The burden of severe asthma in childhood and adolescence: results from the paediatric U-BIOPRED cohorts

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Children with severe preschool wheeze or severe asthma are usually atopic and have impaired quality of life http://ow.ly/RrrGE

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ABSTRACT U-BIOPRED aims to characterise paediatric and adult severe asthma using conventional and innovative systems biology approaches.

A total of 99 school-age children with severe asthma and 81 preschoolers with severe wheeze were compared with 49 school-age children with mild/moderate asthma and 53 preschoolers with mild/moderate wheeze in a cross-sectional study.

Despite high-dose treatment, the severe cohorts had more severe exacerbations compared with the mild/moderate ones (annual medians: school-aged 3.0 *versus* 1.1, preschool 3.9 *versus* 1.8; p<0.001). Exhaled tobacco exposure was common in the severe wheeze cohort. Almost all participants in each cohort were atopic and had a normal body mass index. Asthma-related quality of life, as assessed by the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) and the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ), was worse in the severe cohorts (mean±SE school-age PAQLQ: 4.77±0.15 *versus* 5.80±0.19; preschool PACQLQ: 4.27±0.18 *versus* 6.04±0.18; both p≤0.001); however, mild/moderate cohorts also had significant morbidity. Impaired quality of life was associated with poor control and airway obstruction. Otherwise, the severe and mild/moderate cohorts were clinically very similar.

Children with severe preschool wheeze or severe asthma are usually atopic and have impaired quality of life that is associated with poor control and airflow limitation: a very different phenotype from adult severe asthma. In-depth phenotyping of these children, integrating clinical data with high-dimensional biomarkers, may help to improve and tailor their clinical management.

Introduction

Asthma is one of the most common chronic diseases in childhood. Although many achieve control with currently available therapies, an estimated 5–10% of patients remain symptomatic despite receiving large amounts of treatment. These children with severe asthma [1] have poor quality of life (QoL), frequent asthma attacks and lung function impairment, are at high risk of side-effects from medication and account for significant medical and societal costs.

It is increasingly recognised that asthma, and particularly severe asthma, is not one single disease entity. Data in adults have been available for some time [2] but evidence now exists in children to suggest that there are a number of different clinical manifestations of severe asthma that are driven by a variety of pathophysiological mechanisms [3, 4]. Phenotypic classifications in children have primarily been based on clinical data, lung function measurement and assessment of allergic status. The small number of studies that have included biological samples have described important differences in the underlying pathobiology of severe asthma in children compared with adults [5–7]. Some but not all preschool children with severe wheeze have evidence of airway remodelling and inflammation from an early age [8], consistent with established asthma, but little is known about the underlying mechanisms, which in many cases appear to be very different from school-age and adult asthma. These early changes do not always predict a progression to asthma [9]. These observations are indicative of considerable heterogeneity amongst children with severe school-aged asthma or severe preschool wheeze.

In order to capture the relevant phenotypes of children with severe asthma or severe wheeze, careful and extensive clinical characterisation is required. This provides the basis for future integration with biological disease markers. The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project is a public–private partnership, within the framework of the Innovative Medicines Initiative, bringing together academic institutions and European Federation of Pharmaceutical Industries and Associations partners from across Europe. It was set up in 2009 in order to take advantage of the combination of extensive clinical characterisation and biological fingerprinting by "omics" technologies for the unbiased discovery of phenotypes in both adult and paediatric severe asthma [10]. The paediatric arm of the U-BIOPRED study used the same thorough clinical characterisation and innovative systems biology approach as the adult study [11] to integrate clinical, physiological and inflammatory data and patient/parent-reported outcomes with the high-dimensional data of "omics" technologies (transcriptomic, proteomic, lipidomic and metabolomic) obtained from blood, urine, breath and airway samples [12].

The main objective of this first report of the paediatric U-BIOPRED study was to fully clinically characterise the cohorts of children with severe asthma and preschool wheeze and mild/moderate cohorts based on cross-sectional baseline data. The second objective was to investigate the burden of severe asthma and severe preschool wheeze, as described by QoL, and the clinical factors that relate to this burden.

Methods

This was a multicentre, prospective, observational cohort study following the life course of asthma. Full details of the adult cohorts are described in the companion paper by SHAW *et al.* [11].

Cohorts

Seven centres in five European countries recruited preschool (age 1–5 years) and school-age (age 6–17 years) children. Four paediatric cohorts were recruited by approaching consecutive patients attending respiratory and general paediatric clinics who fulfilled the following inclusion criteria. 1) Severe school-aged asthma (SA): ongoing poorly controlled asthma (persistent symptoms, frequent exacerbations or persistent airflow limitation) despite high-dose inhaled corticosteroids (ICS) and at least two other controller medications [13]. 2) Mild/moderate school-aged asthma (MMA): controlled or partly controlled asthma, prescribed low-dose ICS and no other or one additional controller medication. 3) Severe preschool wheeze (SW): persistent symptoms and frequent exacerbations despite current or failed high-dose ICS and a leukotriene receptor antagonist (LTRA). 4) Mild/moderate preschool wheeze (MMW): controlled or partially controlled symptoms prescribed no treatment or low-dose ICS and/or a LTRA.

Full cohort descriptions, inclusion and exclusion criteria are shown in table S2 in the online supplementary material. All children in the severe cohorts (SA and SW) had been under follow-up with a respiratory paediatrician for ⩾6 months before enrolling in the study. During this time, assessments were undertaken to exclude other diagnoses, treat comorbidities, optimise asthma control, assess medication adherence (*e.g.* checking prescription uptake) and reduce allergen exposure in sensitised individuals [13].

Study design

The study was approved by the local ethics committees (see table S1). Parents/caregivers provided written consent; children gave assent where appropriate. The study is registered with ClinicalTrials.gov (NCT01982162). The study aims and outcomes have been published on the U-BIOPRED website (www.europeanlung.org/en/projects-and-research/projects/u-biopred/home).

All participants were identified and recruited locally and attended a screening and baseline visit. Clinical data and biological samples were collected from all cohorts (figure 1). Full details are provided in the supplementary material.

Study assessments

Baseline data included demographics, current and past medical history (including detailed asthma and atopic disease history), medications, birth history, family history, exposure to environmental tobacco smoke, and known clinical and environmental risk factors. Asthma control was assessed using the Asthma Control Test (ACT) (for children ⩾12 years of age) [14] or the Childhood Asthma Control Test (cACT) for children <12 years [15]. Non-scheduled healthcare utilisation was assessed by documenting exacerbations. QoL was assessed using the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) (school-aged children only) and the Paediatric Asthma Caregiver's Quality of Life Questionnaire

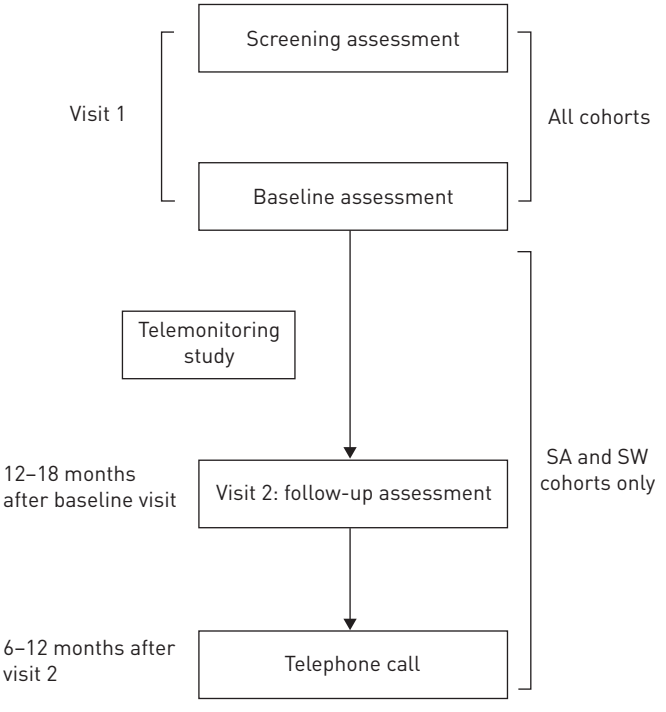

FIGURE 1 Visit schedule. SA: severe school-aged asthma; SW: severe preschool wheeze.

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(PACQLQ) [16, 17]. Adherence was evaluated using the Medication Adherence Report Scale (MARS) [18]. A summary of the assessments carried out in each of the cohorts is shown in table S3.

In all cohorts, total IgE, specific IgE tests and/or skin prick testing (SPT) to six common allergens were undertaken. Atopy was defined as the presence of sensitisation on SPT (wheal ⩾3 mm) or serum specific IgE (⩾0.35 $kU{\cdot}L^{-1}$). Spirometry before and after bronchodilator [19] and exhaled nitric oxide fraction (F_{ENO}) were measured where possible. Sputum induction was performed in the school-aged cohorts and differential cell counts were determined. Exposure to environmental tobacco smoke was assessed by measuring urinary cotinine. In selected centres, forced oscillation technique and plethysmography were undertaken.

Full details of the methods are provided in the supplementary material, including samples collected for future "omic" analysis. A centralised biobank was selected for the study and operated in accordance with its own Standard Operating Procedures, as described in the supplementary material.

Data management and statistics

Data were entered into an electronic clinical record form before being transferred to the tranSMART system for quality control checks [20]. Missing data were not imputed.

The cohort sizes of 97 and 43 (comparing SA and MMA), and 77 and 54 (comparing SW and MMW), both provide 80% power to detect a difference in means of half a standard deviation, assuming standard normally distributed data, in a two-sided t-test at the 5% significance level [21].

Due to the descriptive (as opposed to inferential) nature of the analyses presented, raw, unadjusted p-values are reported throughout the manuscript. Those in tables 1–4 were derived using logistic regression (binary variables) or general linear regression (continuous variables). Continuous variables exhibiting positive skew were summarised by the median and interquartile range (IQR), and were log-transformed prior to association testing. Where appropriate, tests of association were performed both with and without adjustments for age and sex.

Associations between key potential facets of asthma (forced expiratory volume in 1 s (FEV_1) z-score, FEV_1/forced vital capacity (FVC), age of onset/diagnosis, number of exacerbations in preceding 12 months, ACT z-score, body mass index (BMI), MARS, hay fever, eczema, atopy, smoking and white race) were each assessed singly for association with asthma burden, as quantified by QoL, using linear regression. Adjustments for age and sex were not applied at this stage due to a lack of univariate association between either age or sex. QoL contour plots were derived for continuous variables with $p<0.05$, using two-dimensional kernel density estimation with a bivariate normal kernel, evaluated at 50 grid points in each direction [23]. The variables were also modelled jointly in a multivariate general linear model. Backwards stepwise regression using the Akaike Information Criterion was then applied, in order to derive a parsimonious model.

Analyses were performed using R version 2.15.2 (R Core Team; www.r-project.org). The present report is based on cross-sectional analysis of the baseline data.

Results

Participants

A total of 298 children and teenagers with asthma or wheeze were screened to recruit 282 participants. Numbers of participants in each cohort that provided baseline questionnaire data, spirometry, blood samples and sputum samples are detailed in figure 2.

Cohorts SA and MMA were well matched for age (mean 12.21 and 11.26 years, respectively), as were cohorts SW and MMW (mean 3.56 and 3.46 years, respectively). Exposure to environmental tobacco smoke was reported by 15.8–22.8% of each cohort. More of the SW cohort were positive for urinary cotinine than of the MMW cohort (19.4% *versus* 4.3%; p=0.035) (table 1).

Atopy

Most of the school-age participants in both cohorts (SA, MMA) were atopic (85.4% and 89.5%, respectively) (table 1). Rates of atopy were lower in both preschool cohorts (36.5% and 37.5%) (table 1). The majority of the school-age children (SA, MMA) had a diagnosis of eczema, hay fever or allergic rhinitis (table 1). Most of the preschoolers had a co-existing diagnosis of eczema with a third also having allergic rhinitis. In the preschool children, significantly more SW than MMW participants had a diagnosis of hay fever (58.8% *versus* 36.1%, respectively; p=0.04). A sizeable minority of participants reported symptoms of food allergy (40.2% for SA *versus* 32.6% for MMA, p=0.39; 21.1% for SW *versus* 27.8% for MMW, p=0.38).

TABLE 1 Baseline demographic characteristics and medical history

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Demographic details						
Female	46/97 (47.4%)	16/43 (37.2%)	0.263	27/77 (35.1%)	20/54 (37.0%)	0.817
Age years	12.21±0.31 (n=97)	11.26±0.48 (n=43)	0.583[f]	3.56±0.14 (n=77)	3.46±0.16 (n=54)	0.410[f]
White	74/97 (76.3%)	32/43 (74.4%)	0.812	62/77 (80.5%)	48/54 (88.9%)	0.204
Mother smoked during pregnancy	14/94 (14.9%)	6/43 (14.0%)	0.885	10/77 (13.0%)	1/54 (1.9%)	0.052
Smoker	0/97 (0.0%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
Second-hand smoke exposure	21/92 (22.8%)	9/43 (20.9%)	0.805	12/76 (15.8%)	9/54 (16.7%)	0.893
Urinary cotinine present	8/93 (8.6%)	5/38 (13.2%)	0.432	12/62 (19.4%)	2/46 (4.3%)	0.035
Anthropometry[#]						
Height cm	152.82±1.65 (n=97)	148.12±2.58 (n=43)	0.604[f]	102.88±1.13 (n=76)	103.62±1.52 (n=53)	0.068[f]
Height z-score	0.68±0.34 (n=97)	0.58±0.2 (n=43)	0.851	1.14±0.16 (n=76)	1.53±0.18 (n=53)	0.108
Weight kg	51.74±1.85 (n=97)	43.64±2.3 (n=43)	0.067[f]	17.63±0.48 (n=77)	17.27±0.46 (n=53)	0.687[f]
Weight z-score	1.14±0.21 (n=97)	0.66±0.19 (n=43)	0.167	0.94±0.14 (n=77)	0.92±0.13 (n=53)	0.930
BMI $kg \cdot m^{-2}$	21.52±0.5 (n=97)	19.21±0.5 (n=43)	0.035[f]	16.56±0.25 (n=76)	15.99±0.15 (n=53)	0.071[f]
BMI z-score	0.99±0.13 (n=97)	0.56±0.17 (n=43)	0.058	0.26±0.15 (n=76)	−0.04±0.1 (n=53)	0.133
Past medical history						
Mode of delivery						
Normal vaginal	68/97 (70.1%)	28/43 (65.1%)	0.558	59/77 (76.6%)	38/54 (70.4%)	0.422
Instrumental	5/97 (5.2%)	3/43 (7.0%)	0.669	4/77 (5.2%)	3/54 (5.6%)	0.928
Caesarian	24/97 (24.7%)	12/43 (27.9%)	0.693	14/77 (18.2%)	13/54 (24.1%)	0.413
Breast feeding months	5.09±0.8 (n=97)	5.72±1.13 (n=43)	0.659	4.61±0.62 (n=77)	7.43±0.81 (n=54)	0.006
Admitted to neonatal unit	14/97 (14.4%)	3/43 (7.0%)	0.223	7/77 (9.1%)	3/54 (5.6%)	0.457
Other medical problems						
Diagnosed hay fever	75/91 (82.4%)	33/40 (82.5%)	0.991	30/51 (58.8%)	13/36 (36.1%)	0.039
Diagnosed eczema	77/95 (81.1%)	28/40 (70.0%)	0.162	42/57 (73.7%)	32/40 (80.0%)	0.473
Diagnosed allergic rhinitis	61/93 (65.6%)	29/38 (76.3%)	0.232	22/52 (42.3%)	11/36 (30.6%)	0.265
Diagnosed gastro-oesophageal reflux[¶]	19/94 (20.2%)	3/40 (7.5%)	0.081	8/58 (13.8%)	11/40 (27.5%)	0.097
Diagnosed vocal cord dysfunction	2/94 (2.1%)	1/40 (2.5%)	0.894	0/59 (0.0%)	0/40 (0.0%)	NA
Reported food allergy[+]	39/97 (40.2%)	14/43 (32.6%)	0.390	16/76 (21.1%)	15/54 (27.8%)	0.376
Allergic sensitisation						
Positive skin prick test	69/83 (83.1%)	33/37 (89.2%)	0.395	22/65 (33.8%)	18/48 (37.5%)	0.688
Positive specific IgE	40/47 (85.1%)	21/24 (87.5%)	0.784	14/30 (46.7%)	13/26 (50.0%)	0.803
Atopy[§]	70/82 (85.4%)	34/38 (89.5%)	0.540	23/63 (36.5%)	18/48 (37.5%)	0.915

Data are presented as n/N (%) or mean±SE, unless otherwise stated. p-values were calculated using Pearson's Chi-squared test or a Kruskall–Wallis test. BMI: body mass index; NA: not applicable. [#]: anthropometry z-scores generated using the 1990 British growth data [22]; [¶]: gastro-oesophageal reflux was diagnosed on the basis of suggestive symptoms, pH monitoring, endoscopy or response to therapy; [+]: symptoms of reported food allergy represent symptoms of urticaria, angioedema, pruiritis, throat tightness, stridor, chest tightness or wheeze within 2 h of contact with food; [§]: atopy defined as a positive skin prick test (⩾3 mm) or a positive specific IgE (⩾0.35 $kU \cdot L^{-1}$); [f]: p-values adjusted for age and sex.

Asthma history and treatment

The mean age at diagnosis was in the fourth year of life for both school-aged cohorts, whereas for the preschool ones it was in their second year (table 2). There were significant differences in the triggers for respiratory symptoms between the severe and mild/moderate cohorts (table 2). While almost all of cohorts SA, MMA and SW were treated with ICS, they were prescribed for less than half of MMW as most had failed to respond to ICS therapy. Additionally, 23.7% of SA and 5.2% of SW were receiving maintenance oral corticosteroid therapy. Parent/participant-reported adherence to therapy was good in all cohorts (table 2).

Lung function and airway inflammation

Lung function and bronchodilator reversibility in the SA and MMA cohorts were similar at baseline when participants were well (table 3). For preschool participants able to perform spirometry, results were again similar for severe and mild/moderate cohorts. There was a trend towards specific airway conductance being lower in the SA cohort compared with the MMA cohort (1.58 *versus* 1.95 $kPa \cdot s^{-1}$; p=0.054) (table 3). We were only able to collect induced sputum from a minority of school-aged participants so we could not make a meaningful comparison between the cohorts (table 3).

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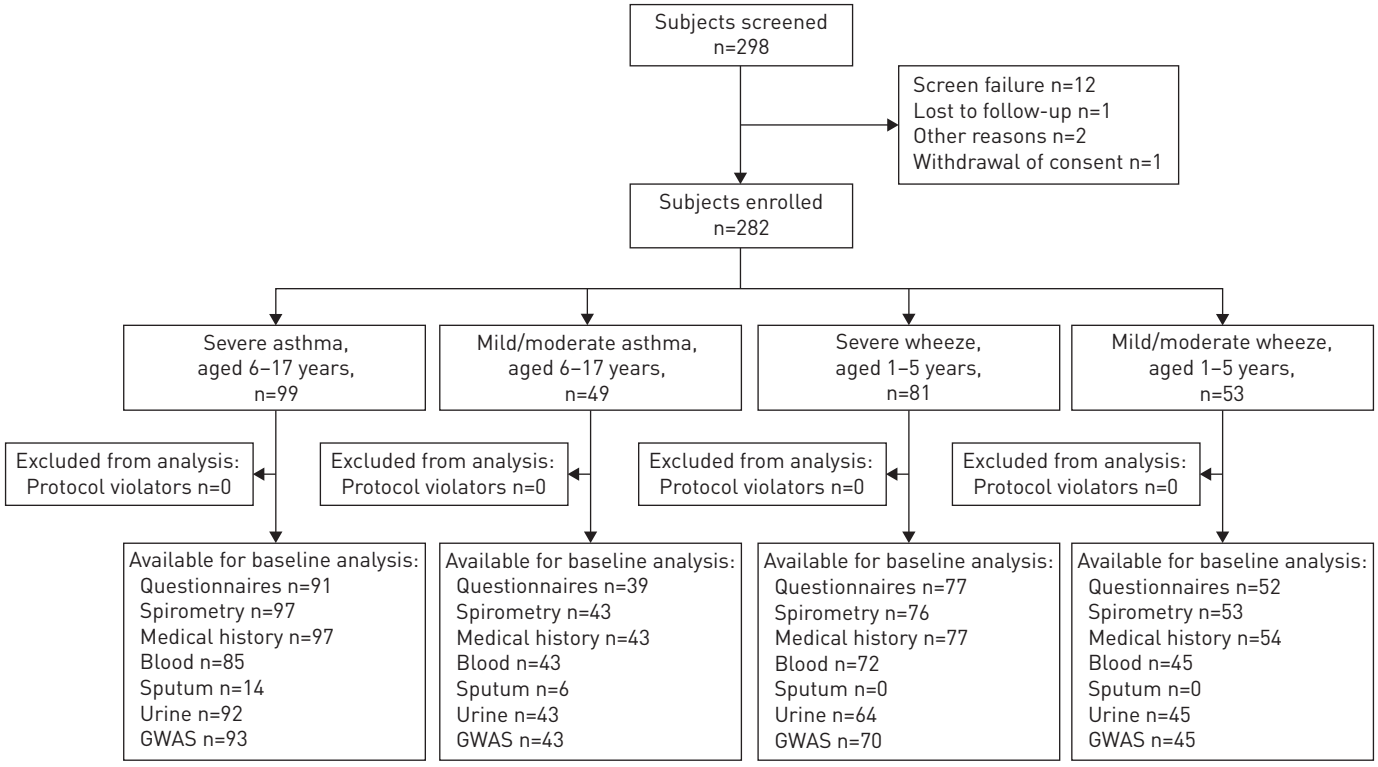

FIGURE 2 Consort diagram for participants in the paediatric Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) study. GWAS: genome-wide association study.

Asthma burden: QoL, control and exacerbations

Asthma-related QoL was used as the primary measure of burden. The mean result for the PAQLQ for the SA cohort was 4.77, equivalent to "somewhat bothered", significantly worse than for the MMA cohort (5.8, equivalent to "bothered a bit"; p<0.001). Similar differences were found for the symptoms, emotions and activity domains (table 4). For the preschool cohorts the PACQLQ was used as a proxy, given that there is no validated QoL tool for preschool wheeze. For SW the mean was 4.27 ("some of the time" or "somewhat worried/concerned"), again significantly worse than for MMW (6.04, "hardly ever" or "hardly worried/concerned"; p<0.001).

The burden of asthma was also illustrated by the ACT results, which assessed ongoing symptoms and rescue medication. Most of the severe cohorts were uncontrolled (74.6% in SA compared with 29.2% in MMA, p<0.001; 78.0% in SW compared with 18.2% in MMW, p<0.001). This was reflected in the number of exacerbations in the year prior to assessment. In the previous year, the SA cohort had a median of three exacerbations (IQR two to five), compared with one (IQR zero to two) in the MMA cohort (p<0.001). A similar difference was seen between the SW and MMW cohorts (table 4). However, there was still an important asthma burden in the mild/moderate cohorts.

Which factors are associated with asthma burden?

Asthma burden is described as asthma-related QoL, with z-scores used to give a combined variable for all age groups. Pre-bronchodilator FEV_1, but not FEV_1/FVC ratio, was significantly related to QoL (regression coefficient 0.151, p=0.002) (table 5). The number of exacerbations in the previous year was significantly inversely associated with asthma QoL (−0.52, p<0.001). Asthma control (measured by ACT and cACT z-score) was significantly related to asthma QoL (0.730, p<0.001). BMI was inversely associated with asthma QoL (−0.036, p=0.011). These are illustrated in figure 3. Results were similar when PAQLQ and PACQLQ were considered separately (table S4).

To assess which factors were independent predictors of asthma-related QoL, a backward stepwise regression analysis was performed for FEV_1 z-score, FEV_1/FVC, age of onset/diagnosis, number of exacerbations in preceding 12 months, ACT z-score, BMI, MARS, hay fever, eczema, atopy, smoking and white race. Significant factors in the reduced model were ACT z-score (regression coefficient 0.76, p<0.001) and FEV_1 z-score (0.11, p=0.036).

TABLE 2 Asthma history and treatment

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Basis of asthma definition						
Airway hyperresponsiveness, PC_{20} <8 mg·mL^{-1}	0/97 (0%)	6/43 (14%)	NA	NA	NA	NA
Bronchodilator reversibility, FEV_1 ⩾12%	66/97 (68%)	19/43 (44.2%)	0.028	NA	NA	NA
Persistent airflow limitation, FEV_1 z-score <−1.96	5/96 (5.2%)	NA	NA	NA	NA	NA
Spontaneous variability, FEV_1 ⩾12%	1/97 (1%)	19/43 (44.2%)	0.994	NA	NA	NA
Diurnal peak flow variability, ⩾15%	8/97 (8.2%)	2/43 (4.7%)	0.452	NA	NA	NA
Basis of severe asthma definition						
High-dose ICS and trials of other controllers	97/97 (100%)	NA	1.000	NA	NA	NA
Persistent symptoms	63/96 (65.6%)	NA	NA	NA	NA	NA
Frequent exacerbations	44/96 (45.8%)	NA	NA	NA	NA	NA
Persistent airflow limitation	5/96 (5.2%)	NA	NA	NA	NA	NA
Maintenance oral corticosteroids	19/96 (19.8%)	NA	NA	NA	NA	NA
Symptom control						
Controlled	NA	22/43 (51.2%)	NA	NA	37/52 (71.2%)	NA
Partially controlled	NA	21/43 (48.8%)	NA	NA	15/52 (28.8%)	NA
Asthma history						
Age at diagnosis years	3.25±0.27 (n=93)	3.78±0.48 (n=41)	0.305	1.74±0.12 (n=73)	1.48±0.13 (n=46)	0.146
ICU admission ever	19/97 (19.6%)	4/43 (9.3%)	0.139	9/77 (11.7%)	2/54 (3.7%)	0.124
ICU admission in past year	5/97 (5.2%)	1/43 (2.3%)	0.458	6/77 (7.8%)	2/54 (3.7%)	0.347
Intubation ever	12/96 (12.5%)	1/43 (2.3%)	0.090	5/77 (6.5%)	2/54 (3.7%)	0.490
Reported triggers for respiratory symptoms						
Respiratory infections	91/96 (94.8%)	41/42 (97.6%)	0.465	77/77 (100.0%)	53/53 (100.0%)	1.000
Pets	62/92 (67.4%)	29/38 (76.3%)	0.315	14/60 (23.3%)	11/49 (22.4%)	0.913
Routine physical activities	44/94 (46.8%)	8/42 (19.0%)	0.003	29/77 (37.7%)	5/54 (9.3%)	<0.001
Physical exercise	86/96 (89.6%)	33/42 (78.6%)	0.090	58/74 (78.4%)	20/51 (39.2%)	<0.001
Aspirin	3/53 (5.7%)	1/22 (4.5%)	0.845	1/51 (2.0%)	0/41 (0.0%)	NA
Cold air	79/97 (81.4%)	24/42 (57.1%)	0.003	61/72 (84.7%)	24/53 (45.3%)	<0.001
Pollutants	55/85 (64.7%)	17/37 (45.9%)	0.055	18/55 (32.7%)	5/47 (10.6%)	0.011
Perfumes	42/90 (46.7%)	23/41 (56.1%)	0.318	20/67 (29.9%)	3/51 (5.9%)	0.003
Wood smoke	41/78 (52.6%)	19/39 (48.7%)	0.695	16/55 (29.1%)	5/46 (10.9%)	0.030
Dust	75/93 (80.6%)	28/42 (66.7%)	0.080	35/70 (50.0%)	13/50 (26%)	0.009
Barns	39/71 (54.9%)	13/28 (46.4%)	0.446	15/50 (30.0%)	4/47 (8.5%)	0.012
Stress	55/92 (59.8%)	18/43 (41.9%)	0.053	24/63 (38.1%)	5/51 (9.8%)	0.001
Menstrual cycle	7/87 (8.0%)	3/40 (7.5%)	0.916	0/72 (0.0%)	0/48 (0.0%)	NA
Pollen	76/93 (81.7%)	31/42 (73.8%)	0.296	34/65 (52.3%)	9/49 (18.4%)	<0.001
Fungus	37/72 (51.4%)	15/34 (44.1%)	0.485	17/53 (32.1%)	6/46 (13.0%)	0.030
Early viral wheeze	0/97 (0.0%)	0/43 (0.0%)	NA	5/77 (6.5%)	18/54 (33.3%)	<0.001
Multi-trigger wheeze	94/97 (96.9%)	43/43 (100.0%)	0.995	72/77 (93.5%)	35/54 (64.8%)	<0.001
Asthma therapy						
Short-acting β-agonist	95/97 (97.9%)	42/43 (97.7%)	0.921	75/77 (97.4%)	40/54 (74.1%)	<0.001
Nebulised β-agonist	22/97 (22.7%)	2/43 (4.7%)	0.019	16/77 (20.8%)	2/54 (3.7%)	0.013
ICS						
Any dose	97/97 (100.0%)	43/43 (100.0%)	1.000	75/77 (97.4%)	24/54 (44.4%)	<0.001
⩽400 µg BUD equivalent	0/96 (0.0%)	42/43 (97.7%)	NA	0/69 (0.0%)	24/26 (92.3%)	NA
⩾800 µg BUD equivalent	96/96 (100.0%)	0/43 (0.0%)	NA	69/69 (100.0%)	2/26 (7.7%)	NA
Oral corticosteroids	23/97 (23.7%)	0/43 (0.0%)	NA	4/77 (5.2%)	0/54 (0.0%)	NA
Any LABA	94/97 (96.9%)	25/43 (58.1%)	<0.001	34/77 (44.2%)	0/54 (0.0%)	NA
Inhaled combination LABA/ICS	89/97 (91.8%)	25/43 (58.1%)	<0.001	32/77 (41.6%)	0/54 (0.0%)	NA
Anti-cholinergic	14/97 (14.4%)	2/43 (4.7%)	0.112	7/77 (9.1%)	3/54 (5.6%)	0.457
Leukotriene modifier	71/97 (73.2%)	10/43 (23.3%)	<0.001	58/77 (75.3%)	20/54 (37%)	<0.001
Xanthine	15/97 (15.5%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
Cromones	10/97 (10.3%)	1/43 (2.3%)	0.140	1/77 (1.3%)	0/54 (0.0%)	NA
Antibiotic therapy	17/97 (17.5%)	1/43 (2.3%)	0.036	10/77 (13.0%)	5/54 (9.3%)	0.511
Mucolytic	2/97 (2.1%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
Anti-IgE therapy	17/97 (17.5%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
SMART regime	23/97 (23.7%)	4/43 (9.3%)	0.055	0/77 (0.0%)	0/54 (0.0%)	NA
Adherence to therapy, MARS total	22.76±0.23 (n=94)	21.3±0.48 (n=43)	0.003	22.85±0.26 (n=73)	22.18±0.43 (n=44)	0.161

Data are presented as n/N (%) or mean±SE, unless otherwise stated. p-values were calculated using a two-sample t-test or a Chi-squared test. PC_{20}: provocative concentration causing a 20% fall in FEV_1; FEV_1: forced expiratory volume in 1 s; ICS: inhaled corticosteroids; ICU: intensive care unit; BUD: budesonide; LABA: long-acting β-agonist; SMART: Symbicort Maintenance and Reliever Therapy (AstraZeneca); MARS: Medication Adherence Report Scale (a five-item self-report scale for assessment of adherent behaviour including unintentional and intentional non-adherence; each item was answered using a five-graded response scale (very often (1) to never (5)), so low scores indicate low levels of adherence); NA: not applicable.

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TABLE 3 Lung function and airway inflammation

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Lung function						
FEV_1 pre-salbutamol						
% predicted	88.68±2.15 (n=96)	93.51±2.47 (n=42)	0.186	104.34±3.21 (n=19)	99.23±5.29 (n=10)	0.390
z-score	−0.92±0.18 (n=96)	−0.53±0.2 (n=42)	0.190	0.33±0.24 (n=19)	−0.03±0.4 (n=10)	0.421
FVC pre-salbutamol						
% predicted	102.15±1.65 (n=96)	104.45±2.02 (n=42)	0.418	107.99±3.5 (n=19)	103.54±5.23 (n=10)	0.473
z-score	0.16±0.14 (n=96)	0.37±0.17 (n=42)	0.381	0.55±0.25 (n=19)	0.25±0.38 (n=10)	0.487
FEV_1/FVC ratio	0.77±0.01 (n=97)	0.8±0.02 (n=42)	0.169	0.91±0.02 (n=19)	0.89±0.02 (n=10)	0.678
Absolute % change in FEV_1 with salbutamol	12.36±1.41 (n=84)	8.98±1.44 (n=42)	0.133	7.89±2.33 (n=15)	9.43±3.39 (n=6)	0.724
Total lung capacity L	4.69±0.12 (n=97)	4.28±0.19 (n=43)	0.073	1.05±0.08 (n=76)	1.09±0.11 (n=53)	0.748
Specific airway conductance $kPa \cdot s^{-1}$	1.58±0.1 (n=54)	1.95±0.18 (n=23)	0.054	2.03±NA (n=1)	3.16±NA (n=1)	NA
Airway inflammation						
Exhaled nitric oxide ppb	33.5 (15.4–62.2) (n=92)	35.84 (14–41) (n=38)	0.152	NA	NA	NA
Sputum eosinophils absolute	5.5 (2.2–14) (n=14)	16.5 (2.8–17.2) (n=4)	0.944	NA	NA	NA
Sputum eosinophils %	1.06 (0.4–2.7) (n=14)	3.34 (0.5–3.5) (n=4)	0.927	NA	NA	NA
Sputum neutrophils absolute	151.5 (77.8–354.5) (n=14)	224.25 (187–306.2) (n=4)	0.645	NA	NA	NA
Sputum neutrophils %	32.55 (16.6–68.7) (n=14)	43.11 (34.5–61) (n=4)	0.670	NA	NA	NA

Data are presented as mean±SE or median (interquartile range), unless otherwise stated. Predictive lung function equations from QUANJER *et al.* [19] were used to generate predicted values and z-scores. p-values were calculated using a Kruskall–Wallis test. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; NA: not applicable.

TABLE 4 Asthma quality of life, exacerbations and control

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Asthma-related quality of life						
PAQLQ						
Total mean	4.77±0.15 (n=91)	5.8±0.19 (n=39)	<0.001	NA	NA	NA
Total z-score	−0.22±0.1 (n=91)	0.51±0.14 (n=39)	<0.001	NA	NA	NA
Symptoms	4.57±0.16 (n=91)	5.77±0.19 (n=39)	<0.001	NA	NA	NA
Emotion	4.91±0.18 (n=91)	6.03±0.19 (n=39)	<0.001	NA	NA	NA
Activity limitation	3.91±0.15 (n=91)	4.57±0.19 (n=39)	0.012	NA	NA	NA
PACQLQ						
Total	NA	NA	NA	4.27±0.18 (n=77)	6.04±0.18 (n=52)	<0.001
Total z-score	NA	NA	NA	−0.46±0.09 (n=77)	0.66±0.12 (n=52)	<0.001
Exacerbations						
Exacerbations in previous year	3 (2–5) (n=97)	1.05 (0–2) (n=43)	<0.001	3.91 (1–6) (n=77)	1.83 (0–2.8) (n=54)	<0.001
Asthma control						
ACT >12 years						
Total	15.49±0.63 (n=67)	20.25±0.81 (n=24)	<0.001	NA	NA	NA
Total z-score	−0.25±0.12 (n=67)	0.69±0.17 (n=24)	<0.001	NA	NA	NA
Total ⩽19	50/67 (74.6%)	7/24 (29.2%)	<0.001	NA	NA	NA
Childhood ACT						
Total	16.38±0.98 (n=29)	19.22±1.01 (n=18)	0.061	15.2±0.79 (n=41)	23±0.67 (n=22)	<0.001
Total z-score	−0.26±0.16 (n=29)	0.23±0.2 (n=18)	0.065	−0.47±0.13 (n=41)	1.01±0.16 (n=22)	<0.001
Total ⩽19	19/29 (65.5%)	7/18 (38.9%)	0.078	32/41 (78%)	4/22 (18.2%)	<0.001
Combined ACT[#]						
z-score	−0.26±0.1 (n=95)	0.47±0.13 (n=41)	<0.001	−0.47±0.13 (n=41)	1.01±0.16 (n=22)	<0.001

Data are presented as mean±SE, median (interquartile range) or n/N (%), unless otherwise stated. p-values were calculated using a Kruskall–Wallis test or Pearson's Chi-squared test. PAQLQ: Paediatric Asthma Quality of Life Questionnaire; PACQLQ: Paediatric Asthma Caregiver's Quality of Life Questionnaire; ACT: Asthma Control Test (used for participants >12 years; children aged 4–11 years completed the childhood ACT); NA: not applicable. [#]: to allow the joint analysis of the ACT and childhood ACT, data were transformed to improve symmetry and then z-scores were calculated.

TABLE 5 Factors associated with asthma burden as measured by quality of life

	Sample size n	Regression coefficient	95% confidence interval	p-value
FEV_1 z-score#	161	0.151	0.05–0.25	0.002
FEV_1/FVC	162	1.113	−0.23–2.46	0.104
Age of onset years	247	0.028	−0.03–0.08	0.311
Specific airway conductance kPa·s^{-1}	78	0.132	−0.16–0.42	0.366
Log exacerbations in previous year	263	−0.523	−0.67–−0.38	<0.001
ACT combined z-score	196	0.730	0.63–0.83	<0.001
BMI kg·m^{-2}	261	−0.036	−0.06–−0.01	0.011
MARS total	249	−0.040	−0.09–0.01	0.097
Hay fever diagnosed	211	−0.281	−0.57–0.01	0.057
Eczema diagnosed	225	−0.116	−0.42–0.19	0.452
Atopy	225	−0.070	−0.34–0.2	0.612
Second-hand smoke	257	−0.022	−0.33–0.29	0.890
White ethnicity	263	0.346	0.04–0.66	0.028

Data represent linear regression analyses looking at the association between each factor and quality of life. Quality of life was assessed by the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) or the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ). To allow the joint analysis of the PAQLQ and PACQLQ, data were transformed to improve symmetry and then z-scores were calculated. In order to make maximum use of the data, plethysmography (specific airway conductance), with 194 missing values, was excluded from joint modelling. Analysis was also performed for PAQLQ and PACQLQ separately (results in the online supplementary material). FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ACT: Asthma Control Test; BMI: body mass index; MARS: Medication Adherence Report Scale. #: predictive lung function equations from Quanjer *et al.* [19] were used to generate z-scores.

Discussion

This article presents the detailed clinical characteristics of 282 children in four paediatric cohorts, including preschool and school-age children with both severe and mild/moderate wheeze and asthma across Europe. Standard Operating Procedures and Good Clinical Practice criteria were used to ensure consistency and quality across sites, with data collected on an online platform and stored in a single online repository. The severe cohorts by definition had a significantly higher treatment burden than the mild/moderate ones, despite which they remained poorly controlled with frequent severe exacerbations and low ACT scores. Children with severe disease, and their caregivers, had significantly lower QoL scores across all domains than the mild/moderate cohorts. Asthma control and airway obstruction were found to be significantly associated with QoL. Exposure to environmental tobacco smoke in the SW cohort was a striking finding and will be an important concomitant factor in future analyses. Otherwise, the severe and mild/moderate cohorts were very similar; this is in contrast to the adult severe and mild/moderate U-BIOPRED cohorts [11] and suggests that paediatricians should be cautious about extrapolating from adult studies. The vast majority of children were atopic. The rates of reported food allergy were high, although the rate of actual food allergy is expected to be much lower [24]. Most had a normal BMI, unlike the typical adult severe asthma phenotype. Also conspicuous was the morbidity in the mild/moderate paediatric groups; although they were clearly differentiated from the severe groups, a number are clearly sub-optimally treated. These data demonstrate that we succeeded in recruiting severe paediatric cohorts and provide a comprehensive view of the clinical burden of severe asthma or wheeze in childhood.

Children in the U-BIOPRED SA and SW cohorts have frequent symptoms and severe exacerbations that adversely impact on QoL and carry a high treatment burden; almost 17% of the SA cohort was prescribed omalizumab and 24% prescribed maintenance oral corticosteroids. This is in keeping with a previous study, which reported a strong association between health-related QoL and ACT score in children with problematic severe asthma [25]. In our study, the impact on QoL was seen to be greatest for the SW cohort. A significant impact on QoL was also seen in the mild/moderate cohorts, highlighting the often overlooked influence of asthma on the lives of children and their families. Allergic sensitisation and other atopic diseases were a frequent finding across all cohorts, more so in school-aged children, adding further to the treatment burden. Lung function was not significantly different in the school-aged cohorts, possibly due to good treatment adherence, being between exacerbations and the fact that FEV_1 is not a good discriminator of severity.

U-BIOPRED builds upon previous severe asthma cohort studies [26–29]. However, to our knowledge, this is the first study to recruit preschool wheeze cohorts on the basis of a consensus definition, which can be

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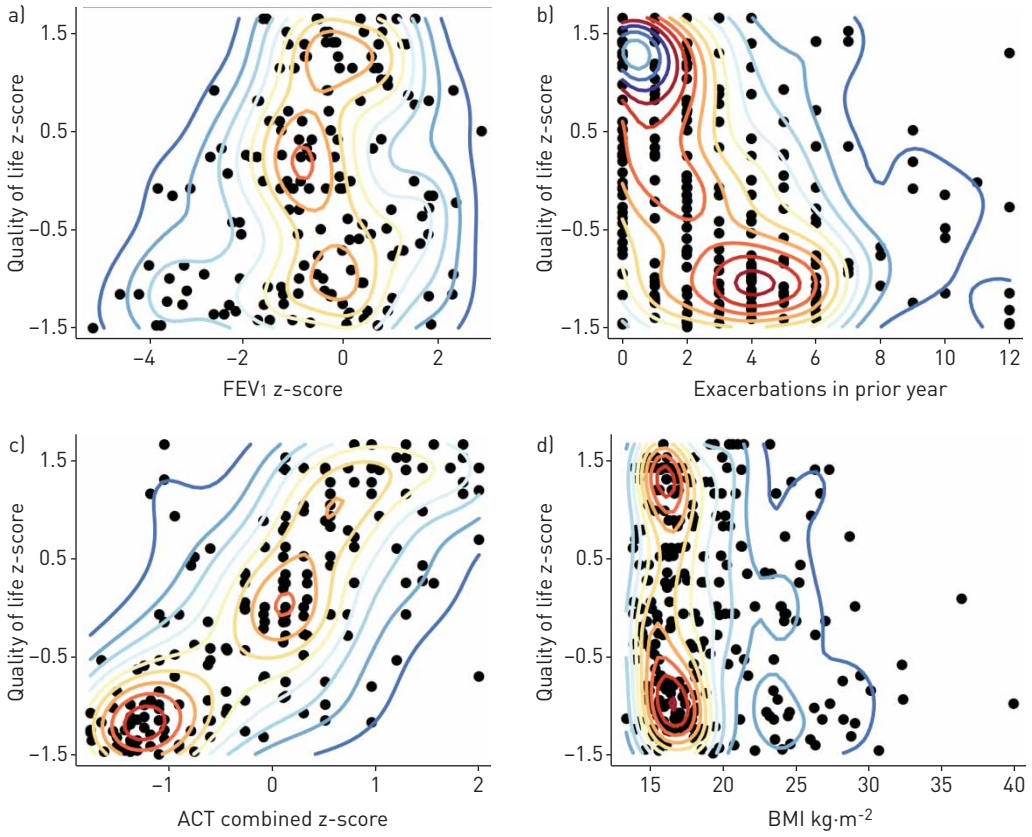

FIGURE 3 Factors associated with asthma burden as measured by quality of life. Figures represent scatter plots describing the relationship between each factor and the combined asthma-related quality of life z-score. The contour lines are coloured blue to red, to indicate increasing density of points in the graph to overcome the issue of overlying data points. The contour plots show c) a strong positive relationship between quality of life and asthma control (Asthma Control Test (ACT)) with a) a weaker positive relationship between quality of life and lung function (forced expiratory volume in 1 s (FEV_1) z-score). Additionally there is b) a strong negative relationship between quality of life and exacerbation rate plus d) a weaker negative relationship between quality of life and body mass index (BMI). The combined z-score merges the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) and the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ). The density was modelled using two-dimensional kernel density estimation.

directly compared with parallel school-age and adult cohorts [13]. Most studies of preschool wheeze have been based on birth cohorts. A small number of studies have focused on severe preschool wheeze [9, 30] and they have provided valuable insights into the underlying pathophysiology and natural history of preschool wheeze. In common with the TENOR (The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens) [27] and SARP (Severe Asthma Research Program) [26, 28] severe asthma cohorts, U-BIOPRED children with severe asthma were commonly atopic, had high healthcare utilisation and a high treatment burden. In the TENOR study there were far more boys than girls (63% *versus* 37%) in the severe cohort but, in common with SARP, we did not see these sex differences. Unlike SARP, children in the SA cohort did not have significantly higher F_{ENO} levels than those in the MMA cohort; however, F_{ENO} measurements were made off-line in SARP, making it difficult to make direct comparisons.

There are a number of limitations to this study. There were no healthy controls recruited to the paediatric cohorts; however, as the aim was to understand what makes asthma severe, the mild/moderate cohorts are the most appropriate comparator. The mild/moderate asthma group were all on prophylactic medication and participants were recruited from general paediatric and respiratory clinics so they are not completely representative of the children with mild/moderate asthma or wheeze seen in primary care. Also, as this is a multicentre pan-European study, it is likely that there were differences in patients recruited into each cohort between centres. Feasibility and safety considerations meant that assessments such as airway hyperresponsiveness were not included. Additionally, preschool children were unable to perform lung function, induced sputum and F_{ENO}. We were not able to reach the target of 100 preschool severe wheeze children; many had not been under tertiary follow-up for $\geqslant$6 months, did not reach the treatment threshold or did not meet the stringent inclusion criteria at screening due to the intermittent nature of

their symptoms. There was no objective measure of adherence during the study; however, this was a pragmatic study of real-life severe asthma where clinics had tried to exclude adherence issues, and the high MARS scores suggest a good level of adherence.

Despite advances in recent years in our understanding and management of severe asthma, the data presented here highlight the ongoing unmet needs. Both severe asthma and severe wheeze are heterogeneous diseases. Single or even clustered biomarkers have had limited impact in predicting clinical course or therapeutic efficacy in children: for example, the SA cohort is not distinguishable from MMA by classical lung function and airway inflammatory phenotypes [5, 7, 9]. Classification of preschool wheeze phenotypes is at an even more basic level, limited to symptom pattern [31, 32] and progression to asthma determined retrospectively. Analysis of samples from these cohorts will provide high-dimensional biological ("omics") data, which can be integrated with clinical characteristics to define multidimensional handprints of severe asthma. This approach has the potential to allow a step change in our understanding of asthma, identify more relevant prognostic and therapeutic targets and enable a personalised, phenotype-driven approach to management to address the unmet burden.

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