clinical asthma control assessment tools and fractional exhaled nitric oxide. Ann Allergy Asthma Immunol. 2008 Aug;101(2):124–9.

22. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

23. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

24. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis. 1978;118(6 Pt 2):1–120.

25. de Jongste J, Vrijlandt EJLE. Astma bijkinderen: samenvatting van de herziene rich-tlijnen van de Sectie Kinderlongziekten vande NVK. [Guideline "Asthma in Children"for pediatric pulmonologists]. Hilversum:SKL, 2007.

26. Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. Am J Hum Genet. 2011 May 13;88(5):586–98.

27. Hall IP, Blakey JD. Genetic association studies in Thorax. Thorax. 2005 May;60(5):357–9.

28. Fleming L, Murray C, Bansal AT, Hashimoto S, Bisgaard H, Bush A, et al. The burden of severe asthma in childhood and adolescence: results from the paediatric U-BIOPRED cohorts. Eur Respir J. 2015 Nov;46(5):1322–33.

29. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med. 2011 Sep 29;365(13):1173–83.

30. Park H-W, Dahlin A, Tse S, Duan Q., Schuemann B, Martinez FD, et al. Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids. J Allergy Clin Immunol. 2014;133(3):664–669.e5.

31. Tantisira KG, Damask A, Szefler SJ, Schuemann B, Markezich A, Su J, et al. Genome-wide association identifies the T gene as a novel asthma pharmacogenetic locus. Am J Respir Crit Care Med. 2012 Jun 15;185(12):1286–91.

32. Hindorff L, Parkinson H, Welter D, MacArthur J, Morales J, Burdett T, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Research, 2014, Vol. 42 (Database issue): D1001-D1006Title.

33. Palmer CNA, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax. 2006 Nov;61(11):940–4.

34. Zuurhout MJL, Vijverberg SJH, Raaijmakers JAM, Koenderman L, Postma DS, Koppelman GH, et al. Arg16 ADRB2 genotype increases the risk of asthma exacerbation in children with a reported use of long-acting β2-agonists: results of the PACMAN cohort. Pharmacogenomics. 2013 Dec;14(16):1965–71.

35. Wechsler ME, Kunselman SJ, Chinchilli VM, Bleecker E, Boushey HA, Calhoun WJ, et al. Effect of beta2-adrenergic receptor polymorphism on response to longacting beta2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, crossover trial. Lancet (London, England). 2009 Nov 21;374(9703):1754–64.

36. Turner S, Francis B, Vijverberg S, Pino-Yanes M, Maitland-van der Zee AH, Basu K, et al. Childhood asthma exacerbations and the Arg16 β2-receptor polymorphism: A meta-analysis stratified by treatment. J Allergy Clin Immunol. Elsevier Ltd; 2016;

37. Carroll CL, Schramm CM, Zucker AR. Severe exacerbations in children with mild asthma: characterizing a pediatric phenotype. J Asthma. 2008 Aug;45(6):513–7.

38. Leusink M, Vijverberg SJH, Koenderman L, Raaijmakers JAM, de Jongste JC, Sterk PJ, et al. Genetic variation in uncontrolled childhood asthma despite ICS treatment. Pharmacogenomics J. 2015;1–6.

39. Vijverberg SJH, Tavendale R, Leusink M, Koenderman L, Raaijmakers JAM, Postma DS, et al. Pharmacogenetic analysis of GLCCI1 in three north European pediatric asthma populations with a reported use of inhaled corticosteroids. Pharmacogenomics. 2014 Apr;15(6):799–806.

40. Depner M, Fuchs O, Genuneit J, Karvonen AM, Hyvärinen A, Kaulek V, et al. Clinical and Epidemiologic Phenotypes of Childhood Asthma. Am J Respir Crit Care Med. 2013 Nov 27;189(2):131127081214005.

41. Owen RP, Altman RB, Klein TE. PharmGKB and the International Warfarin Pharmacogenetics Consortium: the changing role for pharmacogenomic databases and single-drug pharmacogenetics. Hum Mutat. 2008 Apr;29(4):456–60.

42. Paternoster L, Standl M, Chen C-M, Ramasamy A, Bønnelykke K, Duijts L, et al. Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. Nat Genet. 2012 Feb;44(2):187–92.

43. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010 Sep 23;363(13):1211–21.

44. Ferreira MAR, Matheson MC, Duffy DL, Marks GB, Hui J, Le Souëf P, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. Lancet (London, England). 2011 Sep 10;378(9795):1006–14.

1

Table 1. PiCA characteristics: Study design and patient characteristics

Study name (Ref in this article)	Country	Study design	Recruiting centers	Asthmatic patients(N)	Age (Range, yrs.)	Male N, (%)	Mean (SD) FEV1% predicted baseline	Medication				DNA source
								ICS	LABA	SABA	LTRA	
BAMSE[1]	Sweden	General birth cohort	Primary care	420	0-16	242 (57.6)	103 (11.0)	226	57	218	-	Peripheral blood§
BREATHE[2]	UK	Asthma cohort	Primary and secondary care	1570	3-22	1017 (64)	96.6 (15.5)	959	62	1505	210	Saliva§
British Columbia Childhood Asthma Cohort	Canada	Asthma cohort	Tertiary/quaternary referral center	343	1-18	223 (65)	-	343	54	343	79	Buccal cell and Saliva
CAMP[3]	USA	RCT	Tertiary care	1041	5-12	621 (59)	95.6±18	311	-	418	-	Peripheral blood§
COPSAC2000[4]	Denmark	High risk birth cohort	Written invitation	43	0-7	22(51)	94.4 (12.1)	43	*	43	*	Peripheral blood§
COPSAC2010	Denmark	General birth cohort	Written invitation	90	0-5	52(57)	97.1 (12.1)	90	0	90	*	Peripheral blood§
COPSACSevere*	Denmark	Asthma cohort	Registry based	1173	0-25	791 (67)	-	*	*	*	*	Peripheral blood
DUCHA	Greece	Asthma cohort	Tertiary care	193	5-14	179 (92)	101.2 (12.8)	193	56	18	25	Peripheral blood
ESTATe	Netherlands	Case-control	Primary care	111	4-19	67 (60)	-	110	42	111	2	Saliva§
followMAGICS[5]	Germany/Austria	Asthma cohort	Secondary and tertiary care	313	7-25	194 (62)	-	150	104	107	27	Peripheral blood§
GALA II[6]#	USA	Case-control	Secondary care, community and clinic-based recruitment	2377	8-21	1288 (54)	90.8 (16.2)	1174	368	1900	610	Peripheral blood and Saliva§
Generation R#2[7]	Netherlands	Population-based birth cohort	Primary, secondary and tertiary care	399	fetal-ongoing	249 (62.4)	100 (12.8)	200	50	280	10	Umbilical cord blood§

GOASC[8]#	Spain	Asthma cohort	Secondary and tertiary care	125	2-18	76 (60)	94.6 (15.2)	125	78	14	107	Peripheral blood and Saliva
PACMAN[9]	Netherlands	Asthma cohort	Primary care	995	4-12	616 (61)	-	844	229	819	87	Saliva§
PAGES[10]	UK	Asthma cohort	Primary, secondary and tertiary care	701	2-18	519 (74)	94 (16)	648	347	696	286	Saliva
PASS[11]	UK	Asthma cohort	Tertiary care	525	5-18	307 (58)	-	525	395	525	369	Peripheral blood and Saliva§
PIAMA[12]	Netherlands	General birth cohort/ high risk birth cohort	Primary care	428	8	254 (59.3)	105.4 (12.2)	208	28	210	5	
SAGE II[6]#	USA	Case-control	Secondary care, community and clinic-based recruitment	987	8-21	503 (51)	98.7 (14.1)	670	171	822	96	Peripheral blood and Saliva§
Singapore Cross Sectional Genetic Epidemiology Study[13]#	Singapore	Asthma cohort	Tertiary care	1450	18-25	600 (41)	76.9 (12.8)	394*	*	*	*	*
Slovenia[14]	Slovenia	Asthma cohort	Tertiary care	350	5-19	162 (46)	89.9 (14.85)	193	*	*	86	Peripheral blood
Study of asthma in Puerto Rican children (HPR)[15]	USA	Case-control	Tertiary care and population based probabilistic sampling design	593	6-14	320 (53.9)	88.5 (16.5)	213	9	452	133	Peripheral blood§
Total: 21 studies	12 countries			14,227				7,619	2,050	8,571	2,132	

*- Data not available, * Data collection ongoing, # patient inclusion ongoing. §Studies with GWAS data available. #2 Patient follow-up ongoing, numbers based on participation until April 1st, 2015, aged 9 years. BAMSE, Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology. CAMP, Childhood Asthma Management Program. COPSAC, The Copenhagen Prospective Study on Asthma in Childhood. DUCHA, . ESTATe, Effectiveness and Safety of Treatment with Asthma Therapy in children. GALA II, Genes-Environment and Admixture in Latino Americans. GOASC, Genetics of Asthma in Spanish Children. ICS, inhaled corticosteroids. LABA, Long-acting Beta2 agonist. LTRA, Leukotriene Receptor Antagonists. MAGICS, Multicenter Asthma Genetics in Childhood Study. PACMAN, Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects. PAGES, Paediatric Asthma Gene Environment Study. PASS, Pharmacogenetics of adrenal suppression. PIAMA, The Prevention and Incidence of Asthma and Mite Allergy. SABA, Short-acting Beta2 agonists. SAGE II, Study of African Americans, Asthma, Genes Environments. RCT, randomized controlled trial*

Table 2. Studies with GWAS

Study	Asthmatic patients, (n)	Genotyping chip	Genotyped SNPs*
BAMSE	122	Illumina, Infinium 610 Quad Chip	582,892
BREATHE	222	llumina Infinium Exome-24 BeadChip	172,660
CAMP	124	illumina, HumanHap550v3 Genotyping BeadChip	486,706
COPSAC2000	43	Illumina Infinium HumanOmniExpressExome Bead chip	657,699
COPSAC2010	90	Illumina Infinium HumanOmniExpressExome Bead chip	657,699
COPSACsevere	1173	Illumina Infinium HumanOmniExpressExome Bead chip	657,699
ESTATe	103	Illumina, Infinium CoreExome-24 BeadChip	538,267
followMAGICS	311	Illumina Sentrix HumanHap300 BeadChip	309,560
GALA II	1,900	Affymetrix, Axiom™ LAT1 array, World Array 4	742,201
HPR	593	Illumina HumanOmni2.5 BeadChip	1,300,000
PACMAN	842	Illumina, Infinium CoreExome-24 BeadChip	518,648
PASS	403	Illumina Omni Express 8v1	654,246
SAGE II	817	Affymetrix, Axiom™ LAT1 array, World Array 4	759,124
Total	6,743		

**Number of SNPs after quality control: SNPs with MAF >5%, failure rate <5% and Hardy-Weinberg p-value < $1*10^{-4}$.*

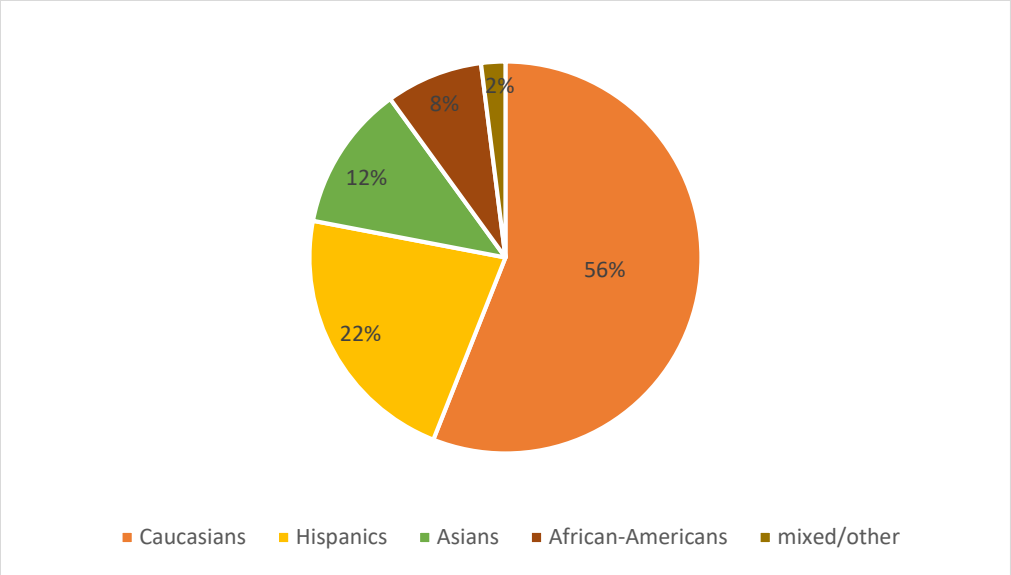

Fig 1. Ethnic backgrounds of the asthmatic patients included in the PiCA consortium

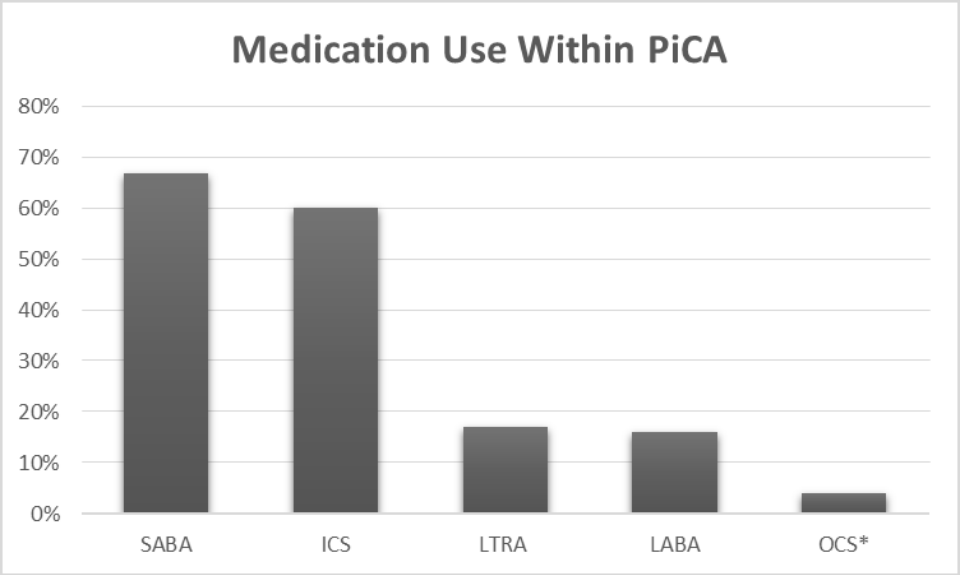

Fig 2. Physician or patient/parental reported medication use in PiCA. *OCS considered as long-term therapy.

1

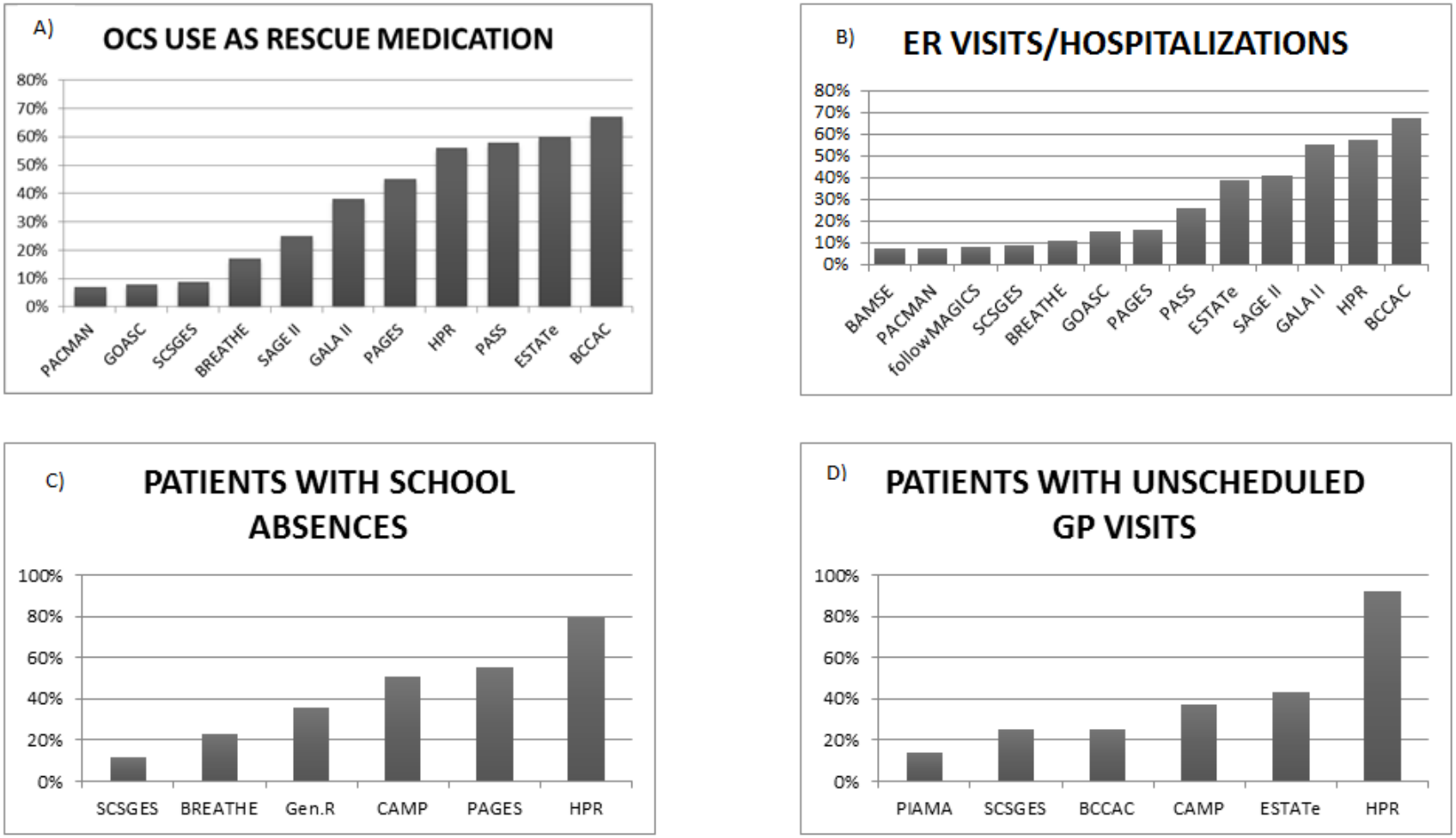

Fig 3. Exacerbations despite regular use of ICS in the preceiding six months or year. A) Percentage of OCS users as a rescue medication in 11 PiCA studies. B) Percentage of patients with ER visit/hospitalization in 13 PiCA studies. C) Percentage of patients with asthma-related school absences in 6 PiCA studies. D) Percentage of patients with unscheduled GP visits in 6 studies. BCCAC; British Columbia Childhood Asthma Cohort, Gen.R; Generation R, SCSGES; Singapore Cross Sectional Genetic Epidemiology Study. In PASS and BREATHE exacerbation data were available in the preceding 6 months.

Supplementary Table 1. PICA characteristics: Treatment outcomes within the total population

Study	Asthma exacerbations					Poor asthma symptoms	
	OCS use[1] N (%)	**ER[1] N (%)**	**Hospitalizations[1] N (%)**	**School absences[1] N (%)**	**GP visits[1] N (%)**	**ACQ N (%)**	**ACT N (%)**
BAMSE	-	**32 (7.6)**	**4 (1.0)**	-	-	-	-
PIAMA	2 (0.4)	3 (0.7)	2 (0.4)	-	75 (17)	-	-
PAGES	**668 (95)**	-	**289 (41)**	**-**	-	-	64 (9)
PACMAN1	60 (6)	61 (6.1)	-	-	61/953 (6.4)	406 (40)	-
followMAGICS	-	**12 (4)**	**6 (2)**	**75 (24)**	**108 (35)**	-	-
GALA II	**745 (31)**	**1144 (48)**	**1195 (50)**	-	-	-	-
SAGE II	**187 (18)**	**335 (33)**	**48 (4)**	-	-	-	-
GOASC	**11 (8)**	**16 (12)**	**2 (1.6)**	-	-	-	-
BREATHE	468 (29)	-	*299(19)*	*605 (38)*	-	-	-
Singapore Cross Sectional Genetic Epidemiology Study	38 (2.6)	**120 (8)**	**34 (2.3)**	**170 (11)**	**309 (21)**	-	92 (6)
DUCHA	-	-	-	-	-	-	56 (29)
British Columbia Childhood Asthma Cohort	**232 (67)**	**214 (62)**	**67 (19)**	-	**91 (26)**	-	-
PASS	*309 (58)*	-	*141 (26)*	-	-	-	-
CAMP	538 (51)	183 (17)		564 (54)	479 946)	-	-
HPR	**229 (38)**	**279 (47)**	**98 (16)**	**355 (59)**	**467 (78)**	**-**	**-**
ESTATE	**39 (35.1)**	**13 (11.7)**	**-**	**-**	**37 (33)**	**-**	**32 (29)**

- Data not available, * data not analyzed. 1 number of children with this outcome during past 6 months (Italic font) or past 12 months of cohort studies (bold font), currently in the birth cohort (underlined), or during the trial (standard font). ACQ-score ≥ 0.75 and ACT–score ≤ 19 is considered not well controlled asthma. #2 patient follow-up ongoing, numbers based on participation until April 2015, aged 9 years. N.a. not applicable at current stage.

Supplementary table 2. Cohen's Kappa values for different definitions of uncontrolled asthma

	Uncontrolled symptoms & OCS use	Uncontrolled symptoms & ER visits and/or hospitalizations
Calculated values of Cohen's Kappa		
HPR		
BREATHE		
GALA II	0.21	0.19
PACMAN	0.03	0.03
SAGE II	0.16	0.2
BAMSE		0.22

1